

## Harvesting of Microalgae *Dunaliella salina* Using Electroflocculation

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### ABSTRACT

One of the important bottle-necks in production of microalgae based biodiesel is the lack of an efficient method for harvesting of microalgae from the culture medium. In this study, the electroflocculation method was developed for harvesting of microalgae *Dunaliella* cells from culture medium. The effect of several parameters such as the current density, electrical conductivity of culture medium, time, electrode gap, and electrode type on harvesting efficiency and energy consumption were also determined. The maximum harvesting efficiency of this method was 97.44% at  $EC= 1.35 \text{ S m}^{-1}$  and current density of  $90 \text{ A m}^{-2}$  during 3 minutes electroflocculation process in a 300 ml beaker. The maximum efficiency was achieved by aluminum electrodes with 1 cm distance between electrodes and the maximum energy consumption for this practice was  $0.621 \text{ kW h m}^{-3}$ . It was concluded that electroflocculation is an efficient and cost effective method for microalgae harvesting.

**Keywords:** Biodiesel, Cell coagulation, Dewatering, Harvesting Efficiency, Microalgae harvesting.

### INTRODUCTION

Recently, production of biodiesel from single cell oil bearing microorganisms (SCO) has become an important challenge for researchers. Due to high photosynthetic efficiency, high biomass production, and rapid growth rate compared to other oil bearing products, microalgae has been introduced as a new feedstock for biodiesel production (Dote *et al.*, 1994; Minowa *et al.*, 1995). Race way ponds and photobioreactors are two equipment extensively used for production of microalgae (Chisti, 2007). There is a lot of experience in microalgae cultivation using both of these equipment. However, an important bottle-neck

is still remaining in finalization of culturing process, which is the heavy cost of separation or harvesting of the produced microalgae from the culture medium.

There are several methods for microalgae harvesting. Centrifugation is a typical method that is widely used for microalgae separation from culture medium (Price *et al.*, 1974), but it is time consuming, complicated, and expensive. The cost of energy, materials, maintenance, depreciation and the service of centrifuge may reach up to 25 percent of total microalgae production cost. The investment cost on centrifuge is also high (Knuckey *et al.*, 2006).

Regarding the small size of microalgal cells (5-30  $\mu\text{m}$ ), physical separation of these cells from culture medium using filtration method is

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difficult. Filter clogging is a common problem in harvesting of microalgae by filtration. The filter clogging substantially increases head loss and requires frequent maintenance.

Horiuchi *et al.* (2003) have investigated the effect of pH changes in harvesting efficiency of *Dunaliella tertiolecta*. They stated that, by increasing the pH of culture medium using NaOH, *Dunaliella* cells coagulate rapidly and settle within a few minutes in the bottom of the container. Froth flotation is another method for separation of microalgae from culture medium (Levin, *et al.*, 1962). In this method, aeration and decreasing the pH of culture medium are used simultaneously to harvest microalgae. Therefore, instead of sedimentation, microalgae cells are floated on the surface of culture medium.

Harvesting of microalgae by adjusting the pH increases or decreases the pH of culture medium, while the high or low pH of culture medium should be neutralized with HCl and NaOH, respectively. This needs extra cost. Also, the culture medium neutralization generates NaCl, which increases the salinity of microalgae culture medium and desalination maybe required. The neutralization and desalination increases the harvesting cost.

The cell flocculation has been investigated by several researchers (Papazi *et al.*, 2010; Harith *et al.*, 2009). In this method, using inorganic chemicals such as ferric, aluminum, and zinc salts, microalgae cells are coagulated and slowly sink to the bottom of the container. Cations such as  $Al^{3+}$  or  $Fe^{3+}$  neutralize the negative charge of microalgae cells. Therefore, the cells aggregate and form large flocs which are then settled and separated from culture medium. Danquah *et al.* (2008) used polymeric flocculants and Oh *et al.* (2001) used bioflocculants for microalgae harvesting. The cost of microalgae harvesting using chemical flocculants is lower than the centrifugation but, in this method, a coagulant is added to the culture medium and remains in the flocs or culture medium after harvesting.

Electroflocculation is an almost new technology for waste water treatment and removing of solid particles from water (Phalakornkule *et al.*, 2009). The main

advantages of electroflocculation over conventional methods are lack of secondary pollution and compact equipment. This method has not been used for microalgae harvesting.

