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Combination of high electric voltage (hev) and nacl as assisted extraction methods for *Nannochloropsis sp*

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The application of high electric voltage (HEV) methods as a pretreatment for cell disruption of microalgae pasta *Nannochloropsis sp* and NaCl assisted method is necessary to microalgae extraction. The purpose of this study was to evaluate the efficacy of HEV combined with the addition of salt in microalgae cell disruption. Pasta *Nannochloropsis sp* was added 0.01 M and 0.1 M NaCl subsequently treated (pretreatment) with high electric voltage 10.71 kV, 11.2 kV and 12.66 kV for 30 seconds and 90 seconds, respectively. The pasta as pretreatment result was continued by Soxhlet extraction. The results showed that HEV effective in microalgae cell disruption. It was indicated by an increase in lipid results after pretreatment. HEV treatment was more efficient than no treatment (NT). The combination treatment HEV and 0.1 M NaCl for 30 seconds was more effective than HEV and 0.01 M NaCl for 90 seconds. An adding NaCl makes the greater electrical conductivity, therefore electroporation increase. Then the available lipid that can be extracted increase as well. Combination of HEV and NaCl can be used as pretreatment method to increase lipid yield of microalgae before solvent extraction.

Keywords: HEV, extraction, microalgae, pretreatment

INTRODUCTION

The idea of utilization fuels from microalgae is not a novel (Sawayama et al. 1995) but it is now being taken seriously because of the escalating price of petroleum, the emerging concern about global warming, and the promising potential of microalgae as biofuels (Gavrilescu and Chisti, 2005; Li et al. 2005; Mubarak et al. 2015). Microalgae biomass can accumulate lipids within their cell similar to vegetable oils with a potential to produce 100 times more oil per acre than any other oil-producing crops (Mubarak et al. 2015). Therefore, biofuels produced from microalgae will be the most suitable alternative fuels in foreseeable future. Microalgae have promise potential as biofuels in the future and considered to be a good candidate for biofuel production because higher photosynthetic efficiency, higher biomass production, and faster growth rate compared to other energy crops such as rapeseed and soybean (Miao, 2006). Microalgae are microscopic photosynthetic organisms that can be found in marine and freshwater environment (Brennan and Owende, 2010; Demirbas, 2010). They have a potential of fixing 1.83 tons CO_2 in an atmosphere when producing 1 ton of algae biomass (Chisti, 2008). The lipid accumulation in the cells of microalgae has ranged from 25–77% of dry weight (Chisti, 2007; Malcata, 2011). Some

advantages of microalgae as a feedstock for biodiesel compared to other energy crops are: the potential for a high yield per hectare (58700 kiloliters/hectare) than other oil crops; able to grow on non-agricultural land; the potential to consume CO_2 and other nutrients in the waste stream during growth; the high oil content of oil depending on the species; the daily crop production throughout the year; and the resource-based non-food raw materials (Chisti, 2007; Gouveia and Oliver, 2009). The production process of biodiesel from microalgae includes the cultivation, harvesting, biomass processing, lipid extraction, and trans-esterification. Among these processes, the extraction of lipids is an important and expensive process because the use of solvents for the extraction of lipids requires extra energy for recovering the same solvents from lipids after the extraction (Iqbal and Theegala, 2013). Economical production of biodiesel from microalgae is mainly dependent on the use of energy used for the processing of biomass and the type of lipid extraction process used (Jungmin et al. 2013). Processing biodiesel from microalgae is similar to biodiesel from other plants oils. All biodiesel process use triglyceride from palm oils. Taking oils from microalgae is still costly; therefore, the alternative solution is necessary if microalgae as feedstock.

