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Research Article

Electrochemical harvesting of the marine microalgae, *Nannochloropsis oculata*: Effect on approximate composition, fatty acid profile, and metals biosorption

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Abstract

The effect of electrochemical harvesting of *Nannochloropsis oculata* by aluminum (Al), iron (Fe), and graphite electrodes on the approximate composition, fatty acids profile, harvesting efficiency, and metals biosorption was investigated. Based on the results, the highest content of crude protein was observed in the control and graphite electrode groups, while the lowest value was measured in Al electrode (p<0.05). The highest content of fat (21.95 % in dry weight) was obtained in the microalgae harvested by Al electrode compared to other treatments (p<0.05). Maximum level of saturated fatty acids was observed in the microalgae harvested by Al electrode (89.68 % of total fat) (p<0.05). However, the lowest levels of mono- and poly-unsaturated fatty acids were recorded in Al electrode treatment (p<0.05). The lowest harvesting efficiency (67.44 %) was observed in graphite electrode treatment (p<0.05). The highest biosorption of Al and Fe were in the microalgae harvested by Al and Fe electrodes, respectively (p<0.05). Overall, electrocoagulation technique using various electrodes caused significant changes in biochemical composition of *N. oculata*. Although the highest biosorption of metals was in the microalgae harvested by sacrificial electrodes and even out of the allowed range of human and animal consumption, they would be suitable for biofuel production.

Keyword: Microalgae harvesting, Fatty acids, Electrochemistry, Electrocoagulation, *Nannochloropsis oculata*

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Introduction

Microalgae are rapidly growing autotrophic microorganisms which can be used in food industry, medicine, cosmetics, and biofuel production (Lauritano *et al.*, 2019; Hosseini Madani *et al*., 2020). *Nannochloropsis oculata* is a marine microalga (Eustigmatophyceae) which has a size of about 2-3 µm. Although this microalga is well-known for its ability to produce polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) (Kagan *et al.*, 2014; Borges *et al.*, 2016), several studies have shown the possibility of growing *N. oculata* on a large-scale for biofuel purposes (Richmond, 2008; Sabzi *et al.*, 2021). However, species belong to *Nannochloropsis* cannot be separated from the culture medium by conventional filtration methods due to their small size (Chua and Schenk, 2017). Therefore, finding a fast and cost-effective method to harvest *N. oculata* with minimal negative impacts on its quality can be a significant step towards industrial production of this microalga.

A bottleneck to the commercial production of microalgae is harvesting methods (Borges *et al.*, 2011, 2016; Yin *et al*., 2020). In fact, utilization of microalgae from the media accounts up to 30% of total processing cost (Al-Yaqoobi *et al.*, 2021; Krishnamoorthy *et al*., 2021). Although various chemical and natural coagulants are used to flocculate microalgae (Anthony *et al.*, 2013), high concentrations of chemical coagulants (*e.g.* aluminum sulfate, iron chloride, and ferrous sulfates) may adversely affect the quality of the cell's compounds and impact the environment (Misra *et al.*, 2014). Recently, harvesting microalgal biomass by electrocoagulation process is introduced as one of the fastest, most efficient, and cheap methods for harvesting microalgae (Matter *et al.*, 2019; Al-Yaqoobi *et al.*, 2021). In this regard, electrochemical harvesting (ECH) method is based on production of metal ions from oxidizing metal electrodes as well as micro-bubbles to facilitate coagulation of microalgae on the surface and/or precipitate at the bottom (Gao *et al.*, 2010; Perreault *et al.*, 2010; Kim *et al.*, 2012a; Matter *et al*., 2019). Moreover, the microalgae membrane has a negative electric charge due to presence of acidic polysaccharides and therefore, neutralizing negative charges of the cells by produced cations from electrodes in the ECH method causing the microalgae cells to be accumulated and precipitate (Safi *et al.*, 2014). Besides, the capital cost of ECH method (0.35 USD m^3) was much lower than flocculation with chemical substances (USD 0.47 m^3) and centrifugation (0.53 USD m^3) methods (Liu *et al*., 2018). In terms of harvesting efficiency, using syntactic flocculants to harvest 1 kg of algal biomass depends on the chemical used, acquire 2.30-15.3 USD compared to 1.15 USD for ECH method (Krishnamoorthy *et al.*, 2021).