Considering the mentioned problems in microalgae harvesting, the main aim of this research work was to suggest a method for microalgae harvesting with high efficiency and low cost. The new method should not add any chemicals to the culture medium so that it could be reused for culturing of microalgae without any extra cost. Therefore, the electroflocculation method was introduced for harvesting of microalgae and the effect of several factors such as current rate, time, culture medium EC, electrode gap, and electrode type on energy consumption and harvesting efficiency were evaluated.

## MATERIALS AND METHODS

### Microalgal Culture

Separation of *Dunaliella Salina* from culture medium is more difficult than other microalgae species due to the small size, low concentration of cells, and high electrical stability. Therefore, *Dunaliella Salina* was selected and used throughout this study. The *Dunaliella* was cultivated in a 250 L photobioreactor and it was harvested at  $OD_{680}$  of 0.333. Cell concentration was determined by Genesys 5 spectrophotometer.

### Electroflocculation Experiments

There are several parameters that affect the harvesting efficiency. In this paper, the effect of different factors such as current rate, time, culture medium EC, electrode gap and electrode type on energy consumption and harvesting efficiency was evaluated. All electroflocculation experiments were carried out in 300 ml beaker in three repetitions.

The averages of repetitions were reported as the results. The initial value of  $EC$  for microalgae solution was  $6.02 \text{ S m}^{-1}$ . Two other  $EC$  were adjusted at  $4.49$  and  $1.35 \text{ S m}^{-1}$  by adding demineralized water to the culture medium. The  $EC$  was measured using  $EC$  meter (Cond 315i WTW). The cultures were stirred at  $100 \text{ rpm}$  by magnetic stirrer with a  $3 \text{ cm}$  magnet. Two aluminum electrodes with  $6 \text{ cm}^2$  surface area were placed in the culture and the distance between electrodes was adjusted to  $3 \text{ cm}$ . A direct current generator (Dazheng PS-305D, China) was used as energy source. Both voltage and current rate were adjustable in this generator. First, the positive and negative cables were connected to electrodes and the current density was adjusted to  $30$ ,  $50$ ,  $70$  and  $90 \text{ A m}^{-2}$ . Then, the generated voltages between electrodes in each culture were recorded. The energy consumption was determined by multiplying current by voltage.

To determine the effect of current intensity on harvesting efficiency, the mentioned procedure was repeated, however, after  $3$  minutes, the energy was switched off but the mixing was continued for  $8$  minutes to complete the flocculation of the cells. Then, the stirrer was switched off and samples were taken from the depth of  $3 \text{ cm}$  after  $2$  minutes. The optical density of samples was measured at  $OD_{680}$ .

Furthermore, the effect of distance between electrodes on energy consumption was studied. The distances were adjusted at  $1$ ,  $2$ ,  $3$  and  $4 \text{ cm}$  and the  $EC$  was set to  $1.35 \text{ S m}^{-1}$ . At current density of  $50 \text{ A m}^{-2}$  for each distance, the generated voltages between electrodes were recorded to evaluate energy consumption. Harvesting efficiency in each case was also determined based on the above mentioned method.

The effect of time on harvesting efficiency was determined by adjusting the  $EC$ , current density and electrode gap to  $1.35 \text{ S m}^{-1}$ ,  $50 \text{ A m}^{-2}$ , and  $3 \text{ cm}$ , respectively. The samples were taken after  $3$ ,  $5$ ,  $7$  and  $9$  minutes.

Finally, the effect of electrode type on energy consumption was investigated by comparing aluminum and iron electrodes in solution with  $EC$  of  $1.35 \text{ S m}^{-1}$ . At current densities of  $30$ ,  $50$ ,  $70$  and  $90 \text{ A m}^{-2}$ , the generated voltages between electrodes was recorded.

