



Optimization of electroflocculation based biomass harvest in microalgae *Dunaliella* sp. and *Nannochloropsis* sp.

Anitha S., K. Vignesh and B. M. Jaffar Ali*

Bioenergy and Biophotonics Laboratory, Center for Green Energy Technology, Pondicherry University, Puducherry- 605014, India

Abstract

Efficiency of biomass harvesting is one of the dominant factors that play the key role in the production of biodiesel from microalgae. It is therefore important to evaluate the harvest process based on type, cost, time and efficiency of the techniques together in order to minimize the cost of biodiesel production. In this study we aim to investigate the electro flocculation technique for harvesting microalgae under given operating parameters and their effects on harvesting efficiency and power consumptions. A 2L capacity electroflocculator is constructed with 10A/24V dc power supply and aluminium as sacrificial electrode. Laboratory cultured *Dunaliella* and *Nannochloropsis* species were electro flocculated to harvest microalgae biomass at various process conditions namely, flocculation time and settling time. At 20 minutes settling time after electroflocculation for 40s, it was observed that for *Dunaliella* species maximum harvesting efficiency of 80% could be attained at a power of 3.2 kWh/kg. However, in terms of power consumed, 77% efficiency appears to be optimal with 2.6 kWh/kg. For *Nannochloropsis* species, optimal harvesting efficiency of 85% was observed with a power consumption of 3.2 kWh/kg at 30 minutes settling time under similar flocculation conditions.

Keywords: Electroflocculation; Biomass harvest; Microalgae.

Introduction

Microalgae are photosynthetic organisms abundant in nature which are capable of growing in various environments. Biomass productivity of the microalgae is significantly greater than higher plants and it can be cultivated in shallow raceway ponds using saline or brackish water [1]. Biomass obtained from microalgae can be used to produce various value added products such as biodiesel, bio ethanol, and biogas, fish feed, animal feed and human food supplements including Omega-3-fatty acids. Various microalgae strains contain high amounts of proteins (43-71% dry matter), carbohydrates (10-30% of dry matter) and oil content in cell which make up 25-77% of dried biomass weight [2]. Apart from using microalgae for food supplement as single-celled proteins, in recent years they are regarded as living-cell factories for the production of bio-fuels and bio-chemicals used in food, poultry and pharmaceutical industries [3].

Microalgae are regarded as the best candidate for the production of biodiesel since they do not compete with edible crops. Application of bio refinery concept to produce biodiesel and other value added products will enhance the economics of biodiesel production. However, processing microalgae into biodiesel requires culturing of the microalgae in a large scale, recovery of the microalgae biomass and the extraction and downstream processing of the oil. However, the major obstacle for using microalgae biomass on an industrial-scale for the production of biodiesel is the dewatering step. They need to be concentrated because they exist as a dilute suspension 0.1 to 1.7 g of dried biomass per litre. Dewatering accounts for 20-40% of the total costs associated with microalgae production and processing [4]. Even though the extraction cost decreases with increased biomass concentration, in order to achieve economically viable production, microalgae recovery process needs to be made less costly and less time consuming.

Dunaliella species belong to the phylum chlorophyta, order volvocales and family polyblepharidaceae, and are unicellular, photosynthetic and motile biflagellate microalgae morphologically distinguished by lack of rigid wall [5]. The microalgae *Dunaliella salina* is the best commercial source of natural beta-carotene [6].

Nannochloropsis represents a genus of marine microalgae with high photosynthetic efficiency and can convert carbon dioxide to storage lipids mainly in the form of transglycerols and to the omega 3 long-chain polyunsaturated fatty acid eicosapentaenoic acid (EPA). Recently, *Nannochloropsis* has received ever-increasing interests of both research and public communities [7]. Because of its great photosynthetic efficiency, high lipid productivity, well established genetic toolbox and relatively mature technology for cultivation system on a large scale, *Nannochloropsis* is considered as a potential oleaginous model [8, 9, 10, 11].

The process involving addition of electrolyte of coagulating metal ions directly from sacrificial electrodes such as Aluminium is called Electro flocculation [12]. The metal ions present in the solution absorb the organic matters like micro algae. These coagulated precipitates then attach it or captured by the gas bubbles released during the process of electrolysis and floated to the top [13]. A micro alga usually prevents the aggregation of the

cells in suspension because of the negative charge on its surface. In electro flocculation method electrode dissolution and deposition takes place in culture medium, while oxidation and reduction takes place on the surface of the electrodes [14]. The anode acts as a sacrificial electrode which donates positive metal ions into the solution. The positive ions in turn combine with the negatively charged surface of the microalgae to initiate flocculation [15]. Several experiments have been published on the use of aluminium electrode and iron electrodes [4, 16].

Each microalgae species represent distinct morphology and also carry different patten of charged patches on their cell surface. This means, the condition for optimal flocculation may be different for different algal strain [17]. To develop the processes for efficient biomass harvest for biofuel, it is imperative to understand the process of electro flocculation completely [18] for which it is necessary to study the factors affecting this process such as current supplied, time of passing current and holding time.

In recent years various experimental investigation on culturing and harvesting particular species of microalgae were studied to understand the effect of current and voltage on the recovery efficiency and power consumption [19].

2. Materials and methods

2.1 Microalgal Culture

Separation of *Dunaliella* from culture medium is more difficult than other microalgae species due to the small size, low concentration of cells, and high electrical stability[6]. Therefore, *Dunaliella salina* was selected and used throughout this study. The *Dunaliella* was cultivated in a 20 L carboy and it was harvested. Cell concentration was determined by spectrophotometer. Similarly *Nannochloropsis* was selected for this study as it is useful for biomass feedstock for the production of fatty acids for biodiesel, biohydrogen and high added-value compounds, in a biorefinery context. It is crucial to study its harvesting efficiency for utilizing its usefulness [20]. It was cultured and harvested using electro flocculation.