Lipid extraction from microalgae is through several common ways that are the pressing, chemical solvents, and supercritical fluid extraction. Other methods that are less well-known but can be used for the extraction of microalgae cells are osmotic shock and ultrasonic. A process of breaking the cell wall or cell wall destruction will be very helpful in removing the contents of the cell so that the extraction will be easier. Evisceration cell can be carried out before the extraction process by conducting pretreatment. Several pretreatment techniques have been applied to improve lipid recovery through cell disruption and lysis (Lai et al. 2014). Pretreatment approaches for lipid extraction from microalgae include mechanical, ultrasound, microwave, osmotic shock, enzymatic lysis, and pulse electric fields (Dejoye et al. 2011; Sheng at al. 2011; Goettel et al. 2017).The most recent technology is a pulsed electric field (PEF) that includes the high electric voltage (HEV) to disrupt biomass. The main effect of high voltage on the biology cell is electroporation on the cell membrane. Electroporation process involves the rapid rearrangement of the membrane structure as a response to the application of a high electric field. Next process is irreversible electroporation so easily occur electro plasmolysis in cell biology (Barbosa et al. 1999). Application of PEF allowed enhancement of the rate of lipid. It was demonstrated that the increase in lipid was due to the electroporation not due temperature effects (Zbinden et al. 2013).

Electroporation provides a significant increase in electrical conductivity and permeability of the cell membrane caused by the application of an external electric field. Electroporation occurs because the cell membrane has a certain dielectric strength that can be transcended by an electric field (Knirsch et al. 2010). The application of an electric field to biological cells causes the build-up of electrical charge on the cell membrane (Schoenbach et al. 1997). Membrane disruption occurs when the membrane potential exceeds a critical value of 1 V in many cellular systems (Castro et al. 1993). An increase in conductivity can be accomplished by the addition of salt because salt contains ions. The high salinity levels of salt will increase the occurrence of electroporation.

Application PEF or HEV for lipid extraction is new methods for microalgae extraction. There are only a few published literatures regarding the use of PEF alone for microalgae lipid extraction (Goettel et al. 2013; Kempkes et al. 2011; Flisar et al. 2014) and use of PEF assisted extraction by solvent extraction (Lai et al. 2014).The purpose of this study to evaluate the efficacy of HEV combined with the addition of salt in microalgae cell disruption to increase extraction yield.

MATERIALS AND METHODS

Microalgae Cultivation and Harvest

Microalgae can be cultivated in open system or closed system. Open system for a large-scale production of microalgae biomass with paddle wheel to agitate the microalgae (Rawat et al. 2013). An alga used in this study was a species *Nannochloropsis sp* (BBPBL, Lampung - Indonesia).The cultivation process was done in an open aquaculture pond with a capacity of 30 m^3. Microalgae cultivation was treated in some stages, started from laboratory scale of 5 L then transferred to the 1000 L capacity tub next to the tub 2000 L next to the tub of 10000 L and the last to the tub of 30000 L. The medium was sea water with 28 ppt salinity. Nutrient for growing microalgae in the laboratory scale was a fertilizer Conway (Fogg and Thake, 1987) and in

the mass scale using urea ZA, TSP and other materials. Growth medium must provide the inorganic elements that constitute the algal cell (Chisti, 2007).The solar light used as illumination, stirring and an addition of CO_2 used pedal aerator continuously. Harvesting *Nannochloropsis sp* used flocculation method of collecting microalgae cells together to form larger particles. Harvesting was done on 4th day by adding NaOH (as a flocculation substance). Cell density about 20 x 10^6 cells per ml. Flocculants will settle after about 24 hours. Furthermore, the sediment was separated from the liquid. *Nannochloropsis sp* precipitate rinsed with clean water and neutralized with citric acid to a pH 7 - 8. Pasta microalgae were obtained by filtering using the plankton net.

Treatment Operations

To determine the weight of dried microalgae, pasta material performed by measuring the moisture content. Drying was done with the oven (Med center) temperature of 103ºC for 3 hours. Then, pasta microalgae was diluted in order to obtain a density 30 g.L^{-1} by adding NaCl 0.01 M and 0.1 M as a pretreatment.

Pretreatment of HEV power generation using the apparatus (the results of laboratory design TPPHP FTP-UB, Malang-Indonesia) with voltage variations 10.71 kV, 11.2 kV and 12.66 kV, electric pulse shaped box with a frequency of 20 kHz. Batch-type treatment chamber is made of 3 mm thick acrylic with dimensions of 5 cm x 5 cm x 3.3 cm. Cathode and anode electrode made of stainless steel (SS) with spacing of 3 cm and dimensions of 5 cm x 5 cm x 0.15 cm until volume of the treatment chamber is 75 cm^3. Liquid microalgae pasta was treated with HEV current according treatment for 30 seconds and 90 seconds.After HEV treatment, liquid pasta dry with oven-dried (Med center) at a temperature of 70-80 ºC. Furthermore, dry pasta crushed and weighed ± 5 g wrapped in filter paper. Soxhlet extraction (Pyrex) was performed using a solvent n-hexane 100 mL and using a water bath with a temperature of 70-80 ºC. The extraction was carried out for at least 5 hours.After the extraction, solvent was eliminated with a vacuum rotary evaporator (Buchi). Extractions were performed in triplicate and the mean values were reported. Lipids that have been obtained subsequently weighed using the analytical balance (Mettler Toledo) to determine the yield.

