

EFFECT OF OHMIC HEATING AS A PRE-TREATMENT METHOD FOR BIODIESEL EXTRACTION FROM MICROALGAE

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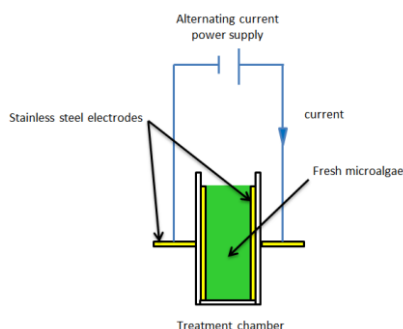
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OHMIC HEATING PRETREATMENT



Abstract

Microalgae are single cell organisms that have the potential to be developed as feedstock for biodiesel oil. One of the problems of using microalgae as feedstock for biodiesel is in the extraction process. Microalgae extraction requires considerable cost. The purpose of this study was to determine the effect of ohmic heating as a method of pretreatment in microalgae oil extraction. The raw materials used were microalgae paste diluted in two levels cell density, 20 g/L, and 30 g/L. The pretreatment using alternating current (AC) electric with two voltage levels (55 V and 110 V), and the duration of pretreatment was 30 seconds and 60 seconds. The next step was drying and extracting microalgae by solvent extraction method of n-hexane. The results showed that the highest oil yields (14.88%) were obtained by cell density treatment 20 g/L, done for 60 seconds of pretreatment and 110 V voltage. This result was higher than without pretreatment, so the use of pretreatment by ohmic heating can improve extracted oil yield than without pretreatment.

Keyword: Pretreatment, microalgae, ohmic heating

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1.0 INTRODUCTION

Microalgae are single-celled organisms that have considerable potential as sources of biodiesel feedstock. Microalgae include microscopic organisms that can perform photosynthesis. Microalgae can be found in marine and freshwater environments [1, 2], and have the ability to produce 1.83 tons of CO₂ in the atmosphere when producing

1 ton of algae biomass [3]. Compared to other oil-producing plants, every hectare of microalgae in the waters can produce 10 to 100 times oil [1, 4].

The use of microalgae as a fuel includes third generation biofuels that have advantages over the previous generation [5]. The advantages of microalgae as biodiesel feedstock are they: (a) can produce higher than food crop, (b) can grow on marginal land, (c) can utilise seawater and

wastewater as culture medium, (d) can produce material burns that are non-toxic, biodegradable and renewable [6].

The process of making biodiesel from microalgae goes through several stages, i.e. cultivation, harvesting, drying, extraction, and esterification. The extraction stage is an important step to take the microalgae oil. This stage required a relatively expensive cost. In the case of biodiesel production using microalgae, 30 - 50% of the production cost for extraction activities includes cell disruption [7]. The greatest challenge to microalgae oil extraction is in the process of lysis or disruption of cells, so the cell contents are easy to remove. Oil is present in the lipid granules inside the cell.

The methods for the extraction of oil from microalgae can be done with mechanical or physically methods (oil expeller, microwave assisted extraction, ultrasonic assisted extraction) and chemically methods (Soxhlet extraction, supercritical fluid extraction, accelerated solvent extraction) [4]. Extraction results can physically remove the oil by about 75%, while chemically removing 90 - 100 % oils. Increased extraction results can be done by damaging cells or lysis. Various methods are being developed by researchers to improve the extraction results, e.g., chemical, mechanical, thermal or enzymatic. A combination of extraction using cell destruction will increase extraction efficiency [8-10].

Various methods of cell destruction continue to be developed such as high-pressure homogenizer (HPH)[11,12], sonication [13,14], microwave assisted extraction [15-17], osmotic shock [8,18], solvent extraction [19-21], supercritical fluid extraction [22-24], and enzymatic [25,26]. Another method of microalgae cell destruction is high voltage electricity [27-35]. The use of high voltage electricity proved to help improve the extraction results.

From some literature has not been found pretreatment method using low voltage electricity to help oil extraction of microalgae. Pretreatment using low voltage electricity in the form of ohmic heating [36]. The purpose of this study was to determine the effect of ohmic heating as a method of pretreatment in microalgae oil extraction.