### Calculation of Harvesting Efficiency

The harvesting efficiency was calculated by comparing the optical density of the culture at  $680 \text{ nm}$  before and after electroflocculation, using the following equation (Papazi *et al.*, 2010).

$$HE (\%) = \frac{OD_0 - OD}{OD_0} \times 100$$

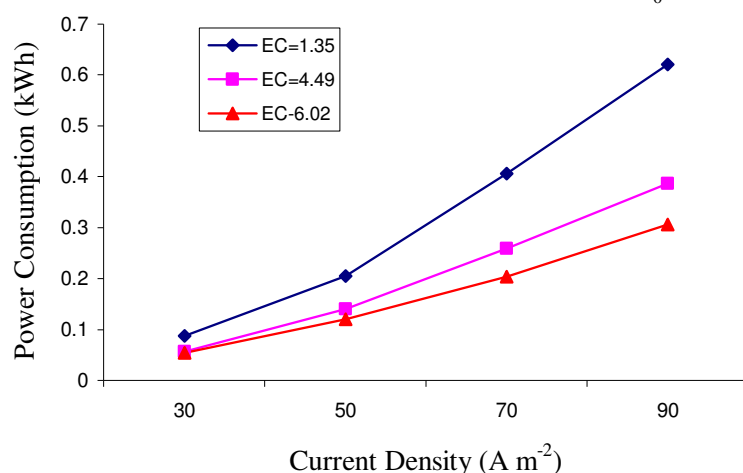


Figure 1. Effect of  $EC$  on energy consumption in  $300 \text{ ml}$  microalgae culture.



Where,  $OD_0$  is optical density of the culture before electroflocculation and  $OD$  is the optical density after electroflocculation process.

## RESULTS AND DISCUSSION

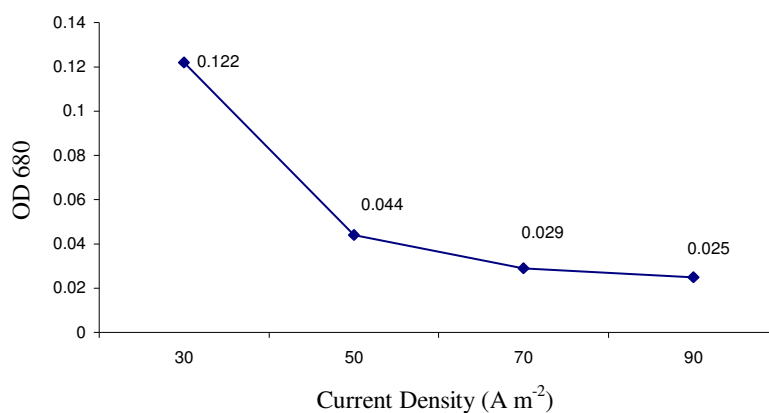
### Effect of $EC$ on Energy Consumption

It was observed that, at each  $EC$ , the energy consumption increased by increasing the current rate, but, at solutions with low salinity and low  $EC$ , the energy consumption was high (Figure 1). Therefore, harvesting of marine and all saline water microalgal cultures by electroflocculation method will consume lower energy compared with harvesting of fresh water microalgal species.

At current density of  $50 \text{ A m}^{-2}$ , the generated voltage between electrodes increased from 1.2 to 2.5V for changing  $EC$  from 6.02 to  $1.35 \text{ S m}^{-1}$ . Therefore, the required electrical energy for harvesting of fresh water microalgae is almost twice the energy required for marine microalgae harvesting.

