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Optimum Extraction of Algae-oil from Microalgae using Hydrodynamic Cavitation

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Abstract- The production of microalgae biodiesel is one solution to solve the energy problem in the future, especially in the transportation sector. Some advantages of the development of the production of biodiesel from microalgae is high productivity, food security friendly and global warming problem-solving . The main problem in the production of microalgae biodiesel is the high production cost due to the high cost of lipid extraction. Several studies have been conducted to find a low cost extraction process. In this research, the *Nannochloropsis sp.* microalgae lipid extraction utilizing hydrodynamic cavitation has been studied. Effects of the concentration of solids, specific energy and time on the extraction have been examined. The study was conducted in a batch with a combined solvent of methanol and hexane. The influence of specific energy given at the same level of cavitation of increasing specific energy would increase the yield where it would be higher at the beginning and remain stable at the end of the process. At the same specific energy and level of cavitation, the value of concentration that provides the lowest extraction energy requirement was 0.105 g/g. The optimum of cavitation levels for the same specific energy and concentration at the value of cavitation number was 0.126. The optimum temperature for the extraction process was 42°C. The lowest extraction energy requirement was 16.743 MJ/kg lipid.

Keywords *Nannochloropsis sp.*, microalgae, lipid extraction, hydrodynamic cavitation, energy.

1. Introduction

The decreasing source of fossil fuel and the increasing demand for fuel in the recent years lead to the energy problem. Other problems arising by using fuel are the increasing combustion flue gas emission that causes greenhouse effect [1] and the ineffective application that cause high energy requirement [2]. These problems require the search for alternative and environmentally friendly renewable energy source to be conducted. Biodiesel is one alternative solution. The first generation of biodiesel has been produced from palm oil. However, the developing biodiesel production from palm oil influences food security [3]. The viable seeking renewable energy source that is not influencing food security can be found in the non food source. Some sources of it has been investigated to develop

second generation of biodiesel. These sources are namely: oil palm empty fruit bunches (EFB) [4], palm fatty acid distillate [5], sugarcane baggase [6], wood, vegetables and fruits waste [7, 8, 9], and non edible seed jatropha, papaya seed [10, 11]. Currently the creation of third generation of biodiesel from microalgae lipids becomes the lime light of some researchers [9].

In the light of its challenge, microalgae has then been estimated as a very good potential source to be used for fuel production (algae fuel) [12, 13, 14, 15]. The advantages of microalgae lie on their higher photosynthetic efficiency, higher biomass production and faster growth when being compared to other energy crops [16]. Microalgae can be cultivated in large scale on non-arable lands and does not need potable water to grow. Consequently, there is no competition with food production that is allocated for

growing human population [17, 18]. Another advantage from microalgae lies on its ability to prevent global warming caused by carbon dioxide emission in the air [19]. This is possible because microalgae needs carbon dioxide to grow. Carbondioxide as a combustion product can be absorbed by microalgae used for photosynthesis to produce lipid, of which then can be converted to biodiesel by transesterification [20].

The key processes involved in the microalgae biodiesel production are cultivation, harvesting, lipid extraction and transesterification. Intensive research on microalgae biodiesel production over these several years is still hindered by high overall production costs. This makes biodiesel production uncompetitive in the market, and it is unsustainable according to several life-cycle assessments (LCA) [21, 22, 23]. Therefore, finding a solution to reduce the production costs has become preponderant [24]. Among these processes, the lipid extraction is the most costly one with the portion of 70-80% of total cost [25]. Direct lipid extraction from wet microalgae is not an easy process due to algal cell walls, which are mainly composed of cellulose. Owing to its high resistance level, it is difficult to break [26].