2.0 METHODOLOGY

2.1 Materials and Methods

The raw material used is fresh microalgae *nannochloropsis* sp. that obtained from the Lampung Sea Fisheries Management Center (BBPBL). Microalgae culture was done in open ponds, whereas the fertiliser was commercial fertiliser N, P, and K with a specific ratio. The cell density of microalgae was 20 g/L (D1) and 30 g/L (D2). The diluent used was aquades. The solvent for extraction was n-hexane.

The equipment used was step down transformer 1000 W using State Electricity Company (PLN) power source. Pretreatment chamber used batch typed with acrylic material. The dimension of the pretreatment chamber was 5 cm x 5 cm x 3 cm with volume 75 cm³. The electrodes used were stainless steel with a thickness of 3 mm, and dimensions of 5 cm x 5 cm. Measuring instrument used was a thermocouple, Avometer, conductivity meter, ampere meter, and stopwatch. Extraction equipment was extractor soxhlet, glassware, water bath, oven, digital analytic, rotary vacuum evaporator.

2.2 Procedure

Pretreatment was done by inserting fresh microalgae pasta with a determined cell density (20 g/L and 30 g/L) into the pretreatment chamber with volume 75 cm³. The next stage was exposure to voltage electricity (55 V and 110 V) according to treatment. Duration of treatment was two levels, 30 seconds and 60 seconds. Each treatment was repeated three times.

After ohmic heating treatment, microalgae paste was dried and extracted with a chemical solvent. Chemical extraction used the Soxhlet method. The chemical solvent was n-hexane. The extraction resulted in an oil and n-hexane mixture. Separation of oil and n-hexane solvent was done using a rotary vacuum evaporator.

The principle of pretreatment work using ohmic heating was the microalgae paste in the treatment room was exposed to the flow of electricity from the positive electrode to the negative electrode which caused the cell to lysis. Lysis cells were due to electricity and heat [36, 37]. With the provision of pretreatment using ohmic heating, the contents of the cell became easy to remove

3.0 RESULTS AND DISCUSSION

3.1 Oil Yield

The yield of pretreatment oils using ohmic heating with microalgae cell density of 20 g/L at two voltage levels and two levels of exposure duration is shown in Figure 1. As can be seen in Figure 1 the highest oil yield of 14.88% occurred at 60 seconds pretreatment and 110 V (D1T2V5), and the lowest oil yield at 30-second treatment and voltage 55 V (D1T1V4).

Pretreatment ohmic heating at 30-second exposures (T1) between 55 V (V4) and 110 V (V5) showed different results. It was found that the greater voltage produced a greater yield. The same pattern occurred at 60 seconds of electrical exposure. Electrical voltage affected the recovery of microalgae oil. The higher voltage produced or electric field strength, the greater the yield [38].

The duration of electric field exposure affects the yield. At the same voltage level, longer electric field

exposures produce greater yield. This increase in yield occurred to V4 and V5. In dilute microalgae, paste (20 g/L) showed that the increase in voltage, and longer pretreatment enhanced the yield of microalgae oil.

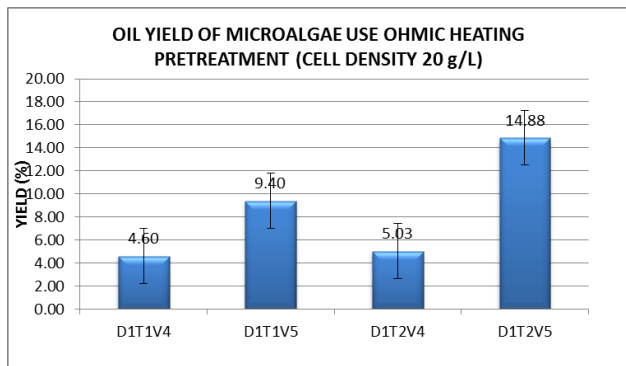


Figure 1 Effect of voltage and exposure duration on oil yield on pretreatment ohmic heating with a density of 20 g/L

Figure 2 shows the result of ohmic heating pretreatment for 30 g/L cell density of microalgae. From Figure 2 it can be seen that the oil yield was influenced by voltage and duration of exposure. A 30-second exposure (T1) showed that an increase in voltage caused a decrease in oil yields. The same pattern also occurred for 60 seconds of exposure (T2). Based on Figure 2, at the same exposure duration, the oil yield is affected by electric voltage. The higher of voltage it will produce a lower lipid yield.