### Effect of Electroflocculation on Optical Density

The effect of electroflocculation on optical

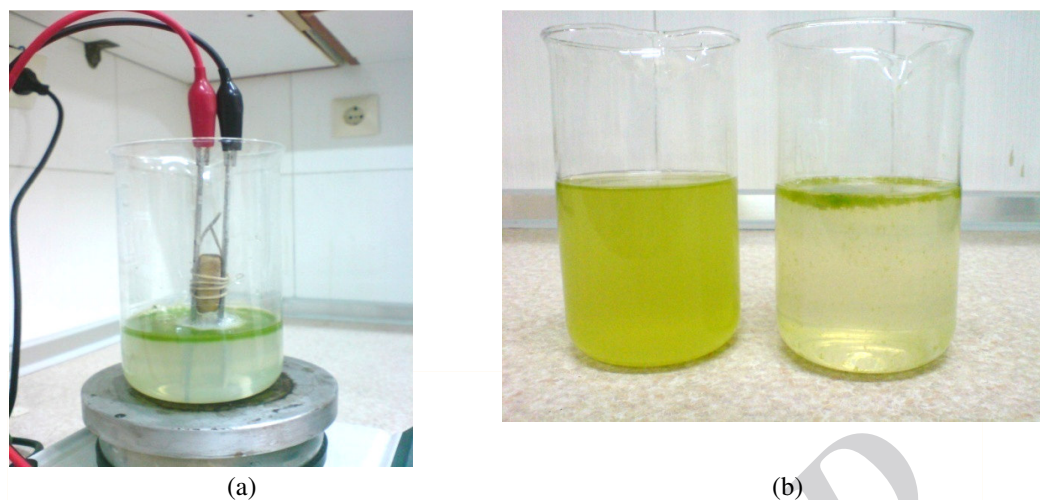


**Figure 2.** Decreasing of  $OD_{680}$  by increase in current density at  $EC = 6.02 \text{ S m}^{-1}$ .

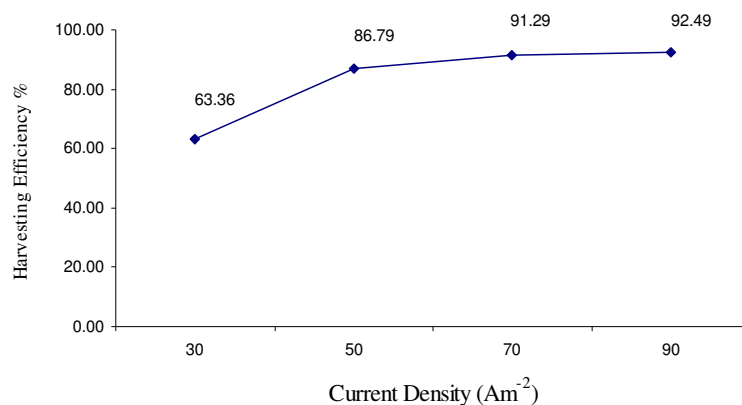
density of microalgae *Dunaliella*, was determined at  $EC = 6.02 \text{ S m}^{-1}$ . By increasing the current rate, the optical density of solution was decreased (Figure 2). This means that at higher current rate, the neutralization of algal cells by  $\text{Al}^{3+}$  cations was increased and, therefore, the cells flocculated and floated on top of the beaker. This phenomenon decreased the turbidity of solution (Figure 3).

### Effect of Electroflocculation on Harvesting Efficiency

By raising the current density from 30 to  $90 \text{ A m}^{-2}$ , the harvesting efficiency increased from 63.36 to 92.49% (Figure 4). To achieve the maximum harvesting efficiency, the current rate was increased to 1.5 A, consequently, the harvesting efficiency reached 98.9%. Although by increasing the current high harvesting efficiency could be obtained, but, considering the following two aspects it is not necessary to reach high harvesting efficiency. Firstly, the algal cells remaining in the culture solution after harvesting can be reused as inoculums for a new culturing process because it was observed that the  $OD$  of the medium culture was increased three days after harvesting. It means that the electroflocculation had no effect on algal cell viability. Secondly, raising the harvesting efficiency from 92.49



**Figure 3.** (a) Flocculation of algal cells by electroflocculation, and (b) Decrease in the turbidity of solution after electroflocculation.



**Figure 4.** Raising the harvesting efficiency by increasing current density at  $EC = 6.02 \text{ S m}^{-1}$ .

to 98.9% consumes extra energy which is not economically justified. Oh *et al.* (2001) have reported a harvesting efficiency of 83% using biofloculants and 72% using aluminum sulfate as flocculant. Also, Harith *et al.* (2009) have reported a harvesting efficiency of higher than 90% by adjusting the pH of microalgae culture to 10.2. Finally, Papazi *et al.* (2010), by comparing twelve salts as coagulants, stated that the harvesting efficiency of 80% was achievable using aluminum chloride during 1 hour. Comparing the results of these research works indicate that the electroflocculation is an efficient method for harvesting of microalgae during short periods of time (10 minutes), without adding any extra

floculants to the culture medium. The amount of electrodes dissolution in the culture medium is negligible compared with the addition of chemical coagulants.