Recently, one of the most important challenges for commercial-scale

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applications of microalgae is related to harvesting process, which is inefficient and costly, so research in this field is much needed and valuable (Krishnamoorthy *et al*., 2021). Utilization of microalgae with ECH method compared to the conventional methods has several advantages in large-scale applications, such as no direct side effects on the target metabolites (e.g., unsaturated lipids, protein, and carbohydrate), lower operation costs and energy consumption, easy to control, high efficiency, and less toxicity (Ghernaout, 2019; Hua *et al.*, 2020; Al-Yaqoobi *et al.,* 2021; Krishnamoorthy *et al*., 2021). However, chief drawbacks of ECH method are short lifetime of electrodes and biomass contamination with metal oxides by excessive doses of dissolved anode material in sacrificial electrode use (Matter *et al*., 2019). Therefore, this study aimed to assess the effect of harvesting *N. oculata* by ECH method using different sacrificial electrodes including aluminum (Al) and iron (Fe) and a non-sacrificial electrode (graphite) on biochemical composition and fatty acids profile.

Materials and methods

Experimental design

N. oculata strain PGBP-abdf1127 (GenBank: KP258172.1) was prepared from Persian Gulf Biotechnology Park (PGBP) and cultivated in F/2 culture medium (Guillard and Ryther, 1962) at Zakariya al-Razi Complex Laboratory Center (Science and Research Branch, Islamic Azad University, Tehran, Iran) based on Sabzi *et al.* (2021) method. The microalgae were grown under 80 µmol photon/m^2s light intensity, 26±0.5°C temperature, and 12:12 h (dark: light) light cycle conditions. After 14 days, algal biomass was separated from the culture medium during the log phase (~ 40×10^6 cells/mL) by ECH technique using different electrodes.

To harvest *N. oculata* by ECH method, a transparent plastic container (cylindrical reactor, 10 L) was equipped with two electrodes (10×4 cm) to clot the algal cells. The electrodes were connected to a direct current (DC) source at a 6-volt adapter and a current of 4 Amps (Misra *et al.,* 2015). Distance from the end of the electrodes to the bottom of the reactor was 8 cm and distance between the electrodes was 1 cm (Fig. 1). Electricity was on for 15 minutes in each treatment. A magnetic stirrer was used to mix the medium inside the reactors at 200 rpm during chemical electrolysis process.

In this study, experimental groups consisted of uniform sacrificial (Al and Fe) and non-sacrificial (graphite) electrodes (1×1×10 cm). Additionally, centrifugation harvesting method (3700×*g* at 4°C; Sigma 3-30K, Osterode, Germany) was considered as control group. Harvested biomass from all groups were lyophilized by a laboratory freez-dryer (Christ Alpha 1–4, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) at -54°C and a pressure of 0.04 mbar for 24 h to minimize the effects of heating on the

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biochemical compounds of the microalgae (Hosseini Shekarabi *et al.*, 2020).

