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Effect of assisted high voltage electricity on *Nannochloropsis oculata* lipid extraction

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Microalgae oil extraction method for biodiesel production has been developed in order to get extraction efficiency. Generally, damaging the cells can be used to increase extraction results. One of the methods to damage cells is using high voltage electricity. The purpose of this study is to determine the effect of electric field strength and exposure duration to the lipid yield of *Nannochlorpsis Oculata*. The method used was to treat the *Nannochloropsis* suspension using high voltage electricity with field strength of 4.3 $kV.cm^{-1}$ - 34.8 $kV.cm^{-1}$ for 10 seconds - 30 seconds and continued chemical extraction. The results showed that giving electric field exposure to microalgae suspension can increase the yield of microalgae oil. The use of high voltage electrical exposure on microalgae suspension and continued by chemical extraction can raise the yield of oil obviously

Keywords: microalgae, high voltage electricity, extraction

INTRODUCTION

Various types of microalgae have been studied by scientists in order to get the oil to be used as biodiesel feedstock. Microalgae contain highly beneficial ingredients such as DHA/EPA and some suitable fatty acids as biodiesel feed stocks. Microalgae have potential as a biodiesel feedstock including *Chlorella, Auxenochlorella protothecodies, Nannochloropsis sp., Chlamydomonas reinhardtii, Dunaliella salina, Isocrysis sp., Scenedesmus spp., Auxenochlorella, Ankistrodesmus falcatus, Synechocystis PCC 6803 (cyanobacteria protothecoides), Botryococcus braunii, Nannochloropsis oculata* (Eing et al. 2013; Goettel et al. 2013; Flisar et al. 2014; Lai et al. 2014).

Nannochloropsis oculata is a single-celled, greenish, non-motile, and nonflagging. In general, the growth rate of *Nannochloropsis occulata* is between 0.11 to 0.2 per day with carbohydrate, protein, beta carotene, lipid and chlorophyll content. Its lipid content is between 37-60% (Ma, 2014).

The ideal microalgae for the production of biodiesel in addition to containing high lipids should also have an appropriate fatty acid composition. *Nannochloropsis* has appropriate fatty acid content for biodiesel feedstock. The fatty acids in *Nannochloropsis* are mostly myristic acid (C14: 0), palmitic acid (C16: 0), palmitoleic acid (C16: 1), stearic acid (C18: 0) and oleic acid (C18: 1) 45% -78% of total fatty acids (Olofsson et al. 2012). The other essential component in *Nannochloropsis* cells is EPA (Hoffmann et al. 2010).

Klopfenstein proposed essential fatty acids for biodiesel production i.e. C16:1, C18:1 and C14:0 with a ratio of 5:4:1 depending on oxidative stability, temperature and quality of diesel fuel combustion (Schenk, 2008). High content C18:0 and C18:1 in microorganisms as a prerequisite in biodiesel production due to the similar high-value oil properties, oxidative stability and greater potential adaptability potential in industrial production. C16:0 has also been proposed as an appropriate fatty acid in renewable solar production (Meng et al. 2009).

Extraction is one of the important stages in the removal of microalgae lipid. This stage is expensive; therefore; the researchers are trying to find extraction methods that provide high efficiency and cheaper. There are various extraction methods for microalgae lipids that is mechanically and chemically (Mubarak et al. 2015). The mechanical process is including pressing, ultrasonic assisted extraction, microwave assisted extraction. While the chemically process is using solvent, supercritical CO_2 extraction, and liquid ionic extraction other than enzymatic and osmotic shock (Adam et al. 2012; Iqbal and Theegala, 2013; Neto et al. 2013).