#### Effect of $EC$ on Harvesting Efficiency

The effect of  $EC$  on harvesting efficiency was determined by measuring  $OD_{680}$  before and after electroflocculation at three different  $EC$  including 6.02, 4.49, and 1.35  $\text{S m}^{-1}$ . Then, using Equation (1), the harvesting efficiency was calculated for each solution (Figure 5). The harvesting efficiency at  $EC$  of 1.35  $\text{S m}^{-1}$  was the highest. Since, according to Figure 1, at low electrical



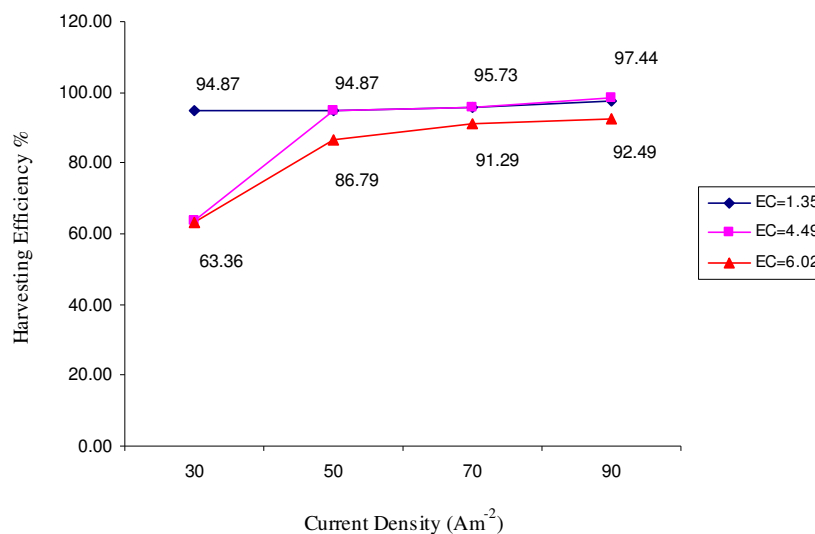


Figure 5. Effect of EC on harvesting efficiency at three different EC.

conductivity high voltage was generated between electrodes which enabled the  $\text{Al}^{3+}$  cations to be departed easily from electrodes, electroflocculation occurred at high efficiency. The amount of electrode dissolution was not measured in this practice, but it should be investigated as another research work from environmental aspects. The effect of EC on harvesting efficiency at current densities of higher than  $90 \text{ A m}^{-2}$  was not considerable because in all solutions the harvesting efficiency reached the highest value of 98.9 % at 1.5 A.

#### Effect of Electrode Gap on Energy Consumption

The effect of distance between electrodes on energy consumption is shown in (Figure 6-a). The EC and current density were  $1.35 \text{ S m}^{-1}$  and  $50 \text{ A m}^{-2}$ , respectively. It is clear that, by increasing the gap from 1 to 4 cm, the energy consumption was raised from 0.13 to  $0.23 \text{ kW h m}^{-3}$ . As the distance was increased, high potential energy was required for transferring the electrons between electrodes. Therefore, the energy consumption was increased. Also, increasing the formation of bobbles around the electrodes by decreasing the electrode gap under 1 cm caused the increase in resistance