Analytical Procedures and Assessments

The yield is the ratio of the amount of lipid produced in the material. Calculation to find the yield can be seen below (AOAC, 1999).

$$Yield = \frac{weight\ of\ lipid\ (g)}{weight\ of\ material\ (g)} \times 100\%.$$

RESULTS

The influence of voltage treatment

Pretreatment for 30 seconds (t1) on microalgae paste mixed with NaCl 0.01 M (M1) and pasta mixed microalgae 0.1 M NaCl (M2) showed the similar trend (Figure 1). The lower voltage was giving higher yield of lipid production. The voltage were VA=12.66 kV, VB=11.2 kV and VC=10.71 kV. Pasta microalgae with molarity M1 and pretreatment t1 indicated that the highest yield occurred in the treatment M1t1VC then M1t1VA and the lowest M1t1VB (Table 1). Similar conditions was observed on pasta with molarity M2. At the pasta microalgae with molarity 0.1 M showed that M2t1VC was the higher yield than M2t1VB, and the lowest M2t1VA. Pretreatment voltage and duration treatment for 90 seconds between pasta with molarity M1 (0.01M) and M2 (0.1 M) showed the same pattern (Figure 2). The lower applied voltage the higher yield, but if the applied voltage lowered again then yield will go down. In the pretreatment for 90 seconds there is voltage which gives the maximum value. At the NaCl molarity M1, the highest yield occurred in M1t2VB followed M1t2VC and M1t2VA (Table 2). The same pattern occurred in molarity M2 was the highest yield on M2t2VB followed M2t2VC and M2t2VA. The yield between M1 and M2 molarity gave different results. Pasta with molarity M1 at pretreatment duration 90 seconds provided a higher yield than the M2 (Figure 2). Treatment M1t2VA had greater results than M2t2VA, while M1t2VB had greater results than M2t2VB and M1t2VC had greater results than M2t2VC.

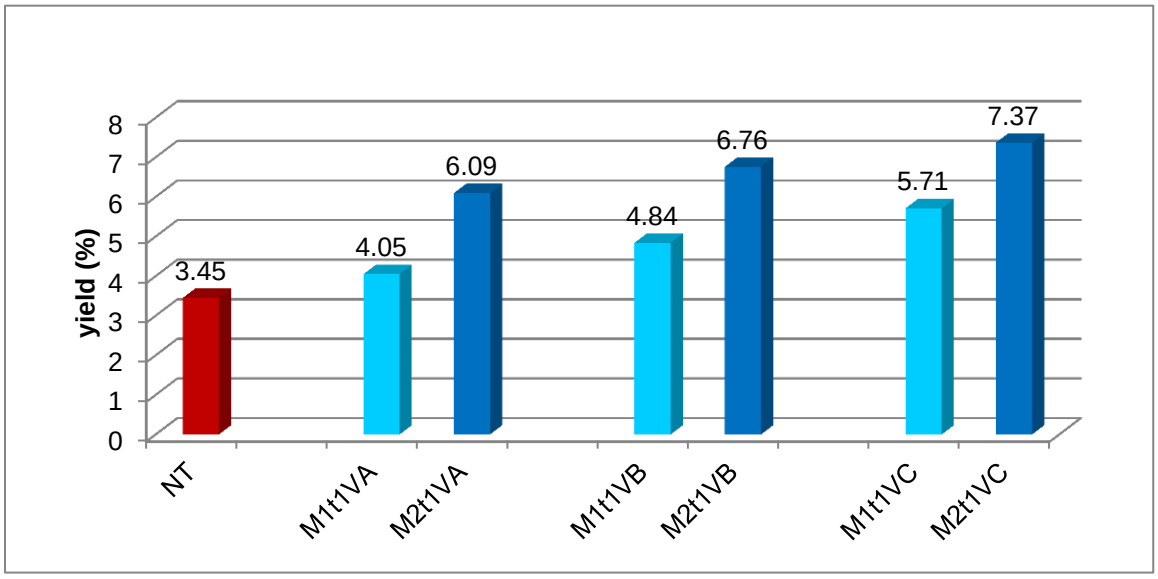

Figure 1.The influence of voltage and molarity NaCl to lipid yield at 30 second treatment