Efforts to improve the efficiency of microalgae extraction have been achieved in various ways. One of them is by damaging microalga cells. It will be easily removed with the condition of damaged cells then the contents of cells including lipid. Cell disruption is done by microwave, sonication and electric field. Microwave assisted extraction method showed higher yield than without treatment (Balasubramanian et al. 2011; Iqbal and Theegala, 2013; Cheng et al. 2014, Dai et al. 2014). Ultrasound-assisted extraction had higher yield than without treatment (Adam et al. 2012;). High voltage electrical extraction also gives higher yields (Sofi'i et al. 2017; Goettel et al. 2013; Grimi et al. 2014).

Application of high voltage will cause electroporation on microalga cell membrane. The contents of the cell will be easy to remove if the cell porous. Factors affecting electroporation are the magnitude of the voltage, the amount of voltage (amplitude), the duration of exposure, the number of pulses, and the frequency (Canatella et al. 2001; Macek-Lebar and Miklavcic, 2001).

There is a limited number of researches on the use of high voltage electricity to the extraction of lipid microalga *Nannochloropsis oculata* as feedstock of biodiesel. This study aims to determine the effect of electric field strength and exposure time to the lipid yield of *Nannochloropsis oculata*.

MATERIALS AND METHODS

Material and apparatus

The material used in this research is the dry microalgae of *Nannochloropsis oculata* obtained from the Center of Brackish Water Aquaculture (BPBAP) Situbondo, East Java, Indonesia. Microalgae culture was done by stratum culture, starting from laboratory culture (erlenmeyer), semi mass culture (500-1000 L), and mass culture (4000-5000 L). Harvesting was precipitated by adding a 75-100 ppm caustic soda and left for 24 hours subsequently separated. The agglomerated deposit was dried naturally or using a 60 ºC oven. After dry, it was meshed and stored. Other materials were aquades and filter paper.

The equipment used for high voltage treatment was high voltage electric generator using ignition coil with output voltage ranging from 13 - 35 kV, frequency 23-24 Hz. Treatment chamber using acrylic with 50 cm^3 volume. Electrode in the treatment chamber was made from stainless steel 2 mm. There were 3 levels of gap between electrodes, that is 1 cm, 2 cm and 3 cm. Equipment for extraction was a soxhlet extractor, water bath, rotary vacuum and analytical scales.

Treatment procedures

Microalgae powder was weighed the and added by aquades to form a microalgae suspension with a concentration of 25 $g.L^{-1}$. High voltage was provided electrical exposure according to treatment. The treatments used voltage level, exposure time and electrode gap. There are 3 levels of voltage that were 13.0 kV, 15.3 kV and 34.8 kV. The exposure time were 3 levels that were 10 seconds, 20 seconds and 30 seconds. The gap of electrode used 3 levels that were 1 cm, 2 cm and 3 cm. Each of the microalgae suspensions were subjected to the appropriate voltage. After application of the voltage treatment on the microalgae suspension, the materials were dried using a natural dryer. The next step was to extract soxhlet using n-hexane solvent. Microalgae lipid was obtained by separating the oil mixture using a rotary vacuum evaporator. The last stage was to weigh the lipid obtained.

Analytical Procedures and Assessments

The oil yield was the ratio between the weight of the lipid divided by the weight of the dry material. Calculation to find the extracted lipids can be seen below (AOAC, 1999).

$$\text{Extracted lipid} = \frac{\text{Weigh of lipid (g)}}{\text{Weigh of material (g)}} \times 100\%$$

The extracted lipid contents from the treatment were compared using graphic method

RESULTS

Electric Field strength

The comparison between the voltage and the gap of the electrode produces a strength electric field. Table 1 and Figure 1 show the magnitude of electric field strength and the electrical jump test, respectively. The largest electric field of 34.8 kV.cm^{-1} occurs at a combination of voltage (V3) 34.8 kV and the electrode spacing (L1) 1 cm (V3L1) and the lowest electric field of 4.3 kV.cm^{-1} occurs at the combination of voltage (V1) 13.0 kV and the electrode gap (L3) 3 cm (V1L3).