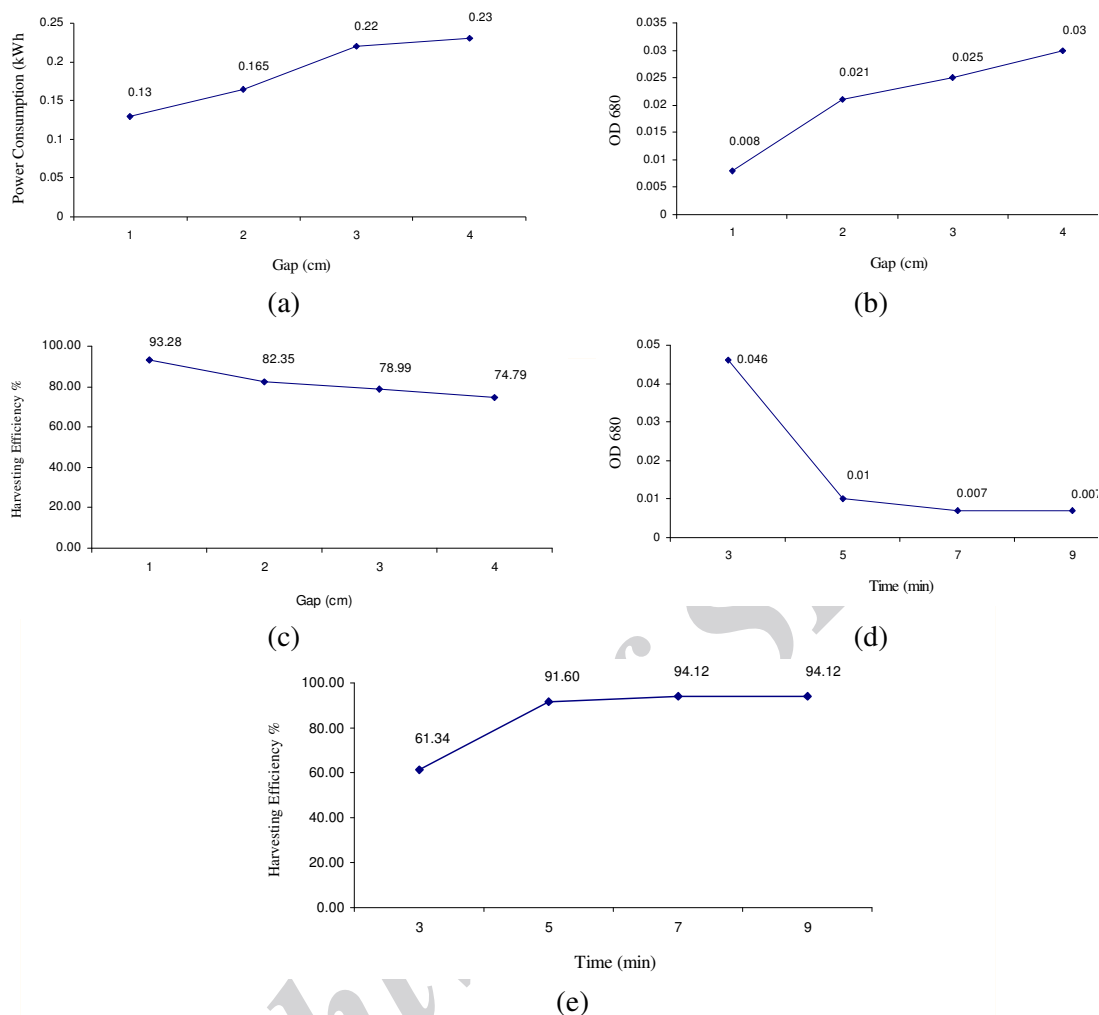
between the electrodes, thereby increasing energy consumption.

#### Effect of Electrode Gap on Optical Density

The optical density of microalgae *Dunaliella* suspension after electroflocculation was measured in four different electrode gaps (Figure 6-b). The lowest OD was achieved when the electrodes were closer to each other (Gap= 1 cm). This means that as the electrodes were adjusted closer to each other, the flocculation process occurred at higher efficiency and the turbidity of algal culture was diminished completely after harvesting. But, at the gaps shorter than 1 cm, the formation of bubbles between electrodes caused a higher electrical resistance and the flocculation efficiency was decreased.

#### Effect of Electrode Gap on Harvesting Efficiency

The harvesting efficiency was determined for samples taken from solution after electroflocculation practice with different electrode gaps (Figure 6-c). Regarding the



**Figure 6.** Effect of (a) electrode gap on energy consumption (b) electrode gap on optical density after electroflocculation (c) electrode gap on harvesting efficiency (d) electroflocculation time on optical density (e) electroflocculation time on harvesting efficiency, at fixed current density of  $50 \text{ A m}^{-2}$ , and a solution with  $EC = 1.35 \text{ S m}^{-1}$ .

optical density (Figure 6-b), it is clear that the harvesting efficiency was diminished when the distance between electrodes was increased. On the other hand, as mentioned in section 3.5, the energy consumption at further electrode gaps was increased. Therefore, shorter electrode gap was suitable for electroflocculation and it had the advantages of both low energy consumption and high harvesting efficiency. But, because of the reasons stated in section 3.6, the gaps shorter than 1 cm are not suitable for electroflocculation.