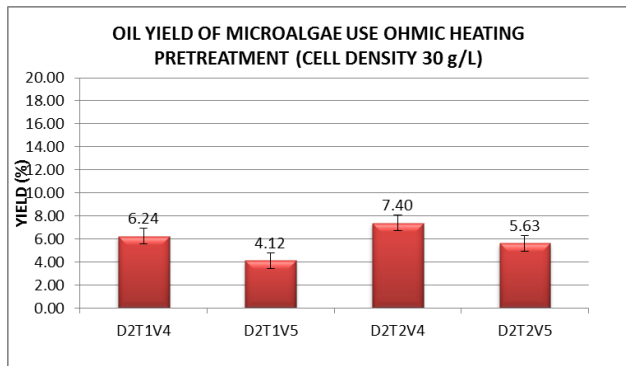


Figure 2 Effect of voltage and exposure duration on oil yield on ohmic heating pretreatment with a cell density of 30 g/L

Voltage treatment and pretreatment duration of microalgae paste with cell density between 20 g/L and 30 g/L produced different results (see Figures 1 and 2). Generally, 55 V and 110 V voltage with 30 seconds and 60-second exposure produced a higher yield of microalgae paste with a cell density of 20 g/L (dilute) than microalgae paste of 30 g/L (more concentrated). In dilute paste, the highest yield was

14.88%, while in the more concentrated paste the highest yield was 7.40%.

The yield of oil produced is influenced by dilution and voltage factors. In a dilute microalgae paste, the oil yield will increase if given a greater voltage but for a more concentrated paste it will be the opposite. This was related to the occurrence of electroporation or cell permeabilization in different cell density conditions [39]. Electroporation is affected by the conductivity of the solution. This means that solutions with lower conductivity required higher voltages, while those with higher conductivity required lower voltages [40].

As dilution affected the yield of microalgae oil, pretreatment using 55 V (low) and 30-second exposure (T1) showed that the higher the microalgae cell density, the higher the yield (see Figure 3). In contrast, the use of 110 V with 30-second exposure, higher microalgae cell density produced a lower yield. The same pattern occurred for 60 seconds of exposure (see Figure 4).

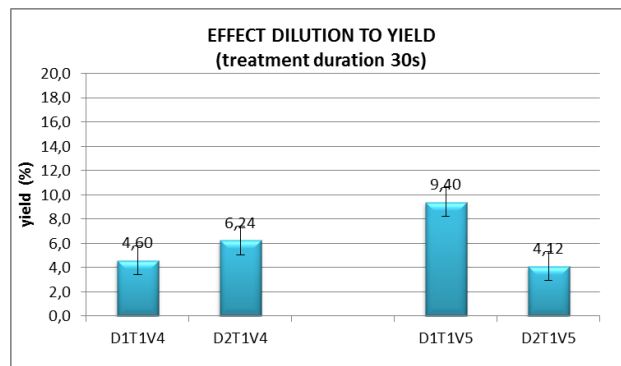


Figure 3 Effect of dilution and voltage on oil yield on ohmic heating pretreatment with 30-second exposure duration

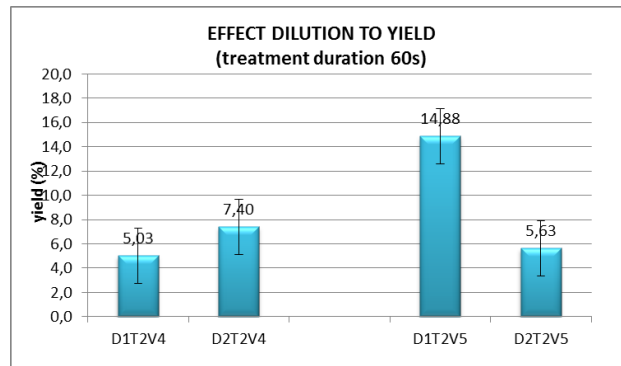


Figure 4 Effect of dilution and voltage on oil yield on ohmic heating pretreatment with 60-second exposure duration

Ohmic heating is heating that occurred due to the flow of electricity in materials that are an electrolyte. Heat occurred because of the movement of electricity. In the pretreatment of microalgae using ohmic heating, there was an increase in the temperature of the liquid. The

temperature changes along with increased time due to exposure to electricity. Temperature change graph during the microalgae pretreatment process at 20 g/L and 30 g/L cell density with 60 seconds pretreatment is shown in Figure 5.