Table 1. Electric field strength

Voltage	gap (cm)	Electric field (kV/cm)	Voltage	gap (cm)	Electric field (kV/cm)	Voltage	gap (cm)	Electric field (kV/cm)
V1	1	13.0	**V2**	1	15.4	**V3**	1	34.8
V1	2	6.5	**V2**	2	7.7	**V3**	2	17.4
V1	3	4.3	**V2**	3	5.1	**V3**	3	11.6

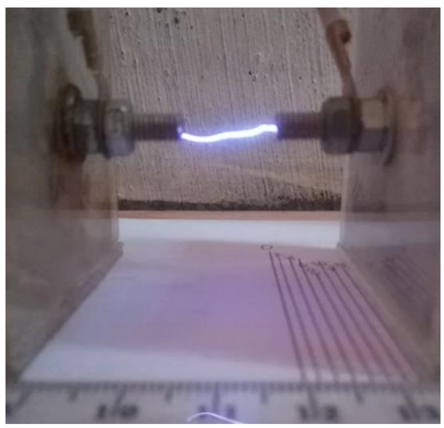

Figure 1. Spark jump test at a gap of 1.4 cm

Treatment

Provision of high-voltage treatment on microalgae suspension with a density of 25 g.L^{-1} show in Fig. 2, Fig. 3 and Fig 4. The treatment provided is the electric voltage (V1, V2, V3) and duration of treatment (T1, T2 and T3) by the gap of the electrode 1 cm.

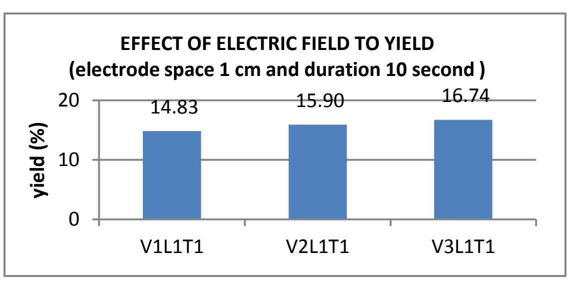

Figure 2. Effect of electric field strength to yield (L = 1 cm and for 10 second)

Based on Figure 2 it can be seen that the treatment of voltage at 1 cm electrode gap for 10 seconds shows the difference. The highest yield occurred on V3L1T1 of 16.74% and the lowest occurred in V1L1T1 of 14.83%. The same pattern occurs in the treatment duration of 20 seconds and 30 seconds. At the voltage treatment with 1 cm electrode gap for 20 seconds (Figure 3),

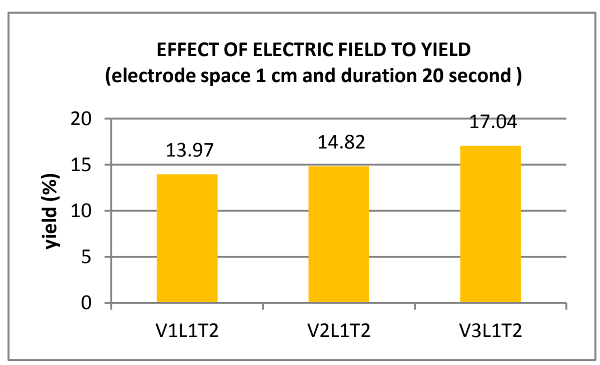

Figure 3. Effect of electric field strength to yield (L = 1 cm and for 20 second)

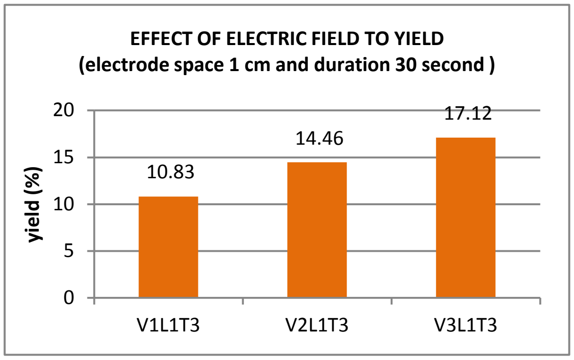

Figure 4. Effect of electric field strength to yield (L = 1 cm and for 30 second)