Several microalgae lipid extractions that use from conventional to advanced methods were reported in some literatures [17, 20, 21, 22]. The conventional extraction used only chemical solvent that had low efficiency. The advanced methods of extraction were employed by assisted cell disruption which got higher efficiency than conventional one. Microalgae cell disruption could be done through chemical, mechanical and enzymatic processes. Extraction that used mixing solvent (organic polar and non polar) could increase the extraction yield compared to the usage of single solvent [27]. Organic non polar solvent could break the protein lipid membrane and the organic polar solvent could break the protein lipid link [28]. But using more solvent would increase purification cost and chemical wastes. Therefore, the mechanical post treatments such as mechanical disruption were needed to ensure the complete extraction of neutral lipid (triglycerides) in particular. The study of lipid extraction assisted by mechanical cell disruption processes have been published by many researchers. Autoclaving, bead-beating, high-pressure homogenization, and ultrasonication were commonly practiced methods [26, 27, 29, 30]. The amount of energy used to disrupt the cell through hydrodynamic cavitation (HC) was the smallest one compared to other processes of mechanical cell disruption [31].

The cavitation phenomenon is occurring microbubbles when the pressure of the fluid drops until lower than its vapour pressure; these microbubbles collapse when the pressure returns to the level above the vapor pressure. The collapse of the microbubbles produces shock waves and increases the momentary pressure (100–5,000 atm) and temperature (500–15,000 K), which mechanically disrupts the microalgal cell [32]. The magnitude of the shock pressure by microbubbles collapse in the cavitation can be calculated. The changing bubble diameter as 1/20 times with difference of pressure at stream (P_{∞}) and vapor pressure (P_v) of 1 bar, can generate pressure shock as big as 1244 atm [33].

Cavitation number is a non dimensional parameter that determines the phenomenon of cavitation. The maximum value of the inception of cavitation number is called σ_{vi} . When cavitation number is smaller than σ_{vi} , it means cavitation has happened, meanwhile when it is higher than σ_{vi} it means no cavitation has occurred. The σ_{vi} for the flow around the circular pipe is around 1.5 [32]. One cause of cavitation is the change of fluid velocity in the closed channel and this phenomenon is called hydrodynamic cavitation (HC). The cavitation is generated by change of liquid velocity which passing through a large cross sectional area into a constriction, such as an orifice plate, venture meter, or throttling valve.

HC has been widely used, among them is the intensification of chemical processing applications [34], while another application is to assist the synthesis of methyl ester leading to reduced energy requirement compared to conventional methods [35]. HC is used as cell disruption technique that was investigated to the *Tetraselmis sp.*, where the level of cell disruption determined by the amount of lipid extracted and chlorophyll released. It was found that for lipids extraction, HC technique requires energy as much as 3 MJ/kg dry mass of microalgae with the microalgae concentration of 1.5-2% w/w [36]. HC that assists the lipid extraction from *Nannochloropsis Salina sp* was reported with varied amount of energy input, time of extraction and comparison with ultrasonic cavitation. The hydrodynamic cavitation has higher extraction yield when being processed at the same energy input. The dry microalgae concentration used is at 2 %w/vol [32].

This study attempts to find the more an energy efficient condition, by using the larger microalgae concentration with the range of 5-11 % mg/l. Another aim intended to achieve is to find out the influence of some operational variables which includes the amount of specific energy, the microalgae concentration, the level of cavitation and the temperature on the extraction yields.

2. Materials and Methods

Dry microalgae *nannochloropsis sp* was purchased from Central Institute of Brackish Water Cultivation (*Pusat Budidaya Air Payau*) Situbondo, East Java, Indonesia. Hexane technical grade (Density of n-hexane = 0.66 kg/l and normal boiling point = 68 °C) was purchased from Brataco chemicals (PT. Brataco), a local chemical company and store at Yogyakarta, Indonesia. Methanol technical grade (density of methanol = 0.791 kg/l, molecular weight of methanol = 32.034 g/mol and normal boiling point = 64.7 °C) was purchased from Multi Kima chemicals (CV Multi Kimia), a local chemical store at Yogyakarta, Indonesia.

2.1. Equipment

The experimental has been carried out on extraction cavitation batch. A compressor was used to provide compressed air to drive the mixture of solvent and microalga. Venturi was equiped to generate cavitation as shown in Figure 1. Sample and product chamber were used to store

feedstock and collected raw product. The separation equipments being utilized were sedimentator and distilator. Sedimentator was used to separate the fluid from solid product by gravitational force, and distilator was used to separate the solvent from lipid by evaporating the solvent.