### Effect of Electroflocculation Time on Optical Density

In order to determine the effect of electroflocculation time on optical density of algal culture, sampling was started after 3 minutes from beginning of electroflocculation process and it was repeated every 2 minutes for three times (Figure 6-d). The optical density of the solution decreased with time. After 7



minutes, the *OD* became stable and the electroflocculation process was stopped.

### Effect of Electroflocculation Time on Harvesting Efficiency

By applying electricity to electrodes for 7 minutes, the maximum harvesting efficiency is achievable, and longer electroflocculation process is not necessary (Figure 6-e). But, the best results were achieved by applying electricity for 3 minutes and mixing the solution for 5 minutes. This is because, firstly, low electrical energy is consumed and, secondly, all of the  $Al^{3+}$  cations are used for flocculation of algal cells and there is no extra  $Al^{3+}$  cations in the solution after harvesting. Also, the fouling of electrodes increases by increasing the time of electroflocculation.

### Effect of Electrode Type on Energy Consumption

Regarding high cationic property of  $Fe^{3+}$  and  $Al^{3+}$ , these two metals were compared to determine the effect of electrode type on energy consumption. Figure 7 shows the difference between energy consumption in each metal. The Fe electrodes consume more electrical energy than Al electrodes. Also, by using Fe electrodes, the color of algae cells turns to dark brown and the wall of

beaker is covered by Fe oxides and becomes opaque. But, in using Al electrodes, no changes are observed in the color of algal cells or the beaker wall because  $Al^{3+}$  is colorless, while  $Fe^{3+}$  is brown.

### CONCLUSIONS

The electroflocculation process for harvesting of microalgae *Dunaliella* cells was investigated in this study. Test results showed that, as the electrical conductivity of algal solution increased, lower electrical energy was required for microalgae harvesting. Therefore, harvesting of marine microalgae by this method seems to be cheaper than fresh water microalgae. The harvesting efficiency of 98.9% is simply achievable by electroflocculation process. The distance between electrodes is a major factor in harvesting efficiency and energy consumption. Results showed that short distance between electrodes increased harvesting efficiency and diminished energy consumption. Also, the effect of electroflocculation time on harvesting efficiency was investigated in this paper. The harvesting efficiency of 94% could be easily achieved during 7 minutes.

Comparison of Al and Fe electrodes showed that the Fe electrodes changed the color of microalgae cell to dark brown. Also, Fe electrodes consumed more electrical energy than Al electrodes for

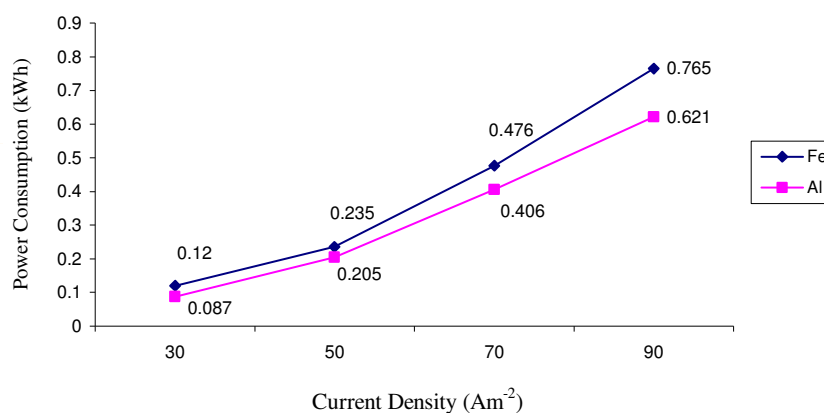


Figure 7. Effect of electrode type on energy consumption at a solution with  $EC = 1.35 S m^{-1}$ .



electroflocculation. Therefore, Al electrodes were introduced as suitable type for electroflocculation process.

Finally, it was concluded that microalgae harvesting by electroflocculation process was an efficient method that could harvest microalgae from the culture medium during a short time, with high efficiency and without adding any extra chemical materials to the culture medium.