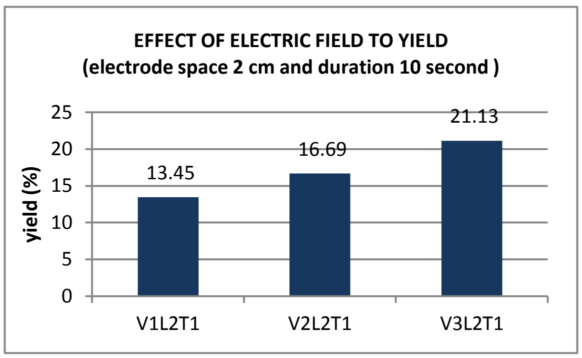

Figure 5. Effect of electric field strength to yield (L = 2 cm and for 10 second)

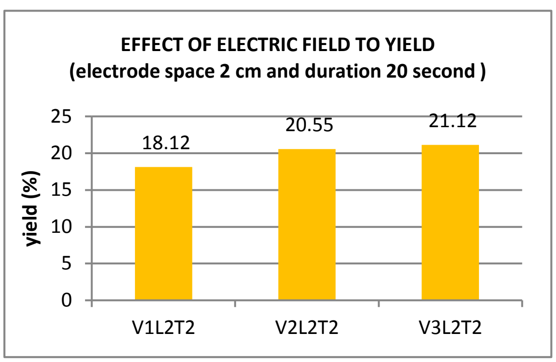

Figure 6. Effect of electric field strength to yield (L = 2 cm and for 20 second)

The highest yield of V3L1T2 was 17.04% and the lowest V1L1T2 is 13.97%. At the voltage treatment with 1 cm electrode spacing for 30 seconds (Figure 4), the highest yield of V3L1T3 was 17.12% and the lowest V1L1T3 is 10.83%.

Fig. 5, Fig. 6 and Fig. 7 are graphs of the effect of high-voltage treatment on the recovery of lipid of microalgae at a gap of 2 cm with a duration of exposure of 10 s, 20 s and 30 s. The three pictures show the same pattern that increase electric field caused increase the lipid yield.

Based on Figure 5 it is clearly seen that the highest yield occurred in V3L2T1 of 21.13% and the lowest occurred in V1L2T1 of 13.45%. In Figure 6 it shows that the highest value of 21.12% occurs in the treatment of V3L2T2 while the lowest occurred in the treatment of V1L2T2 of 18.12%. Figure 7 shows that the highest oil yield obtained by V3L2T3 treatment was 21.52% and the lowest V1L2T3 was 14.32%.

Fig. 8, Fig. 9 and Fig. 10 show a graph of the effect of the electric field on the extraction results at a gap of 3 cm electrode with a duration of exposure of 10 s, 20 s and 30 s. The three images show the same pattern that there is a greater tendency of electric field will be the increase the yield of lipid.

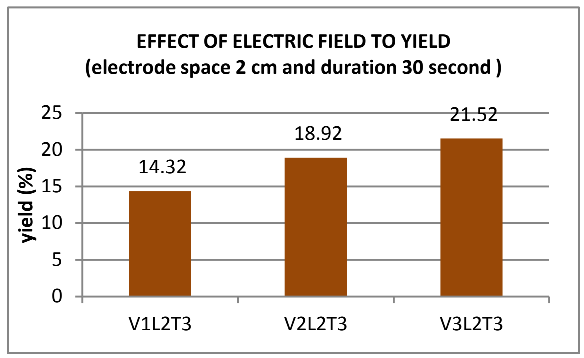

Figure 7. Effect of electric field strength to yield (L = 2 cm and for 30 second)