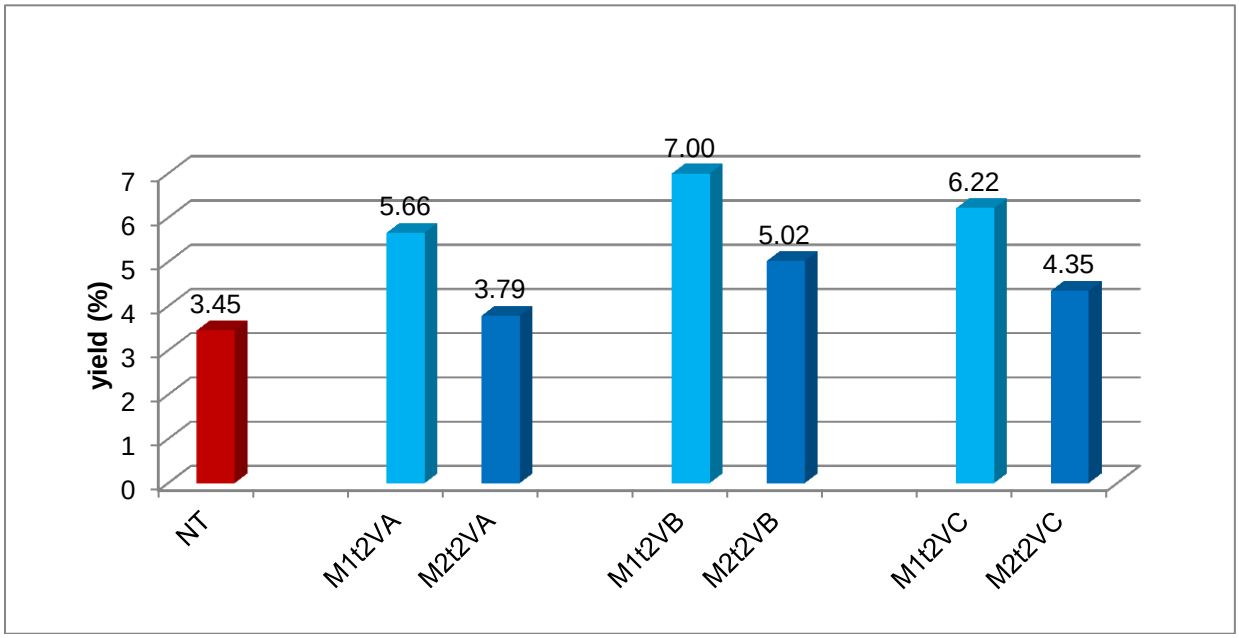

Figure 2.The influence of voltage and molarity NaCl to lipid yield at 90 second treatment