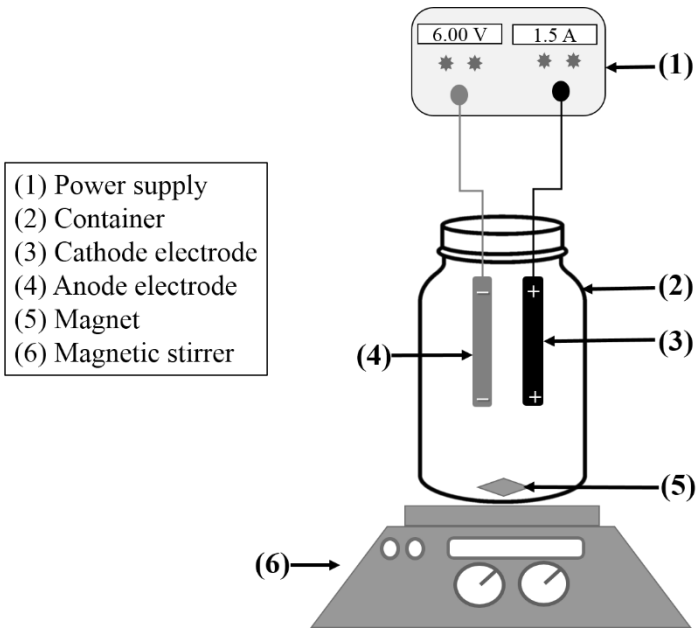


Figure 1: Schematic diagram of electrochemical technique for harvesting *N. oculata* biomass by different electrodes.

Approximate composition

To measure lipid content, 10 g of the harvested biomass (dry weight) from each treatment was gently homogenized in a mortar to obtain a fine powder and then, the lipid content was extracted by method of Folch *et al.* (1957). One gram of the lyophilized algae was placed in oven at 105°C for 6 hours to calculate moisture content (AOAC, 2000; method 976.05). Ash content was obtained using electric oven at 550°C until the samples' color was completely white (AOAC, 2000; method 923.03). To measure crude protein content, one gram of the lyophilized algae was digested with sulfuric acid. Then, nitrogen was extracted and titrated with 0.5 N hydrochloric acid using a semi-automatic Kjeldahl apparatus (V50, Bakhshi, Tehran, Iran) and converted to protein content (N × 6.25) (AOAC, 2000; method 976.05).

Determination of fatty acid composition

To determine the composition of fatty acids, derivatization of fatty acids was first performed by boron trifluoride in methanol for 150 mg of the extracted oil (Hosseini Madani *et al.*, 2021). The mixture was heated in water bath at 80ºC for 2 min and cooled to ambient temperature. The obtained upper organic phase (fatty acid methyl esters) was injected into a gas chromatograph (ACME 6100, Young Lin Instrument Co., Anyang, Korea), equipped with a DB-WAX capillary column (30 m length, 0.25 mm inner diameter, and 0.25 mm thickness) and a thermal conductivity detector. Temperature reached to 180°C and maintained for 5

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min and then increased to 220°C at 4°C/min rate and was maintained at this temperature for 25 min. Helium was used as a carrier gas at a rate of 1.5 mL/min. Obtained peaks of fatty acid methyl esters were identified by comparing their retention time with those of authentic standards (Fatty Acid Methyl Ester Mix, 18917AMP; Supelco, Sigma-Aldrich, USA).

Determination of metals residual

To determine heavy metals concentration, 10 mg of dried algal biomass was first digested by nitric acid. Then, according to atomic absorption spectrometer's guidelines (Varian SpectrAA-200 atomic absorption apparatus, Varian Co., Melbourne, Australia) as well as calibration curves of Al and Fe, the concentrations of the elements were calculated in the solutions (Ahmad and Shuhaimi-Othman, 2010).

Harvesting efficiency

To measure harvesting efficiency, optical density (OD) of the samples in pre-harvest medium (OD_i) and post-harvest medium (OD_f) was measured by a UV-VIS spectrophotometer (Varian Cary 50, Varian Inc., Palo Alto, California, USA) at 680 nm after 5 min from the end of each electrochemical harvesting method as follows (Vandamme *et al.*, 2011):

$$\text{Harvesting efficiency (HE, \%)} = \left[\frac{(ODi - ODf)}{ODi}\right] \times 100$$

Statistical Analysis

All experiments were performed with three replicates (n=3) and the data were presented as mean ± standard deviation (SD). After checking the normality and homogeneity of the data by Kolmogorov-Smirnov and Barlett's tests, respectively. One-way analysis of variance (ANOVA) was performed followed by Tukey's post-hoc test to compare the means at 95% (p<0.05). Data were analyzed using SPSS software version 22.