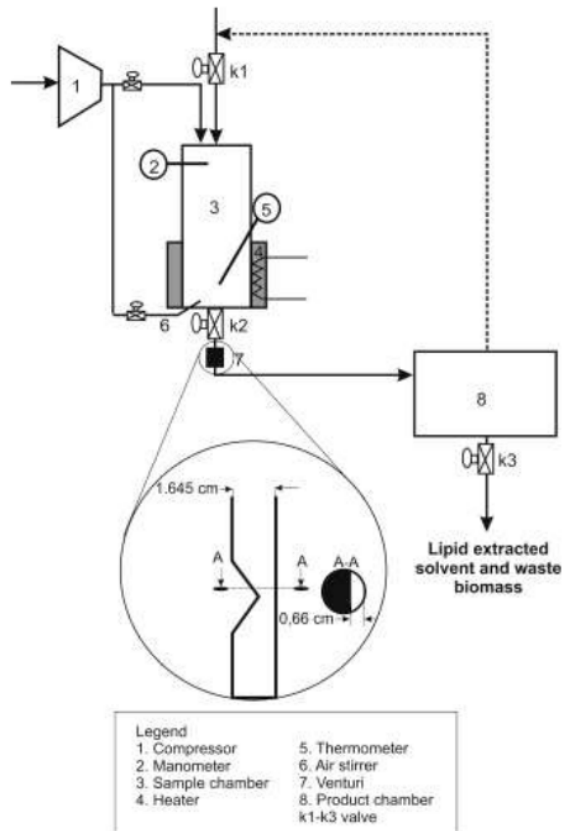


Fig 1. The scheme of experiment equipment

2.2. Experimental Procedures

The experiments were performed by mixing 10 grams of *Nannochloropsis sp.* with 95 ml of hexane and 41 ml of methanol into the sample chamber. Then, compressed air stream was inserted into the sample chamber until the pressure achieved the level of 6.8 atm. The valve (k2) was then opened in order to make way for the microalgae and solvent to flow through the venturi and accommodate the product chamber. This procedure was repeated for several rounds (2, 3, 4, etc.) to attain variables of extraction. After the extraction processes were done, the product in the beaker glass being stood for 10 minutes until the solid and liquid layers being formed. The liquid phase of the solids was then separated and the remaining solids were washed with 25 ml methanol and 25 ml of hexane separately. The washing of the methanol and hexane was done by combining solvent that has been separated in the first step. The next step was separating lipid from solvent through distillation process in order to obtain a residue with a constant weight. The residue

was then weighed and recorded as w_1 . It was washed with hexane 5 ml and the remaining solids were dried up until the weight remained constant and recorded as w_2 . Lipid weight (w_p) and extraction yield (y) which were obtained from microalgae were obtained from equations (1) and (2), respectively,

$$w_p = w_1 - w_2 \quad (1)$$

where w_1 and w_2 are the weight of lipid after separated from solvent and after separated from solvent and the solid debris, respectively

$$y = \frac{w_p}{w_{mi}} \quad (2)$$

where y is extraction yield, w_{mi} is initial weight of dry microalgae

2.3. Energy for Cavitation

The amount of energy used to drive the mixture can be calculated by adopting equation (3):

$$E = f \cdot S \quad (3)$$

where E is energy (Joule), f is power (Newton) that can be calculated using pressure (kg/cm^2) multiplied by cross sectional area (cm^2) and S is length of power trajectory in this case is the depth of sample (cm).

Specific energy (E_s) is a comparison between the amount of energy input and the feed weight. The E_s was computed using equation (5)

$$E_s = \frac{E}{(w_{mi} + w_{solvent})} \quad (4)$$

where E_s is specific energy and $w_{solvent}$ is solvent weight

Specific yield (y_s) is a comparison between the weight of extracted lipids and the amount of energy input. The y_s was computed using equation (5)

$$y_s = \frac{w_p}{E} \quad (5)$$

2.4. Cavitation number

The cavitation number must be calculated to ensure that cavitation was occurred, and in the case of gate the cavitation number can be calculated by the following equation [25], as determined with equation (6)

$$\sigma_v = \frac{P_{downstream} - P_v}{P_{upstream} - P_{downstream}} \quad (6)$$

where σ is cavitation number, P is pressure. Flow around the 10 mm diameter circular cylinder, and the cavitation inception number is around 1.5.