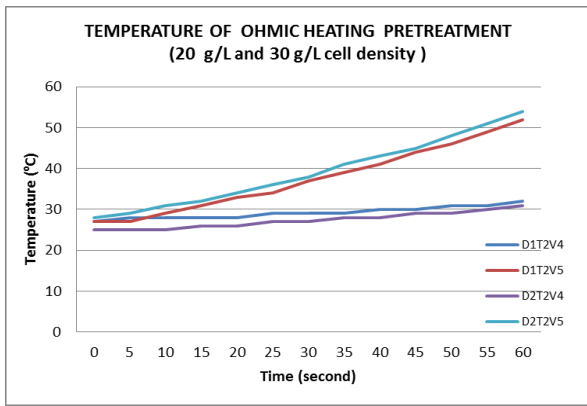


Figure 5 Temperature changes during ohmic heating pretreatment

Temperature changes in ohmic heating with 60 seconds pretreatment are shown in Figure 5. As can be seen in Figure 5, the longer pretreatment time resulted in an increase in temperature. A faster increase in temperature occurred at a higher voltage of 110 V (V5). At a voltage of 55 V (V4), the temperature increased more slowly. Cell density also influenced the rise in temperature. The cell density of 30 g/L (D2) was greater than the cell density of 20 g/L (D1). The higher the density of the cell; the faster the temperature increased.

3.2 Pretreatment Energy

The consumption of electrical energy during the pretreatment process was related to the amount of electric current flowing, and the voltage used during the pretreatment. Electric power is the multiplication of voltage, electrical current and time. The change in electric current during pretreatment ohmic heating is shown in Figure 6. The amount of electric current was influenced by the duration of pretreatment, the magnitude of the voltage, and the level of cell density.

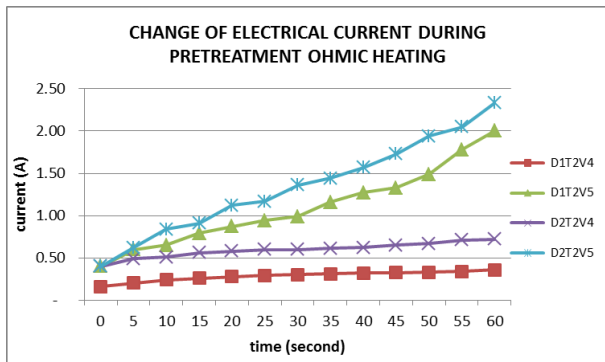


Figure 6 Development of electric current during pretreatment ohmic heating

During the ohmic heating process there was a change in temperature, which is a function of time. The longer duration of exposure will be increasing the temperature. These temperature changes will affect electric conductivity. The greater of electric conductivity then will result in a greater current electric. Thus, increased treatment time resulted in changes in temperature, conductivity, and electric current [41].

Changes in electric current were also influenced by electrical voltage and cell density. The greater voltage resulted in a greater current. Likewise, the more concentrated the solution, the greater the conductivity, which resulted in a greater electric current. The change in electric current was more dominantly influenced by the voltage than the cell density. In shown in Figure 7, most of the electric current changes occurred to the treatment of D2T2V5 (cell density 30 g/L, 60 second exposure time and voltage 110 V).

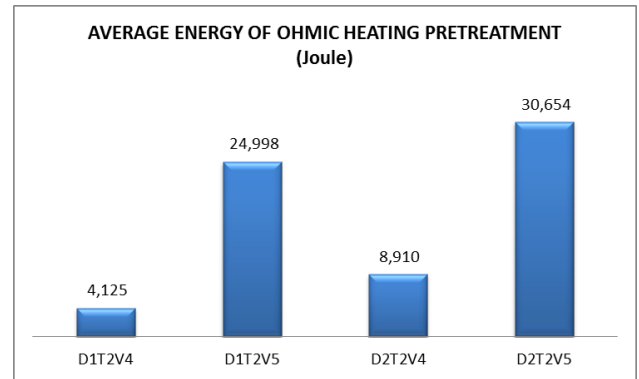


Figure 7 Electrical energy averaged 60 seconds pretreatment ohmic heating

The amount of electrical energy during pretreatment ohmic heating was affected by the voltage of electricity, electric current, and the duration of exposure. Figure 7 shows that the greatest energy requirement occurred to the treatment of D2T2V5 (30 g/L cell density, 60 second exposure time, and 110 V voltage) 30654 Joule, while the lowest electrical energy occurred to D1T2V4 treatment (20 g/L cell density, exposure 60 seconds, and voltage 55 V) of 4125 Joule. Electric energy is the multiplication of voltage, current, and exposure duration of electricity.