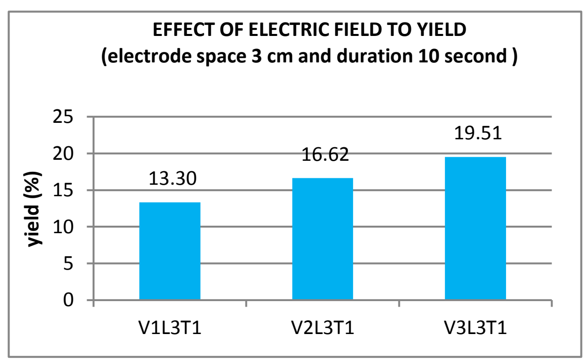

Figure 8. Effect of electric field strength to yield (L = 3 cm and for 10 second)

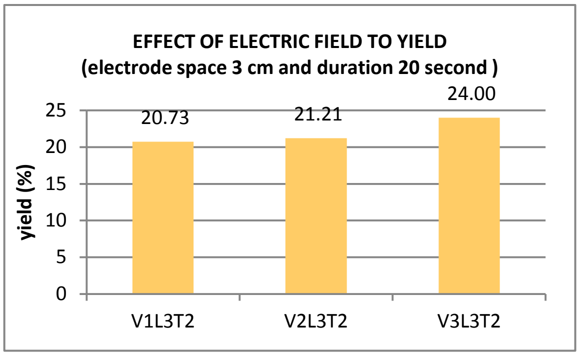

Figure 9. Effect of electric field strength to yield (L = 3 cm and for 20 second)

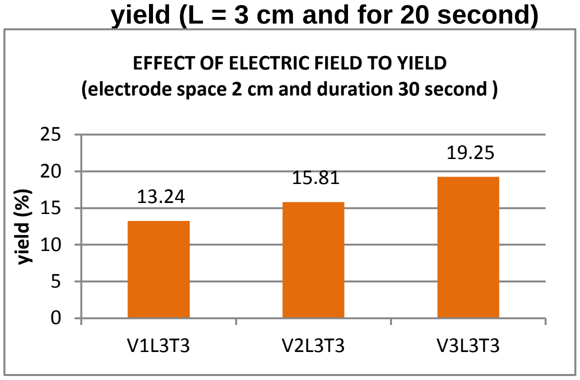

Figure 10. Effect of electric field strength to yield (L = 3 cm and for 30 second)

Effect of electric field with 3 cm electrode gap and 10 seconds of exposure time as shown in Figure 8. The highest oil yield occurred at 19.51% V3L3T1 treatment and the lowest occurred at 13.30% V1L3T1. Figure 9 shows a graph of electric field influence at 3 cm electrode gap and 20 seconds of exposure time. The highest yields occurred in the treatment V3L3T2 of 24.00% and the lowest occurred in V1L3T2 of 20.73%. Figure 10 shows a graph of electric field effect on yield at treatment with a gap of 3 cm and 30 seconds of exposure time. The highest yield of lipid was produced by V3L3T3 of 19.25% and the lowest was in V1L3T3 of 13.24%.

Energy

The energy required for the treatment depends on electrical power and the duration of exposure. Table 2 shows the amount of energy for each treatment duration. The highest energy of 1487.4 J occurs on the use of voltage V3 with a duration of 30 second while the lowest energy of 218.2 J occurs on the use of voltage V1. Energy requirement per unit of yield as shown in Table 3. The highest energy occurred at treatment of 77.27 J V3L3T3 and the lowest energy occurred on V1L1T1 of 14.72 J.

Table 2. Energy per treatment duration

CODE	INPUT VOLTAGE (V)	INPUT CURRENT (A)	POWER (W)	DURATION (s)	ENERGY (J)
V1	8.80	2.48	21.82	10	218.2
				20	436.4
				30	654.6
V2	11.00	3.06	33.66	10	336.6
				20	673.2
				30	1009.8
V3	13.40	3.70	49.58	10	495.8
				20	991.6
				30	1487.4