2.5. Lipids extraction and identification

The lipids were extracted from dry microalgae by using the mixture of solvent methanol and hexane. 5 mg of dry microalgae was mixed with 20.5 ml of methanol and 47.5 ml of hexane and stirred at 1000 rpm for 2 hours. Then, the methanol and hexane containing lipids were separated using

centrifugal process. This step was then repeated three times. The lipids were recovered by evaporating the methanol and hexane, then were weighed and recorded as w_1 . The next step was the lipids were washed with hexane 5 ml and the remaining solids were dried up until the weight remains constant and recorded as w_2 and lipid weight (w_p) obtained from microalgae was calculated using equation (1). Lipids content was analyzed using gas chromatography-mass spectrometry (GCMS).

3. Results and Discussion

3.1. Total lipid content

The result of the process of determining total lipid content of microalgae *Nannochloropsis sp* that was used as the sample was of 10.46% weight of lipid / weight of dry microalgae. Figure 2 shows the composition determining by GCMS. It shows some high peaks at the retention time of 26.598 with 24.76% area of this peak indicated a compound with a formula of $C_{20}H_{38}$. Peaks at retention time of 27.097 and 27.62 indicated $C_{20}H_{40}O$ compound. And the four major compounds could be found in the lipids are listed in the Table 1.

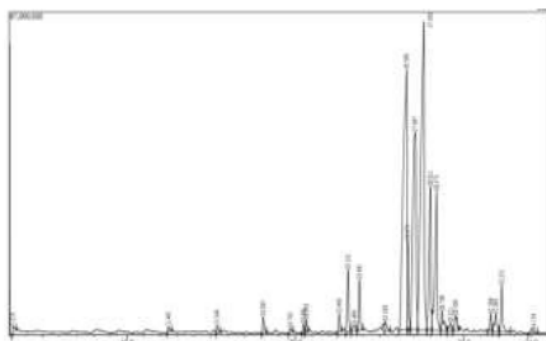


Fig 2. Result of GCMS lipid analysis

Tabel 1. Lipid composition of *Nannochloropsis sp*

Fatty acid	Composition, %
$C_{20}H_{40}O$	45.05
$C_{20}H_{38}$	24.76
$C_{17}H_{34}O_2$	7.64
$C_{17}H_{32}O_2$	6.99
Others	15.56

Table 1 shows that the lipids of *Nannochloropsis sp.*, composed of fatty acids with C20 atoms chain with a composition of 69.81%. The content of fatty acids in these lipids shows that lipids from microalgae *Nannochloropsis sp.* potentially be processed into biodiesel.

3.2. Effect of amount of specific energy

Figure 3. shows the influence of specific energy against the level of extraction which was measured by the lipid yield. It shows the extraction yields were increased by the

increasing of the specific energy. The first extraction process formed specific energy of 0 to 91.3 J/g, while the increasing extraction yield formed a percentage of 0 to 4.42%. This was the highest extraction yield increase, and then plateau off with only a slight of increase and a fix specific energy increase. And the extraction yields with energy specific above 1200 J/g tended to be constant at the value of total lipids content.

This phenomenon was similar with the extraction curve of hydrodynamic cavitation extraction which was done by Lee and Han [32]. The extraction yields (g extracted lipid/g of total lipid, in wt%) of this experiment was 93% at the specific energy 1186 kJ/kg, microalgae concentration 9.5% and temperature 30°C. It was higher than another hydrodynamic cavitation extraction which was around 42% at specific energy 2000 kJ/kg and microalgae concentration 2% at the same temperature [32]. The higher extraction yield was caused by higher microalgae concentration.

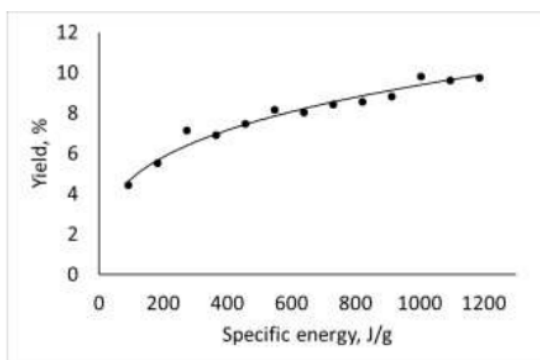


Fig 3. Effect of specific energy on yield.