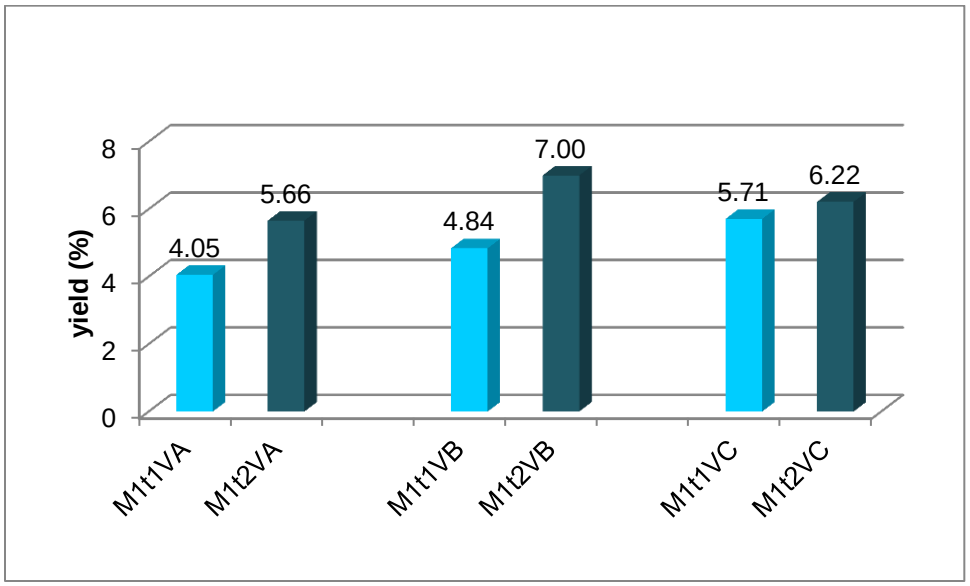

Figure 3. Influence of duration pretreatment to lipid yield at NaCl 0.01M

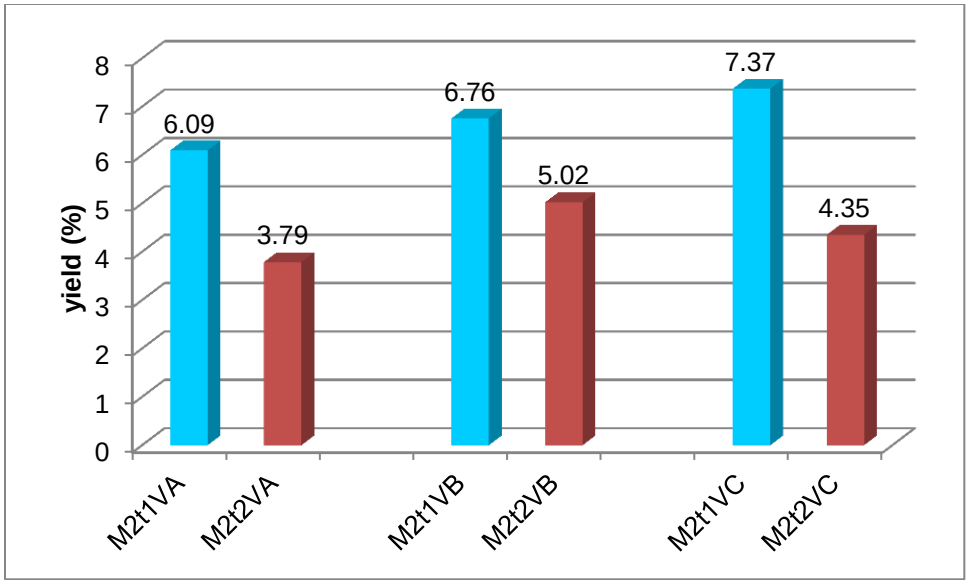

Figure 4. Influence of duration pretreatment to lipid yield at NaCl 0.1M

The influence of treatment duration

Pretreatment HEV on microalgae pasta with 0.01 M NaCl molarity treatment showed an increase between exposures to 30 seconds with 90 seconds. Difference highest yield occurred at a voltage VB at 2.16%, then the VA at 1.61% then VC at 0.51% (Figure 3). In conditions low NaCl molarity, increase of duration pretreatment will be able to increase the yield. This happens at a voltage VA, VB and VC.

Increase of molarity is not directly proportional to the increase in yield. At a higher molarity (0.1 M) precisely with increased duration exposure will reduce the amount of lipid yield. The higher voltage and the longer exposure it will decrease

lipid yield. This condition occurs in all of the voltage between the VA, VB and VC (Figure 4).

Table 1. The yield difference between treatments at 30 second treatment

Code	Yield (%)	Difference
M1t1VA	4.05	2.04
M2t1VA	6.09	
M1t1VB	4.84	1.92
M2t1VB	6.76	
M1t1VC	5.71	1.66
M2t1VC	7.37	
NT	3,45	

Table 2. The yield difference between treatments at 90 second treatment

Code	Yield (%)	Different
M1t2VA	5.66	1.87
M2t2VA	3.79	
M1t2VB	7.00	1.98
M2t2VB	5.02	
M1t2VC	6.22	1.87
M2t2VC	4.35	
NT	3,45	