Table 1 Ratio of energy to yield during ohmic heating

Treatment	Energy(J)	Yield (%)	Ratio
D1T2V4	4125	5.03	820
D1T2V5	24998	7.40	3378
D1T2V5	8910	14.88	599
D2T2V4	30654	5.63	5445

In this study, the energy of pretreatment per percent of the yield of oil produced was done by looking at the energy ratio to yield (see Table 1). The lowest ratio was in the treatment of D2T2V4 of 599, which means that to produce 1% oil required pretreatment energy of 599 J. The biggest ratio was in the treatment of D2T2V5 of 5445 which means that to produce 1% of oil needed energy 5445 J. The low ratio indicates a more efficient use of energy to produce oil.

3.3 Fatty Acid Composition

The extracted *nannochloropsis* composition is known from the analysis using GC-MS (see Figure 8 and Table 2). The GC-MS analysis resulted in 31 different chemical compounds shown by the peak chromatogram. These compounds were short chain fatty acids up to long chains. The compounds may be used for various purposes including fuels, industry, cosmetics, pharmaceuticals, and foodstuff.

The GC-MS analysis showed that *nannochloropsis* is suitable as a biodiesel feedstock because it contains suitable fatty acids. Suitable fatty acids for fuel are myristic acid (C14: 0), palmitic acid (C16: 0), palmitoleic acid (C16: 1), stearic acid (C18: 0), and oleic acid (C18: 1) [42]. Some fatty acids suitable for biodiesel are about 17% of the total fatty acid. It also contained other important chemical compounds as food and pharmaceuticals such as Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA).

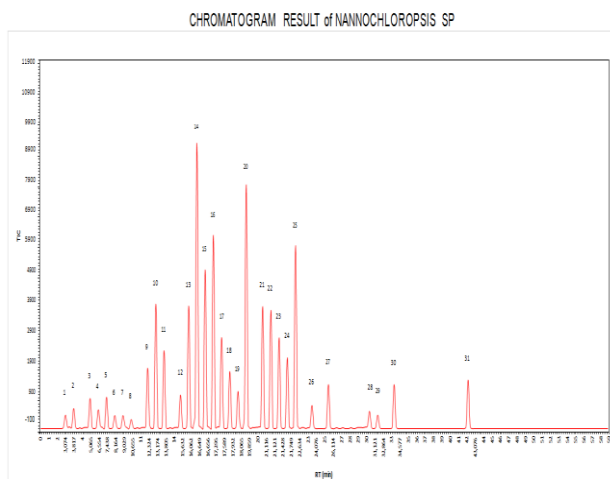


Figure 8 Chromatogram of GC-MS analysis results

Table 2 Fatty acid composition of *nannochloropsis* sp

No	Component	Composition (%)
1	Isovaleric acid	0.56
2	Hexanoic acid	0.84
3	Octanoic acid	1.25

No	Component	Composition (%)
4	Nonanoic acid	0.78
5	Decanoic acid	1.30
6	Undecanoic acid	0.54
7	Dodecanoic acid	0.54
8	Tridecanoic acid	0.38
9	Myristic acid	2.51
10	Palmitoleic acid	5.16
11	Palmitic acid	3.24
12	Heptadecanoic acid	1.39
13	Stearidonic acid	5.09
14	α Linolenic acid	11.84
15	γ Linolenic acid	6.58
16	Linolenic acid	8.02
17	Oleic acid	3.78
18	Octadecanoic acid	2.37
19	Nonadecanoic acid	1.55
20	Eicosapentaenoic acid	10.11
21	Arachidonic acid	5.06
22	Eicosatetraenoic acid	4.91
23	Eicosadienoic acid	3.77
24	Gondoic acid	2.94
25	Docosahexaenoic acid	7.59
26	Erucic acid	0.96
27	Tetracosanoic acid	1.82
28	β Sitosterol	0.72
29	γ Tocopherol	0.56
30	α Tocopherol	1.82
31	β Carotene	2.01

4.0 CONCLUSION

The pretreatment method using ohmic heating can be used to help extract microalgae oil. Factors that influence ohmic heating pretreatment methods are electrical voltage, duration of treatment and cell density. During the pretreatment using ohmic heating, the temperature of the solution changes.