The reduction of increasing extraction yield with constant addition of specific energy showed that energy utilization was more inefficient when specific energy increased. This phenomenon is explained in Figure 4.

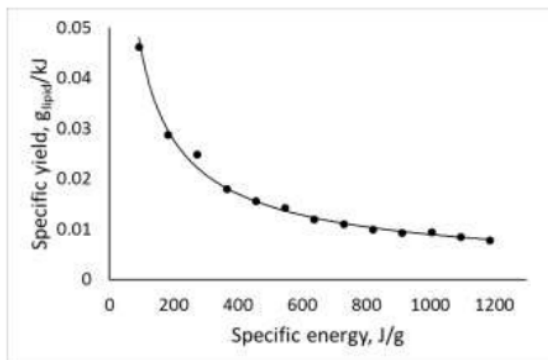


Fig 4. Effect of specific energy on specific yield

Figure 4 shows that the specific yield tended to decrease along with the increasing of specific energy. At the beginning of the process, the specific energy was of 91.3 J/g and the specific yield was of 0.046 g_{lipid}/kJ . Further, specific yield tends to decrease along with the increasing of specific energy. It means the energy efficiency decreased as the

specific energy increased. This phenomenon shows that hydrodynamic cavitation was effectively assisting the lipid extraction at the beginning of the process. It caused the cell disruption of microalgae. The reduction of energy efficiency during the extraction process causing a lower cell disruption at the later cavitation as compared to the beginning of the process. In this process, the increasing of specific energy means the increasing of processing time or the increasing of the repetition of cavitation. This is due to every cavitation was using the equal specific energy, therefore the impact of the next cavitation to the microalgae was not as high as at the beginning. It is due to the existence of broken cells, and therefore the cavitation impacting them. Another cause of the efficiency reduction was the decreasing mass transfer driving force. It caused the microalgae lipid concentration at the next process decreased and the lipid concentration in the solvent increased. Furthermore, the limitation of energy input or of cavitation repetition number is needed to be set in order to optimize the energy extraction.

The lowest energy requirement for lipid extraction assisted by hydrodynamic cavitation at the cavitation number 0.068, microalgae concentration 9.5% and temperature 30°C was 22.13 MJ/kg lipid at the specific energy 98 kJ/kg dry microalgae and the highest was 130.89 MJ/kg lipid at the specific energy 1211 MJ/kg dry microalgae. The amount of energy available from the combustion of biodiesel was around 42 MJ/kg [36]. So this extraction process was feasible with once through cavitation.

3.3. Effect of the concentration

Figure 5 shows how the solids concentration of microalgae affected the extraction yield. The yield increased along with the increasing of microalgae concentration that was from 0.05 to 0.073 g microalgae/g (microalgae + solvent), however at the concentration above 0.073 the extraction yield tended to decrease. Figure 6 shows the specific yield tended to increase along with the increasing of microalgae concentration. The increasing specific yield at the microalgae concentration from 0.05 to 0.073 g/g was higher than the increasing specific yield at the value above 0.073 and then it was tend plateau off. This phenomenon might occurred due to the influence of the solid to flow and the cavitation events did not affect all of the solids. With the input of specific energy was relatively fixed, therefore the increase of solids concentration would affect the flow of microalgae and solvent mixtures, and their potential to slow down the flow and the cavitation became smaller. However the increasing concentration of solids caused the amount of microalgae which was affected by the cavitation becoming higher. When it was compared to the amount of microalgae feed, the fraction of affected microalgae was tend to decrease, consequently the yield tended to decrease at the concentration above 0.073 g/g.

Figure 6 shows that specific yield tended increase from microalgae concentration 0.049 to 0.095 and then plateau off after that. It indicated that energy efficiency was reaching the maximum value at the point that specific yield began tend to plateau off. This phenomenon shows that optimal microalgae

concentration values in order to obtain optimal extraction process. In this case at the specific energy 470 J/g, temperature 30 °C and cavitation number 0.068 with the optimum microalgae concentration at 0.105 g/g.