Results

As shown in Table 1, the contents of fat, protein, and ash of *N. oculata* harvested by different electrodes were significantly different (p<0.05). The highest level of crude protein was observed in control and graphite electrode groups (p<0.05), while the lowest value was measured in Al electrode treatment (p<0.05). Regarding fat and ash contents, the highest values were obtained in the microalgae harvested by Al electrode (p<0.05).

The composition of fatty acids in the microalgae harvested by ECH method using different types of electrodes is presented in Table 2. Based on the results, 13 fatty acids were detected and the dominant saturated fatty acid (SFA) was palmitic acid (16:0). The content of oleic acid (18:1) as the major monounsaturated fatty acid (MUFA) was decreased in experimental groups and the lowest value was obtained in Al electrode treatment (p<0.05). The highest EPA was measured in control and graphite electrode groups, while the

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lowest value was observed in Al electrode treatment (p<0.05).

According to Figure 2, the highest and lowest levels of SFA were measured in Al electrode treatment (75.96±1.33% of total fat) and control group (47.32±0.86 % of total fat), respectively (p<0.05). However, the lowest levels of MUFA and PUFA were recorded in Al electrode treatment (p<0.05). Maximum amount of MUFA was also seen in control group (34.68±0.74 % of total fat) (p<0.05).

Table 1: Effect of electrochemical harvesting technique using different electrodes on approximate composition of *N. oculata* (% in dry matter).

Parameter (% in dry matter)	Treatments			
	Control	Al electrode	Fe electrode	Graphite electrode
Protein	48.48±0.19 [a]	41.55±0.10 [c]	44.89±0.15 [b]	48.02±0.31 [a]
Lipid	18.30±0.25 [c]	21.95±0.07 [a]	20.36±0.12 [b]	18.82±0.33 [c]
Ash	20.02±0.23 [c]	23.79±0.19 [a]	21.10±0.04 [b]	19.26±0.04 [c]
Moisture	5.61±0.14 [a]	5.64±0.23 [a]	5.60±0.08 [a]	5.59±0.01 [a]

Values are presented as mean ± standard deviation. Different letters in each row indicate significant differences (n=3, p<0.05). Harvesting method in control group was centrifugation method (3700 ×g, 4°C, 10 min).

Table 2: Fatty acids profile of *N. oculata* after harvesting by electrochemical method using different electrodes (% of total fat).

Fatty acids	Treatments			
	Control	Al electrode	Fe electrode	Graphite electrode
Butyric acid (C4:0)	8.18±1.10 [a]	7.77±0.06 [a]	2.06±0.17 [b]	8.16±0.30 [a]
Caproic acid (C6:0)	2.59±0.01 [b]	3.40±0.06 [a]	0.74±0.05 [d]	2.24±0.08 [c]
Caprylic acid (C8:0)	12.93±0.11 [c]	20.86±0.15 [a]	6.36±0.54 [d]	15.69±0.40 [a]
Decanoic acid (C10:0)	7.85±0.08 [c]	8.42±0.49 [b]	9.38±0.20 [a]	5.14±0.02 [d]
Myristic acid (C14:0)	3.06±0.54 [a]	1.28±0.30 [b]	1.88±0.18 [b]	3.43±0.41 [a]
Palmitic acid (C16:0)	12.71±0.50 [d]	34.23±1.03 [a]	32.53±0.37 [b]	19.23±0.47 [c]
Stearic acid (C18:0)	3.94±0.03 [b]	5.70±0.29 [a]	5.86±0.14 [a]	3.76±0.03 [b]
Palmitoleic acid (C16:1)	2.05±0.08 [a]	0.31±0.04 [d]	0.56±0.01 [c]	1.42±0.06 [b]
Oleic acid (C18:1; n-9)	31.53±0.40 [a]	14.01±0.30 [d]	29.60±0.67 [b]	27.35±0.03 [c]
Erucic acid (C22:1)	1.10±0.09 [a]	0.36±0.08 [c]	0.83±0.05 [b]	1.20±0.02 [a]
Linoleic acid (C18:2; n-6)	10.07±0.14 [a]	2.30±0.01 [d]	7.65±0.63 [c]	8.52±0.60 [b]
Linolenic acid (C18:3; n-6)	0.50±0.05 [a]	0.10±0.00 [b]	0.49±0.02 [a]	0.13±0.00 [b]
Eicosapentaenoic acid (EPA; C20:5; n-3)	3.10±0.10 [a]	0.73±0.08 [c]	1.64±0.05 [b]	3.38±0.24 [a]
Total	99.36	99.28	99.37	99.45