The highest pretreatment efficiency occurred at 55 V, for 60 seconds with a cell density of 20 g / L.

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References

- [1] Demirbas, A. 2010. Use of Algae as Biofuel Sources. *Energy Conversion and Management*. 51: 2738-2749. <https://doi.org/10.1016/j.enconman.2010.06.010>.
- [2] Brennan, L. and Owende, P. 2010. Biofuels from Microalgae: A Review of Technologies for Production, Processing, and Extractions of Biofuels and Co-products. *Renewable and Sustainable Energy Reviews*. 14: 557-577. <https://doi.org/10.1016/j.rser.2009.10.009>.
- [3] Chisti, Y. 2008. Biodiesel from Microalgae Beats Bioethanol. *Trends in Biotechnology*. 26: 126-131. <https://doi.org/10.1016/j.biotechadv.2007.02.001>.
- [4] Mubarak, M., Shaija, A., and Suchithra, T. V. 2015. A Review on the Extraction of Lipid from Microalgae for Biodiesel Production. *Algal Research* 7: 117-123. <https://doi.org/10.1016/j.algal.2014.10.008>.
- [5] Daroch, M., Geng, S., and Wang, G. 2013. Recent Advances in Liquid Biofuel Production from Algal Feedstocks. *Applied Energy*. 102: 1371-1381. <https://doi.org/10.1016/j.apenergy.2012.07.031>.
- [6] Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., Kruse, O., and Hankamer, B. 2008. Second Generation Biofuels: High-efficiency Microalgae for Biodiesel Production. *BioEnergy Research*. 1: 20-43. <https://doi.org/10.1007/s12155-008-9008-8>.
- [7] Teixeira, R. E. 2012. Energy-efficient Extraction of Fuel and Chemical Feedstocks from Algae. *Green Chemistry*. 14(2): 419-427. <https://doi.org/10.1039/C2GC16225C>.
- [8] Lee, J. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., and Oh, H. M. 2010. Comparison of Several Methods for Effective Lipid Extraction from Microalgae. *Bioresource Technology*. 101: S75-S77. <https://doi.org/10.1016/j.biortech.2009.03.058>.
- [9] Prabakaran, P., and Ravindran, A. D. 2011. A Comparative Study on Effective Cell Disruption methods for Lipid Extraction from Microalgae. *Letters in Applied Microbiology*. 53(2): 150-154. <https://doi.org/10.1111/j.1472-765X.2011.03082.x>.
- [10] Ranjan, A., Patil, C., and Moholkar, V. S. 2010. Mechanistic Assessment of Microalgal Lipid Extraction. *Industrial and Engineering Chemistry Research*. 49(6): 2979-2985. <https://doi.org/10.1021/ie9016557>.
- [11] Samarasinghe, N., Fernando, S., Lacey, R., and Faulkner, W. B. 2012. Algal Cell Rupture Using High Pressure Homogenization as a Prelude to Oil Extraction. *Renewable Energy*. 48: 300-308. <https://doi.org/10.1016/j.renene.2012.04.039>.
- [12] Halim, R., Harun, R., Danquah, M. K., and Webley, P. A. 2012. Microalgal Cell Disruption for Biofuel Development. *Applied Energy*. 91(1): 116-121. <https://doi.org/10.1016/j.apenergy.2011.08.048>.
- [13] Lee, A. K., Lewis, D. M., and Ashman, P. J. 2012. Review Disruption of Microalgal Cells for the Extraction of Lipids for Biofuels: Processes and Specific Energy Requirements. *Biomass and Bioenergy*. 46: 89-101. <https://doi.org/10.1016/j.biombioe.2012.06.034>.
- [14] Wang, M., Yuan, W., Jiang, X., Jing, Y., and Wang, Z. 2014. Disruption of Microalgal Cells Using High-frequency Focused Ultrasound. *Bioresource Technology*. 153: 315-321. <https://doi.org/10.1016/j.biortech.2013.11.054>.
- [15] Biller, P., Friedman, C., and Ross, A. B. 2013. Hydrothermal Microwave Processing of Microalgae as a Pre-treatment and Extraction Technique for Bio-fuels and Bio-products. *Bioresource Technology*. 136: 188-195. <https://doi.org/10.1016/j.biortech.2013.02.088>.