DISCUSSION

A pasta microalga with no treatment (NT) was the lowest yield than microalgae with pretreatment. This result shows that the higher voltage applied to the pasta microalgae because more cells were damaged by HEV (Figure 1). Damage cell or electroporation is influenced by the applied voltage. Electric field strength is the amount strength (V) over distance (cm) to lysis the cell membrane of a microorganism or cell. It is very obvious that the amount of field strength applied directly proportional to the treatment efficiency. Increasing field strength provides better efficiency rather than increasing the pulse duration. Amplitude value is the maximum peak of electric field strength applied in PEF (Heinz et al. 2003). The increase in voltage pulse produces a larger area of the membrane electroporation with electroporation smaller level, while increasing the number of pulses or the duration does not affect the membrane area but increase the level of electroporation (Rols, 2006).

The result shows that different voltages provide different lipids yield, particularly for pasta microalgae with different molarity. The higher molarity provide greater yield. Mixture pasta with NaCl molarity 0.01 M (M1) have less electrolyte solution ions when compared mixture pasta with NaCl molarity 0.1 M (M2). NaCl will decompose to Na^+ and Cl^- ions. The more ions make the greater electrical conductivity; therefore, the rates of the cell microalgae increase during electroporation process. The more cells undergoing electroporation will provide the more lipids extracted in the cell; hence, the lipid yield also increased. The yield of M2t1VA is greater than M1t1VA, M2t1VB is greater than M1t1VB, and M2t1VC is greater than M1t1VC. This indicates that electroporation influenced by conductivity of the material in addition affected by magnitude of the voltage.When high electric fields applied to the cell membranes, it become more permeable and allow the entrance of various molecules. The process called electroporation as it is believed that the pores formed in the membrane as the trans membrane voltage induced above the critical voltage (between 0.2 and 1 V), but the term is also used to stress electro permeabilization increased permeability of the membrane was observed (Neumann et al. 1989; Tsong, 1991; Weaver and Chizmadzhev, 1996).
Molarity will effect to the pretreatment process. The higher molarity of NaCl was given provide the greater yield of lipid produced at each voltage. In the pretreatment time of 30 seconds, the highest yield difference between M1 (0.01 M) and M2 (0.1 M) was in treatment VA. Difference in the highest yield in the treatment of VA voltage at 2, 04%, and then the VB amounted to 1.92% and the lowest in treatment VC voltage of 1.66%. The higher voltage (VA) made the greater occurrence of electroporation which causes the greater damaged cells. The amount of damaged cells was caused by some contents of cells including lipid which came out. In this study, after pretreatment is drying, so that the loss can be caused by evaporation of lipid due to the drying mainly in cells that have high levels of electroporation. The presence of the salt content in a pretreatment

process is very profitable. Salt is a powerful electrolyte that helps simplify the process of electroporation. The higher salt contents or the higher molarity are more ions in solution.

At treatment duration 90 seconds indicated that increased molarity tends to lower yield. High molarity and high duration pretreatment will damage the cell and its contents including lipid content. The occurrence of electroporation excess lipids and it will damage would lower the yield. Yield optimum occurs at 11.2 kV and molarity 0.01 M. The difference yield on molarity treatment at duration 90 second occurred in the voltage VB treatment of 1.98%, further treatment of the voltage VA and VC respectively by 1.87%. Pretreatment has a better result than no-treatment. Electroporation is affected electrical pulse parameters and the chemical composition of the medium used and on the other hand influenced the characteristics of cells exposed to an electric field. Electrical pulse parameters of the most important are the amplitude/voltage, duration, amount, and frequency (Gabriel and Teissi´e, 1995; Bilska, 2000; Canatella et al. 2001).The level of microalgae cell damage is affected by long exposure to an electric field to the cell. The longer the cells exposed to electric field, the greater occurrence of electroporation, which damage cells. The more cells are damaged, the greater yield of lipid obtained. In the pretreatment using 0.1 M NaCl solution, the longer time electrical current is given it will decrease the yield, because a lot of cells undergoing electroporation and lipid partially damaged. Exposure duration affects the yield. At low NaCl molarity and the longer exposure, lipid yield was the highest but in high NaCl molarity has different result. When the electric field is too high, for a specific duration and number of pulses, physiological changes in the cells become too large to be repaired, good cells lose too much of the content or swell too much, which ultimately leads to cell death (Pavlin et al. 2005) but for the microalgae cells will further lower the lipid yield**.**.