Values are presented as mean ± standard deviation. Different letters in each row indicate significant differences (n=3, p<0.05). Harvesting method in control group was centrifugation method (3700×g, 4°C, 10 min).

Concentrations of Al and Fe in the dried algal biomass are illustrated in Table 3. The highest accumulation of Fe by microalgae cells was recorded in Fe electrode treatment compared to the other groups (p<0.05), while no

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significant difference was found among experimental groups to uptake Fe (p>0.05). In terms of Al concentration, it was only detected in Al electrode treatment.

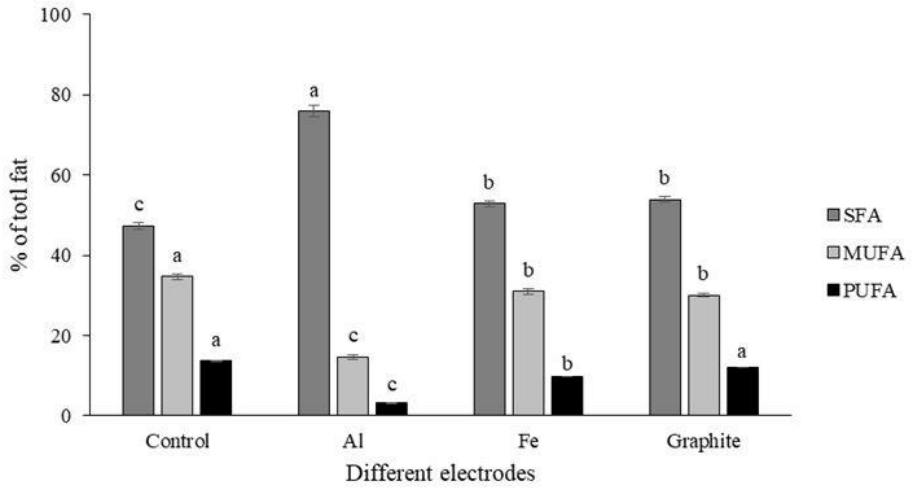


Figure 2: Changes in saturated fatty acids (SFA), monounsaturated fats (MUFA), and polyunsaturated fatty acids (PUFA) of harvested *N. oculata* by an electrochemical technique using different electrodes. Different superscripts indicate significant differences (n=3, p<0.05). Error bars show standard deviation.

Harvesting efficiency of the microalgae was influenced in different treatments and the highest value was obtained in Al electrode treatment which was not significantly different from control group (p>0.05; Fig. 3). However, the lowest harvesting efficiency value was observed in graphite electrode (p<0.05; Fig. 3).