- [16] Cheng, J., Yu, T., Li, T., Zhou, J., and Cen, K. 2013. Using Wet Microalgae for Direct Biodiesel Production via Microwave Irradiation. *Bioresource Technology*. 131: 531-535.
- [17] Iqbal, J., and Theegala, C. 2013. Microwave Assisted Lipid Extraction from Microalgae Using Biodiesel as Co-solvent. *Algal Research*. 2: 34-42. <https://doi.org/10.1016/j.algal.2012.10.001>.
- [18] Yoo, G., Park, W. K., Kim, C. W., Choi, Y. E., and Yang, J. W. 2012. Direct Lipid Extraction from Wet Chlamydomonas Reinhardtii Biomass Using Osmotic Shock. *Bioresource Technology*. 123: 717-722. <https://doi.org/10.1016/j.biortech.2012.07.102>.
- [19] Boyd, A. R., Champagne, P., McGinn, P. J., MacDougall, K. M., and Melanson, J. E. 2012. Switchable Hydrophilicity Solvents for Lipid Extraction from Microalgae for Biofuel Production. *Bioresource Technology*. 118: 628-632. <https://doi.org/10.1016/j.biortech.2012.05.084>.
- [20] Balasubramanian, R. K., Doan, T. T. Y., and Obbard, J. P. 2013. Factors Affecting Cellular Lipid Extraction from Marine Microalgae. *Chemical Engineering Journal*. 215-216: 929-936. <https://doi.org/10.1016/j.cej.2012.11.063>.
- [21] Wahlen, B. D., Willis, R. M., and Seefeldt, L. C. 2011. Biodiesel Production by Simultaneous Extraction and Conversion of Total Lipids from Microalgae, Cyanobacteria, and Wild Mixed-Cultures. *Bioresource Technology*. 102: 2724-2730. <https://doi.org/10.1016/j.biortech.2010.11.026>.
- [22] Cheng, C. H., Du, T. B., Pi, H. C., Jang, S. M., Lin, Y. H., and Lee, H. T. 2011. Comparative Study of Lipid Extraction from Microalgae by Organic Solvent and Supercritical CO₂. *Bioresource Technology*. 102: 10151-10153. <https://doi.org/10.1016/j.biortech.2011.08.064>.
- [23] Mouahid, A., Crampon, C., Toudji, S. A. A., and Badens, E., 2013. Supercritical CO₂ Extraction of Neutral Lipids from Microalgae: Experiments and Modelling. *Journal of Supercritical Fluids*. 77: 7-16. <http://dx.doi.org/10.1016/j.supflu.2013.01.024>.
- [24] Sovová, H. 2012. Steps of Supercritical Fluid Extraction of Natural Products and Their Characteristic Times. *Journal of Supercritical Fluids*. 66: 73-79. <https://doi.org/10.1016/j.supflu.2011.11.004>.
- [25] Horst, I., Parker, B. M., Dennis, J. S., Howe, C. J., Scott, S. A., Smith, A. G. 2012. Treatment of Phaeodactylum Tricornutum Cells with Papain Facilitates Lipid Extraction. *Journal of Biotechnology*. 162: 40-49. <http://dx.doi.org/10.1016/j.jbiotec.2012.06.033>.
- [26] Fu, C. C., Hung, T. C., Chen, J. Y., Su, C. H., and Wu, W. T. 2010. Hydrolysis of Microalgae Cell Walls for Production of Reducing Sugar and Lipid Extraction. *Bioresource Technology*. 101: 8750-8754. <https://doi.org/10.1016/j.biortech.2010.06.100>.
- [27] Sofi'i, I., Sumarlan, S. H., Wignyanto, and Susilo, B. 2017. Combination of High Electric Voltage (Hev) and NaCl as Assisted Extraction Methods for *Nannochloropsis* sp. *Boscience Research*. 14(2): 386-394. <http://doi.org/10.5281/zenodo.2557043>.
- [28] Flisar, K., Meglic, S. H., Morelj, J., Golob, J., and Miklavcic, D. 2014. Testing a Prototype Pulse Generator for a Continuous Flow System and Its Use for E. Coli Inactivation and Microalgae Lipid Extraction. *Bioelectrochemistry*. 100: 44-51. <https://doi.org/10.1016/j.bioelechem.2014.03.008>.
- [29] Goettel, M., Eing, C., Gusbeth, C., Straessner, R., and Frey, W. 2013. Pulsed Electric Field Assisted Extraction of