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### REFERENCES

1. Chisti, Y. 2007. Biodiesel from Microalgae. *Biotechnol. Advan.*, **25**: 294–306.
2. Danquah, M. K., Ang, L., Uduman, N., Moheimani, N. and Forde, G. M. 2008. Dewatering of Microalgal Culture for Biodiesel Production: Exploring Polymer Flocculation and Tangential Flow Filtration. *J. Chem. Technol. Biotechnol.*, **84**:1078-1083.
3. Dote, Y., Sawayama, S., Inoue, S., Minowa, T. and Yokoyama, S. 1994. Recovery of Liquid Fuel from Hydrocarbon-rich Microalgae by Thermochemical Liquefaction. *Fuel*, **73**: 1855–1857.
4. Harith, T. H., Yuosoff, F. M., Mohamed, M. S., Mohamed Din, M. S. and Ariff, A. B. 2009. Effect of Different Flocculants on the Flocculation Performance of Microalgae, *Chaetoceros Calcitrans*, Cells. *African J. Biotechnol.*, **8(21)**: 5971-5978.
5. Horiuchi, J., Ohba, I., Tada, K., Kobayashi, M., Kanno, T. and Kishimoto, M. 2003. Effective Cell Harvesting of the Halotolerant Microalga *Dunaliella* with pH Control. *J. Biosci. Bioengineer.*, **95(4)**: 412-415, 2003.
6. Knuckey, R., Brown, M., Robert, R. and Frampton, D. 2006 Production of Microalgal Concentrates by Flocculation and Their Assessment as Aquaculture Feeds. *Aquacul. Engin.*, **35**: 300–313.
7. Levin, G. V., Clendenning, J. R., Gibor, A. and Bogar, F. D. 1962. *Harvesting of Algae by Froth Flotation*. Resource Research Ins., Washington, PP. 169-174.
8. Minowa, T., Yokoyama, S. Y., Kishimoto, M. and Okakurat, T. 1995. Oil Production from Algal Cells of *Dunaliella tertiolecta* by Direct Thermochemical Liquefaction. *Fuel*, **74**: 1735–1738.
9. Oh, H-M., Lee, S. J., Park, M-H., Kim, H-S., Kim, H-C., Yoon, J-H., Kwon, G-S. and Yoon, B-D. 2001. Harvesting of *Chlorella Vulgaris* Using a Bioflocculant from *Paenibacillus* sp. AM49. *Biotechnol. Letters*, **23**: 1229-1234.
10. Papazi, A., Makridis, P. and Divanach, P. 2010. Harvesting *Clorella minutissima* Using Cell Coagulants. *J. Appl. Phycol.*, **22**: 349-355.
11. Phalakornkule, C., Polgumhang, S., and Tongdaung, W. 2009. Performance of Electrocoagulation Process in Treating Directy Dye: Batch and Continuous up Flow Process. *World Academy Sci. Technol.*, **57**: 277-282.
12. Price, C. A., Mendiola-Morgenthaler, L. R., Goldstein, M., Breden, E. N. and Guillard, R. R. L. 1974. Harvest of Planktonic Marine Algae by Centrifugation into Gradients of Silica in the CF-6 Continuous Flow Zonal Rotor. *Biol. Bull.*, **147**: 136–145.



## برداشت ریزجلبک دونالیلا سالیبا با استفاده از انعقاد الکتریکی

ع. زنوزی، ب. قبادیان، م. ا. حجازی و پ. رهنمون

### چکیده

یکی از مهم ترین گلوگاهها در تولید سوخت بیودیزل از ریزجلبک، عدم وجود روشی مناسب برای برداشت ریزجلبک از محیط می باشد. در این مقاله روش انعقاد الکتریکی برای برداشت سلولهای ریزجلبک دونالیلا مورد استفاده قرار گرفت. همچنین تاثیر پارامترهای مختلف از قبیل شدت جریان، هدایت الکتریکی محیط کشت، زمان، فاصله الکترودها و نوع الکترودها بر بازده برداشت و توان مصرفی تعیین شد. بازده برداشت پیشینه در هدایت الکتریکی ۱.۳۵ زیمنس بر متر و چگالی جریان ۹۰ آمپر بر مترمربع در طی ۳ دقیقه اعمال جریان الکتریسیته با استفاده از الکترودهای آلومینیومی با فاصله ۱ سانتی متر برابر با ۹۷.۴۴ درصد بدست آمد و انرژی مصرفی این روش برابر با ۰.۶۲۱ کیلووات ساعت بر متر مکعب بود. نتیجه گرفته شد که روش انعقاد الکتریکی، روشی موثر و اقتصادی برای برداشت ریزجلبک می باشد.