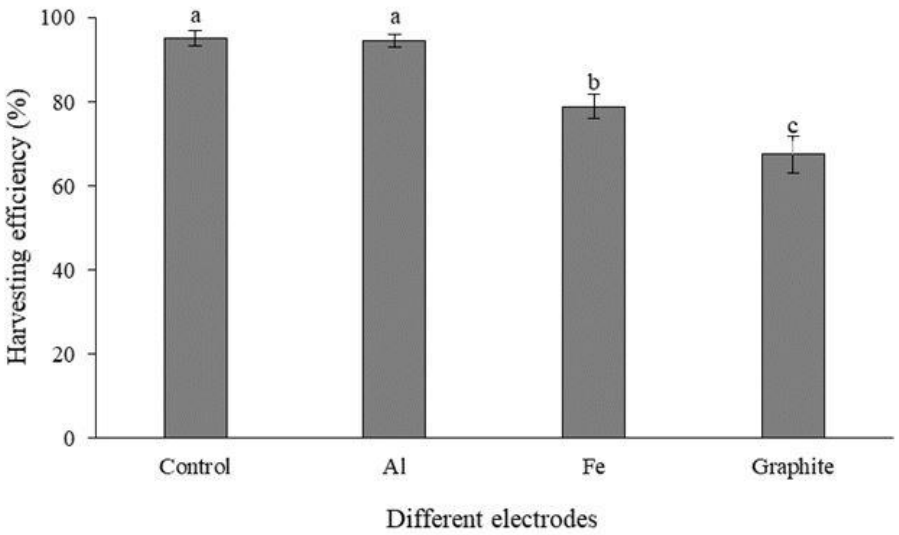


Figure 3: Effect of electrochemical technique using different electrodes in harvesting efficiency (%) of *N. oculata*. Different superscripts indicate significant differences (n=3, p<0.05). Error bars show standard deviation.

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Discussion

The ability to accumulate lipids in different microalgae species is highly dependent on cultivation condition and composition of nutrients in the culture medium (Borges *et al.*, 2011; Gao *et al.*, 2013; Rastar *et al*., 2018; Sabzi *et al.*, 2021). However, quantity and quality of microalgae lipids can be altered by different harvesting methods (Borges *et al.*, 2011, 2016; Hua *et al.*, 2016). In the present study, higher content of lipid was observed in *N. oculata* harvested by Al and Fe electrodes compared to graphite electrode and control groups. This increase in extraction of lipids may be related to higher cellular damages due to osmotic shock and oxidative destructions of released Al ions in the reactor (Yoo *et al.*, 2012; Lee *et al.*, 2013).

Based on our results, crude protein content of the microalgae harvested by graphite electrode and control groups was significantly higher than other treatments. Higher protein degradation ratio in the microalgae harvested by sacrificial electrodes may be due to extensive oxidative damages (e.g. Fenton-active metal ions oxidation process) and hydroxyl radicals products (Stadtman, 1990). However, more sensitive methods rather than Kjeldahl method in direct protein detection are recommended to compare with our results.

The highest level of ash was seen in *N. oculata* harvested by metal electrodes, especially in Al electrode treatment. This can be attributed to biosorption of released metals from sacrificial electrodes in the microalgae cells during ECH process. Baierle *et al.* (2015) represented that ECH techniques of microalgae with inorganic flocculants, especially Al, lead to significant decrease in heavy metals concentration of wastewater. This can easily approve that microalgae had ability to bio-absorb released metals from sacrificial metal electrodes and subsequently increase the ash content.

Our findings showed that maximum content of unsaturated fatty acids was in *N. oculata* harvested by graphite electrode compared to sacrificial electrodes, while maximum level of SFA was obtained in the microalgae harvested by Al electrode. Misra *et al.* (2014) similarly showed that harvesting *Chlorella sorokiniana* and *Tetradesmus obliquus* by graphite electrodes did not have a negative effect on composition of fatty acids compared to conventional centrifugation method. Using non-destructive electrodes in ECH method not only released no metal ions to the environment but also oxidative processes would be at the lowest rate, which can prevent breaking down the chains and carbon double bonds of unsaturated fatty acids in post harvested microalgae (Singh *et al.*, 2014; Guldhe *et al.*, 2016).

Saturated fatty acids from microalgae are used to produce biodiesel due to their high thermal and oxidative stability (Singh *et al.*, 2014). However, long-chain unsaturated fatty acids are suitable for pharmaceutical and food applications (Borges *et al.*,