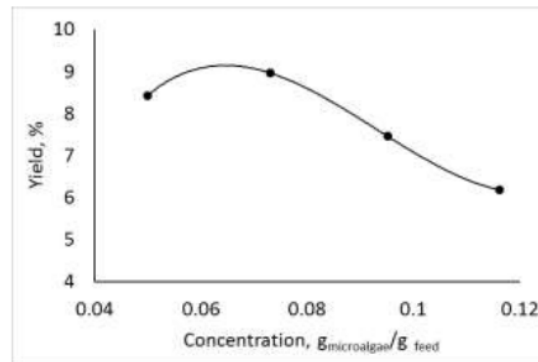


Fig 5. Effect of microalgae concentration on yield

The study of extraction *Jatropha* oil using mixture methanol and hexane solvent was tend to decrease the extraction efficiency with the decreasing solid concentration [37]

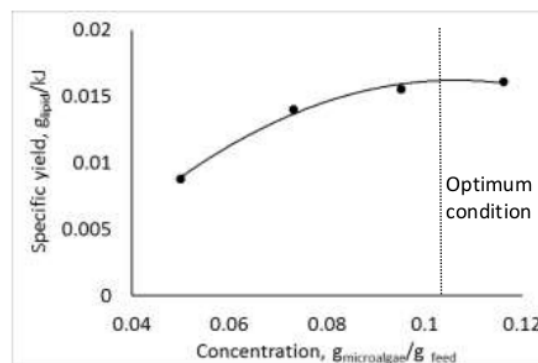


Fig 6. Effect of microalgae concentration on specific yield

3.4. Effect of Cavitation Numbers

Figure 7 shows the effect of cavitation number on yield. From this figure we may see that the yields increased along with the increasing cavitation number from 0.068 to 0.126 and then decreased at the cavitation number larger than 0.126. In this experiment, various cavitation numbers were done at the same specific energy resulting in various pressure booster and number of repetition. The values of cavitation number, pressure booster and number of repetition are shown at the Tabel 2.

Tabel 2 shows that to increase cavitation number, the decreasing pressure booster and increasing number of repetition needed to be performed. Figure 7 illustrates and explains that at the smallest cavitation number was of 0.068, the phenomenon of the cavitation was at its highest, but the effect to the extraction was apparently less than the value of 0.1 and 0.126.

Tabel 2. Cavitation number related with pressure booster and number of repetition

Cavitation number	Pressure booster, atm	Number of repetition, times
0.068	6.80	4
0.100	5.00	5
0.126	4.17	6
0.156	3.57	7
0.188	3.13	8

It occurred because at the smallest cavitation number the contact time between cavitation and microalga was the shortest. And at the value of 0.1 and 0.126 the time contact became longer and the cavitation amount was sufficient enough. At the value above 0.126, the impact of the cavitation in the extraction process was decreased, although the contact time became longer but the cavitation became weaker. The cavitation number related to the droplet size of bubble, the smaller cavitation number caused smaller droplet size and mixing of the droplets with the fluid caused the turbulence of flow. However at the cavitation number lower than 0.2 the droplets size are not significant reduced. So it is not make higher of extraction yield because the contact time is shorter [38].

The optimal cavitation number value of hydrodynamic cavitation extraction is 0.126 at the temperature 30 °C, specific energy 470 J/g and microalgae concentration 0.073 g/g. This optimum cavitation number for extraction is lower than that to make stable emulsion oil and water which the values are 0.17 to 0.2 [38]. It is because at the extraction process the energy from cavitation shock pressure is used for cell disruption. The lowest energy requirement to lipid extraction at the cavitation number 0.126, microalgae concentration 0.095 and temperature 30 °C is 18.723 MJ/kg lipid.