- Intracellular Valuables from Microalgae. *Algae Research*. 2: 401-408.
<https://doi.org/10.1016/j.algal.2013.07.004>.
- [30] Foltz, G. 2012. Algae Lysis with Pulsed Electric Fields. Master Thesis, California State Polytechnic University, San Luis Obispo Follow.
- [31] Kempkes, A. K., Roth, I., and Gaudreau, M. P. J. 2011. Pulsed Electric Field (PEF) Method for Continuous Enhanced Extraction of Oil and Lipids from Small Aquatic Plants. US Patent 2011/0107655 A1, 12 May 2011.
- [32] Lai, Y. S., Parameswaran, P., Li, A., M. Baez, and B. E. Rittmann. 2014. Effects of Pulsed Electric Field Treatment on Enhancing Lipid Recovery from the Microalga, *Scenedesmus*. *Bioresource Technology*. 173: 457-461.
<https://doi.org/10.1016/j.biortech.2014.09.124>.
- [33] Eing, C., Goettel, M., Straessner, R., Gusbeth, C., and Frey, W. 2013. Pulsed Electric Field Treatment of Microalgae-Benefits for Microalgae Biomass Processing. *IEEE Transactions on Plasma Science*. 41(10): 2901-2907.
<https://doi.org/10.1109/TPS.2013.2274805>.
- [34] Zbinden, M. D. A., Strum, B. S. M., Nord, R. D., Carey, W. J., Moore, D., Shinogle, H., and Stagg-Williams, S. M. 2013. Pulsed Electric Field (PEF) as an Intensification Pretreatment for Greener Solvent Lipid Extraction from Microalgae. *Biotechnology and Bioengineering*. 110: 1605-1615.
<https://doi.org/10.1002/bit.24829>.
- [35] Sheng, J., Vannela, R., and Rittmann, B. E. 2011. Evaluation of cell-disruption effects of pulsed-electric-field treatment of *Synechocystis* PCC 680. *Environmental Science & Technology*. 45: 3795-3802.
<https://doi.org/10.1021/es103339x>.
- [36] Islam, M. S., Aryasomayajula, A., and Selvaganapathy, P. R. 2017. A Review on Macroscale and Microscale Cell Lysis Methods. *Micromachines*. 8: 83.
 DOI: 10.3390/mi8030083.
- [37] He, Z., and Lin, J.-M. 2018. *Recent Developmnet of Cell Analysis on Microfluidics (Chapter 2) in Cell Analysis on Microfluidics*. Integrated Analytical System. ISSN 2196-4483 (electronic), ISBN 978-981-10-5394-8 (eBook).
<https://doi.org/10.1007/978-981-10-5394-8>.
- [38] Lakkakula, N., Lima, M., and Walker, T. 2004. Rice Bran Stabilization and Rice Bran Oil Extraction using Ohmic Heating. *Journal of Bioresource Technology*. 92: 157-161.
<https://doi.org/10.1016/j.biortech.2003.08.010>.
- [39] Pucihar, G., Kotnik, T., Kanduser, M., and Miklavcic, D. 2001. The Influence of Medium Conductivity on Electroporabilization and Survival of Cells in Vitro. *Bioelectrochemistry*. 54: 107-115.
[https://doi.org/10.1016/S1567-5394\(01\)00117-7](https://doi.org/10.1016/S1567-5394(01)00117-7).
- [40] Ivorra, A., Villedojade, J., and Mir, L. M. 2010. Electrical Modeling of the Influence of Medium Conductivity on Electroporation. *Physical Chemistry Chemical Physics*. 12: 10055-10064. DOI: 10.1039/c004419a.
- [41] Icier, F., and Ilcali, C. 2005. Temperature Dependent Electrical Conductivities of Fruit Purees during Ohmic Heating. *Food Research International*. 38: 1135-1142.
<https://doi.org/10.1016/j.foodres.2005.04.003>.
- [42] Olofsson, M., Lamela, T., Nilsson, E., Bergé, J. P. I., Pino, V. del, Uronen, P., and Legrand, C. 2012. Seasonal Variation of Lipids and Fatty Acids of the Microalgae *Nannochloropsis oculata* Grown in Outdoor Large-Scale Photobioreactors. *Energies*. 5(5): 1577-1592.
<https://doi.org/10.3390/en5051577>.