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2011). In the present study reduction of PUFAs, especially EPA, in the microalgae harvested by sacrificial electrodes may be due to the fact that unsaturated fatty acids are highly vulnerable to oxidative processes (Singh *et al.*, 2014; Hosseini Shekarabi and Shamsaie Mehrgan, 2021). Indeed, oxidation and degradation of anode electrode is main drawback of ECH method using sacrificial electrodes (Kim *et al.*, 2012a, 2012b). However, technology of using non-destructive electrodes in ECH method can be used to harvest algal biomass for various industrial and food purposes (Perreault *et al.*, 2010; Misra *et al.*, 2015). On the contrary, Guldhe *et al.* (2016) pointed out that ECH process did not affect the fatty acid composition of *Ankistrodesmus falcatus* and *T. obliquus* biomass by sacrificial electrodes compared to non-sacrificial electrodes (graphite). This conflict in the results can be related to various factors such as environmental conditions, technology used in ECH process, algal biomass density and species (Misra *et al.*, 2014).

The results of this study showed that concentrations of Al and Fe were dramatically increased in the microalgae harvested by sacrificial electrodes and in terms of Al concentration, it exceeded standard limits for aquaculture, irrigation, and even indirect human contact (maximum 10 mg/L) (Uduman *et al.*, 2010). Although concentration of Fe was much higher than that in the algal biomass harvested by centrifugation method (control group), low concentration of Al can cause greater environmental and human hazards compared to Fe (Ribes *et al.*, 2008). In agreement with our results, Baierle *et al.* (2015) demonstrated that residual level of Al in *Desmodesmus subspicatus* harvested by Al electrode was more than that harvested by centrifugation method. Since concentration of Al is directly associated with health risks (Bondy, 2010), using Al electrodes in ECH is not an appropriate technique for human or animal consumption, while it is recommended to harvest microalgae with sacrificial electrodes for biodiesel production.

The results of our study showed that harvesting efficiency for *N. oculata* in sacrificial electrodes was higher than graphite electrode, while harvesting efficiency in control group (centrifugation method) was more than all experimental groups. Although filtration and centrifugation methods have higher harvesting efficiency than ECH methods, these common harvesting methods require more energy and time (Baierle *et al.*, 2015). The process of harvesting microalgae by ECH is influenced by various parameters such as species, size of microalgae, biomass density, and electrochemical conditions (*i.e.* current intensity, voltage, and type of electrodes) (Misra *et al.*, 2014). From our results, harvesting efficiency of *N. oculata* in Al electrode treatment was higher than that of Fe electrode. Similarly, Baierle *et al.* (2015) reported 95.4% harvesting efficiency using Al

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electrodes compared to Fe electrodes (64.7%) for *D. subspicatus*. In general, Al electrodes cause higher harvesting efficiency than Fe and graphite electrodes for three main reasons: (1) Al is more reactive to participate in electrolysis reactions and generate more cations (Al^{3+}) than other metals, which can increase flocculation rate of microalgae by binding to the cells (Wong *et al.*, 2017); (2) electrical conductivity of Al is more than iron (Cañizares *et al.*, 2005; Zongo *et al.*, 2009); and (3) higher toxicity of produced aluminum hydroxide accelerates the cell death and consequently sedimentation rate (Duan and Gregory, 2003; Gao *et al.*, 2010).

In the present study, harvesting efficiency of *N. oculata* in graphite electrode treatment was 67.66%, which was significantly lower than that of metal electrodes. This may be due to absence of metal cations. In consistence with our results, Misra *et al.* (2014) showed that minimum harvesting efficiency for *C. sorokiniana* and *T. obliquus* was recorded in graphite electrodes treatment in comparison with that of metal electrodes. Also, Vandamme *et al.* (2011) reported a higher harvesting efficiency (about 92%) for *Chlorella vulgaris* by sacrificial electrodes.

This study showed that using sacrificial and non-sacrificial electrodes for ECH of *N. oculata* caused significant changes in lipid and protein contents as well as fatty acids profile. The highest crude protein content and unsaturated fatty acids were seen in the microalgae harvested by graphite electrode. However, the highest level of oils with a high content of saturated fatty acids was extracted from the microalgae harvested by Al electrodes. According to high accumulation of Al in the microalgae cells harvested by Al electrodes, this harvesting technique is recommended for biofuel production, however, further research is required to evaluate its environmental pollution.

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