CONCLUSION

Combination of high voltage and NaCl can be used as pretreatment method to increase lipid yield of microalgae before solvent extraction. Pretreatment duration, NaCl molarity and size of voltage have significant effect of lipid microalgae yield. The low voltage (10.71 kV) can increase yield for 30 second duration both in NaCl molarity of 0.01 M and 0.1 M. Voltage 11.2 kV provides the highest yield at NaCl 0.01 M and 0.1 M molarity for 90 seconds pretreatment. Pretreatment for 90 seconds is more suitable for microalgae with NaCl molarity 0.01 M. Pretreatment for 30 seconds is more suitable for microalgae with molarity of NaCl 0.1 M.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

IMS design and perform the experiments and also write the manuscript. SHS, WJT, BMS review, correct and edit the manuscript. All authors read and approve the final version.

REFERENCES

AOAC, 1999. Official methods of analysis (15th Ed.). K. Helrich (Ed.). Virginia.

Barbosa-Cánovas GV, Góngora-Nieto MM, Pothakamury UR, Swanson BG, 1999. Preservation of foods with pulsed electric fields. Academic Press, San Diego, USA. (pp. 4-47, 108-180).

Bilska A, DeBruin KA, Krassowska W, 2000. Theoretical modeling of the effects of shock duration, frequency and strength on the degree of electroporation. *Bioelectrochemistry.* 51:133-14. https://doi.org/10.1016/S0302-4598(00)00066-0

Brennan L, P Owende, 2010. Biofuels from microalgae: A review of technologies for production, processing, and extractions of

biofuels and co-products. *Renew. Sust.Energ*. Rev. 14: 557–577. https://doi.org/10.1016/j.rser.2009.10.009

Canatella PJ, Karr JF, Petros JA, Prausnitz M, 2001. Quantitative study of electroporation-mediated molecular uptake and cell viability. *Biophysical Journal*. 80:755-764. https://doi.org/10.1016/S0006-3495(01)76055-9

Castro AJ, Barbosa-Canovas GV, Swanson BG, 1993. Microbial inactivation of foods by pulsed electric fields. *Journal of Food Processing and Preservation.* 17:47–73**.** https://doi.org/10.1111/j.1745-4549.1993.tb00225.x

Chisti Y, 2007. Biodiesel from microalgae. *Biotechnology Advances.* 25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chisti Y, 2008. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol*. 26:126–131. https://doi.org/10.1016/j.tibtech.2007.12.002

Dejoye C, Vian MA, Lumia G, Bouscarle C, Charton F, Chemat F, 2011. Combined extraction processes of lipid from chlorella vulgaris microalgae: microwave prior to supercritical carbon dioxide extraction. *Int. J. Mol. Sci. 12*(12). 9332-9341; https://doi.org/10.3390/ijms12129332

Demirbas A, 2010. Use of algae as biofuel sources. *Energy Convers Manag*. 51:2738–2749. https://doi.org/10.1016/j.enconman.2010.06.010

Flisar K, SH Meglic, J Morelj, J Golob, D Miklavcic, 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

Fogg GE and Thake B, 1987. Algae culture and phytoplankton ecology. Second Edition.The University of Winconsin Press. London.

Gabriel B, Teissi´e J, 1995. Spatial compartmentation and time resolution of photooxidation of a cell membrane probe in electropermeabilized chinese hamster ovary cells. *European Journal of Biochemistry*. 228:710-718.

Gavrilescu M, Chisti Y, 2005. Biotechnology a sustainable alternative for chemical industry. *Biotechnol Adv*. 23:471-99. https://doi.org/10.1016/j.biotechadv.2005.03.004

Goettel M, C Eing, C Gusbeth, R Straessner, W Frey, 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

Gouveia L, Oliver AC, 2009. Microalgae as a raw material for biofuels production. *J Ind Microbiol Biotechnol*. 36(2):269-274. https://doi.org/10.1007/s10295-008-0495-6

Heinz V, S Toepfl, D Knorr, 2003. Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatment. *Innovation Food Science & Emerging Technology*. 4:167-175. https://doi.org/10.1016/S1466-8564(03)00017-1

Iqbal J, C Theegala, 2013. Microwave assisted lipid extraction from microalgae using biodiesel as co-solvent. *Algal Res.* 2: 34–42. https://doi.org/10.1016/j.algal.2012.10.001