Table 3. Energy per lipid yield

NO	CODE	ENERGY/ YIELD	NO	CODE	ENERGY/ YIELD	NO	CODE	ENERGY/ YIELD
1	V1L1T1	14.72	10	V1L2T1	16.22	19	V1L3T1	16.40
2	V2L1T1	21.16	11	V2L2T1	20.17	20	V2L3T1	20.25
3	V3L1T1	29.63	12	V3L2T1	23.46	21	V3L3T1	25.41
4	V1L1T2	31.25	13	V1L2T2	24.09	22	V1L3T2	21.05
5	V2L1T2	45.43	14	V2L2T2	32.76	23	V2L3T2	31.73
6	V3L1T2	58.21	15	V3L2T2	46.96	24	V3L3T2	41.31
7	V1L1T3	60.46	16	V1L2T3	45.71	25	V1L3T3	49.42
8	V2L1T3	69.85	17	V2L2T3	53.38	26	V2L3T3	63.86
9	V3L1T3	86.88	18	V3L2T3	69.11	27	V3L3T3	77.27

DISCUSSION

The magnitude of the electric field strength is influenced by the voltage and the gap of the electrode. The higher the voltage and the closer the inter-electrode gap will produce a strong electric field. At that gap the flame jump is more dense. Conversely, the lower the voltage and the farther the electrode gap the electric field will be lower and the spring electric sparks are little. In an electric spark plug test indicates that a maximum spacing of 1.4 cm of electric sparks occurs at a voltage of 34.8 kV (Figure 1).

The electric field exposed to the microalgae suspension at 25 g.L^{-1} density gives effect to the microalgae cell. As a result of exposure to electric fields then the cell will be damaged that happens electroporation. Electrical field strength is the amount of force (voltage) from a certain gap (cm) for the process of cell membrane lysis of microorganisms. The amount of electric applied field strength is directly proportional to the treatment efficiency (Heinz et al. 2003). If the cell is exposed to exposure to a high voltage electric field it will produce a high trans membrane voltage (0.5 V - 1 V), this causes the phospholipid molecular arrangement of the membrane to change (Frey et al. 2013). As a result of the change, the cell membrane will lose the barrier function and become permeable; this phenomenon is often referred to as electroporation or Electro permeabilization (Frey et al. 2013; Ganeva et al. 2003).

The higher electric field causes the greater cell disruption. If the cell membrane is disrupted then the contents of the cell will come out or easily removed. Thus it will be able to increase oil yield. In general there is a tendency that the extraction results increase by increasing the electric field. Increasing the electric field provides better efficiency than the increase in the exposure time of the electric field. The amplitude value is the maximum peak of electric field strength in the application of pulsed electric field (PEF) (Huang and Wang, 2009).

In addition to voltage (amplitude) factors affecting cell damage rates are prolonged exposure (duration), number of pulses that pertain to cell and repetition frequencies (Canatella et al. 2001; Macek-Lebar and Miklavcic, 2001). The longer the cell is exposed to the electric field then the number of pores will be greater so that the contents of cells that can be issued also the greater. Figure 1 shows the pattern that the greater voltage and the longer exposure more extractable lipid content will be.

The use of energy associated with cost, the greater energy the greater cost incurred. Based on table 2 it will be better to use low energy as in the treatment of V1L1T1 of 14.72 J per unit of yield compared with the use of large enough energy as in V3L1T3 of 86.88 J per unit of yield. The higher voltage and the longer treatment will require greater energy.

CONCLUSION

Electric field strength can be seen from its ability to skip positive and negative electrode at a certain gap. Exposure of electric field to microalgae cells and continued chemical extraction can increase the efficiency of extraction lipid microalgae. The yield of microalgae lipid is influenced by electric field strength and long exposure. The stronger the exposure of the electric field to the microalgae cell will increase the electroporation and increase the yield of the microalgae lipid.

CONFLICT OF INTEREST

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

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AUTHOR CONTRIBUTIONS

The division of tasks in this study: IMS design, perform the experiments and write the manuscript, RBT edit and correct the manuscript. All authors read and approve the final version.

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