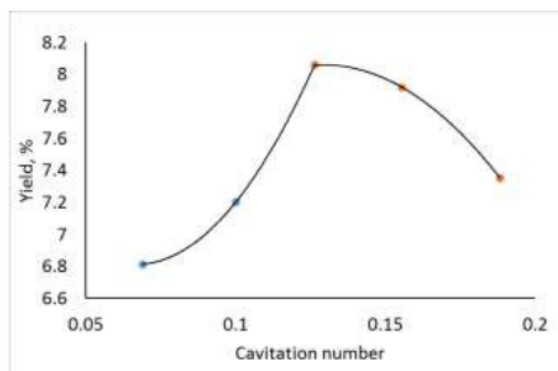


Fig 7. Effect of cavitation numbers on yield

3.5. Effect of Temperature

Figure 8 shows the effect temperature on the yield. From this figure we may see that generally increasing temperature tended to increase yields and at one point would turn back to the decrease in yields. Experiment of cavitation number of

0.1 showed that increasing the yields took place from 30 to 42 °C, and then decrease. The increasing temperature from 30 to 42 °C resulting in the yields increased from 7.2 to 9.53% with a slope of changing at 0.19 %/°C.

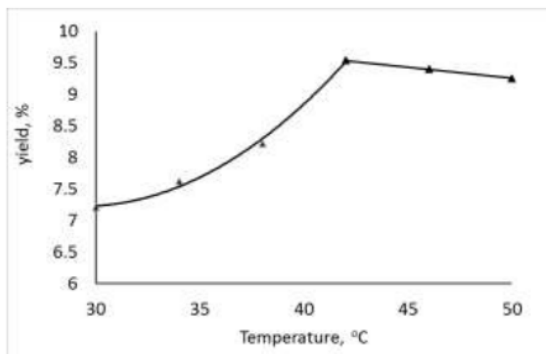


Fig 8. Effect of temperature on yield

This phenomenon occurred because the increasing temperature caused the increasing of distribution coefficient as well as mass transfer driving force. The temperature effect on the distribution coefficient is determined according to the Van't hoff equation [39].

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (7)$$

where K is distribution coefficient, ΔH° is enthalpy change at the standard condition (J/mol), ΔS° is entropy change at the standard condition (J/mol/K) and R is the universal gas constant (J/mol/K). With the value of ΔH° and ΔS° in the common extraction process both are positive [39]. So the increasing temperature will cause increasing distribution coefficient and mass transfer driving force, as shown for the larger lipid concentration difference between on the microalga surface and the fluid bulk. The increasing temperature also caused increasing diffusivities lipid through cell wall and film layer resistance [40]. But in this case diffusivities factor was ignored because the very thin layer and cell wall was disrupted.

The process of cavitation was also greatly affected by the temperature. This was because the cavitation occurred when the pressure level of the system was under the pressure level of vapor phase, where at the higher temperature the vapor pressure was higher so the system became easier to reach the condition under the vapor pressure. Consequently, the evaporation process became easier to happen. However, at higher temperatures, the collapse of bubble was not as easy as at low temperatures, so the cavitation effect at higher temperatures is decrease. This phenomenon shows the need for temperature optimization to obtain an energy-efficient extraction process with maximum results. In this experiment the optimum temperature was 42 °C for processes with cavitation numbers 0.1 and the microalgae concentration 0.073 g/g. The study of extraction Jathopa oil using mixture methanol and hexane solvent was found the optimum temperature was 36 °C [37], and the boiling point of

methanol and hexane was around 52 °C at the atmospheric pressure. The lowest energy requirement for this condition is 18.577 MJ/kg lipid.

4. Conclusion

The microalgae lipid extraction process assisted by hydrodynamic cavitation was influenced by the amount of specific energy input, the microalgae concentration, the level of cavitation, the temperature and the amount of cavitation passes. The increasing specific energy at the same cavitation level tend to decrease the energy efficiency. The temperature, microalgae concentration and level of cavitation at the optimum condition were giving the lowest extraction energy requirement. The optimum condition was found at the specific energy 91.3 kJ/kg, temperature 42 °C, cavitation number 0.126 and the microalgae concentration 0.105 g/g. The lowest energy requirement at the optimal condition was 16.743 MJ/kg lipid.

Form the above fact we may conclude that the lipid extraction from *Nannochloropsis sp* assisted by hydrodynamic cavitation was feasible to develop.

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