Jungmin Kim GY, Hansol Lee, Juntaek Lim, Kyochan Kim, Chul Woong Kim, Min S. Park, Ji-Won Yang, 2013. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnol. Adv*. 31(6): 862–876. https://doi.org/10.1016/j.biotechadv.2013.04.006

Kempkes AK, I Roth, MPJ Gaudreau, inventors. May 12, 2011. Pulsed electric field (PEF) method for continuous enhanced extraction of oil and lipids from small aquatic plants. US Patent 2011/0107655 A1

Knirsch M, Santos CA, Vicente AM, Penna TC, 2010. Ohmic heating a review. *Trends Food Sci Tech.* 21(9):436-441. https://doi.org/10.1016/j.tifs.2010.06.003

Lai YS, Prathap P, Ang Li, Maria Baez, Bruce E Rittmann, 2014. Effects of pulsed electric field treatment on enhancing lipid recovery from the microalgae Scenedesmus. *Bioresource Technology.* 173:457–461. https://doi.org/10.1016/j.biortech.2014.09.124

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N, 2008. Biofuels from microalgae. *Biotechnology Progress*. 24:815–820. https://doi.org**/**10.1021/bp070371k

Malcata FX, 2011. Microalgae and biofuels: a promising partnership. *Trends Biotechnol*. 29:542–549. https://doi.org/10.1016/j.tibtech.2011.05.005

Miao X, Q Wu, 2006. Biodiesel production from heterotrophic microalgal oil. *Bioresources Technology.* 97:841–846. https://doi.org/10.1016/j.biortech.2005.04.008

Mubarak M, A Shaija, TV Suchithra, 2015. A

review on the extraction of lipid from microalgae for biodiesel production. *Algal Research*. 7:117–12. https://doi.org/10.1016/j.algal.2014.10.008

Neumann E, AE Sowers, CA Jordan, 1989. Electroporation and electrofusion in cell biology. Plenum Press, New York, USA.

Pavlin M, Masa Kanduser, Matej Rebersek, Gorazd Pucihar, Francis X. Hart, Ratko Magjarevic, Damijan Miklavcic, 2005. Effect of cell electroporation on the conductivity of a cell suspension. *Biophysical Journal*. 88:4378-4390. https://doi.org/10.1529/biophysj.104.048975.

Rawat I, R Ranjit-Kumar, T Mutanda F Bux, 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy.* 103:444–467. https://doi.org/10.1016/j.apenergy.2012.10.004

Rols MP, 2006. Electropermeabilization, a physical method for the delivery of therapeutic molecules into cells. *Biochimica et Biophysica Acta*. 1758:423–428. https://doi.org/10.1016/j.bbamem.2006.01.005

Sawayama S, Inoue S, Dote Y, Yokoyama SY, 1995. CO2 fixation and oil production through microalgae. *Energy Convers Manag*. 36:729–31. https://doi.org/10.1016/0196-8904(95)00108-P

Schoenbach KH, Peterkin FE, Alden RW, Beebe SJ, 1997. The effect of pulsed electric fields on biological cells: Experiments and applications. *IEEE Transactions on Plasma Science.* 25(2):284–292. https://doi.org/10.1109/27.602501

Sheng J, R Vannela, BE Rittmann, 2011. Evaluation of cell-disruption effects of pulsed electric field treatment of *Synechocystis PCC 6803. Environ. Sci. Technol*. *45* (8):3795–3802 https://doi.org/10.1021/es103339x

Tsong TY, 1991. Electroporation of cell membranes. *Biophys. J*. 60:297-306. https://doi.org/10.1016/S0006-3495(91)82054-9

Weaver JC, YA Chizmadzhev, 1996. Theory of electroporation: a review. B*ioelectrochem*. *Bioenerg*. 41:135–160. https://doi.org/10.1016/S0302-4598(96)05062-3

Zbinden, BSM Strum, RD Nord, WJ Carey, D Moore, H Shinogle, SM Stagg-Williams, 2013. Pulsed electric field (PEF) as an intensification pretreatment for greener solvent lipid extraction from microalgae. *Biotechnolology and Bioengineering*. 110:1605-1615. https://doi.org/10.1002/bit.24829