2.2 Electroflocculation Experiments

2.2.1 Electroflocculator

A commercially available power source connected with constant built volt meter, ammeter and regulators was used for the electro flocculation experiments in a 2000 mL plastic container (17×12 cm) with wall thickness of 2mm. The aluminium electrode plate had an area of 18 ×7 cm and a thickness of 2mm. They were placed in the container parallel and vertically. The distance between anode and cathode was kept at 2cm. 1500 ml microalgae culture broth was added to this container, and each electrode was submerged about 14 cm deep. Fig. 1 gives the schematic arrangement of the flocculator.

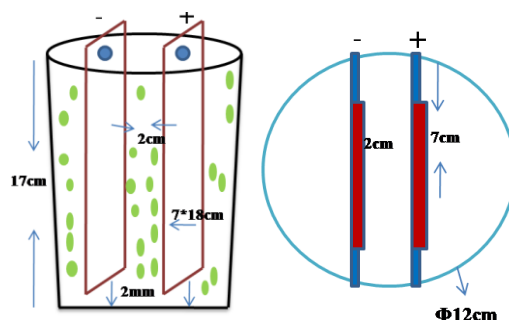


Figure 1: Schematic of custom-built electroflocculator for algal biomass harvest

2.2.2 Flocculation protocol

Electro flocculation current of 10A for a fixed electrode distance of 2cm, rated current was supplied at 24V. The rated current was administered for 10s, 20s, 30s, 40s and 60s of time periods. The electro flocculated culture was held at different “Settling/holding” time of 5min, 10min, 20min and 30min to enable completion of flocculation process. The clear solution was studied for residual algae by means of optical density at 680nm [21].

2.2.3 Calculation of Harvesting Efficiency and energy consumption

The harvesting efficiency was calculated by comparing the optical density of the culture at 680 nm before and after electro flocculation, using the following equation [22].

$$\eta (\%) = [\text{OD}_0 - \text{OD}] / \text{OD}_0 \times 100$$

Where, OD₀ is the optical density of the culture before electro flocculation and OD is the optical density after electro flocculation process. For given applied voltage (U), ratted current (I), time of the harvest process (t), volume of the harvest chamber (V in m³), efficiency of harvest (η) and concentration of algae (C in g/L), the amount of energy consumed for the harvest is given by,

$$E = \frac{1000VnC}{\eta}$$

3. Results and discussion
 As shown in Fig. 2(a), the microalgae recovery efficiency of *Dunaliella* species reached 42%, 60% and 80% for the electro flocculation conducted with constant current and voltage but varying settling time viz., 5min, 10min and 20min respectively. Settling time significantly affects the harvest efficiency when comes to commercial harvesting at large scale. From the graph it is clearly seen that for settling time of 20 min, recovery efficiency reached 77% for 30 s of current supply, and for 40 s current supply, it reaches 80%. Beyond 40s, there is no significant increase in efficiency. From power consumption point of view, supplying current for 30 s is optimal to the harvest the *Dunaliella sp.*

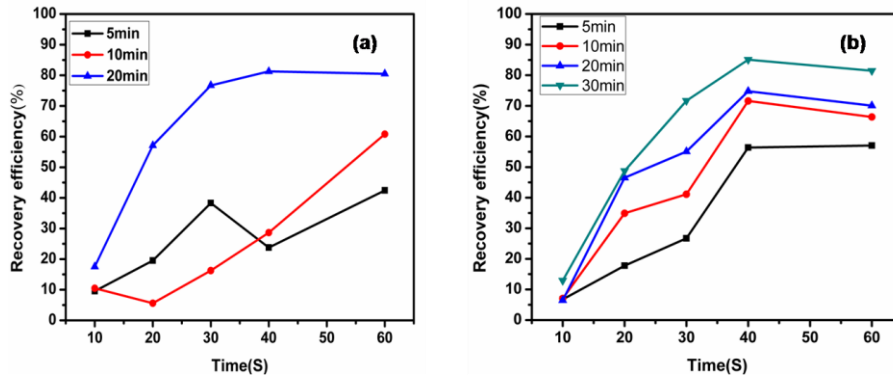


Figure 2: Biomass harvest efficiency determined at varying duration of 10A current supply for three indicated settling times after flocculation of microalgae a) *Dunaliella sp.*; b) *Nannochloropsis sp.*

In case of *Nannochloropsis* species, the efficiency were 57%, 66%,70% and 81% for 5min, 10min, 20min, 30min settling time respectively for 60 s flocculation as shown in the Fig. 2(b). However, in all the settling time periods, flocculation time of 40 s of appears to be optimal for harvest, beyond which no increase in efficiency takes place. The maximum efficiency of 85% was observed at 40 s flocculation for 30 minutes settling time.

As shown in Fig. 3(a), to attain 77% harvest efficiency for *Dunaliella* culture, 2.6 kWh/kg power was consumed. We notice that for further 4% increase in recovery efficiency require a power of 3.2 kWh/kg. In the case of *Nannochloropsis* culture, harvesting efficiency reaches its peak of 85% at 3.2 kWh/kg, as shown in Fig. 3(b). In a commercial harvest of micro algae for biofuel, it is necessary to reduce the cost of harvesting wherein power consumed is the key factor affecting the production cost.

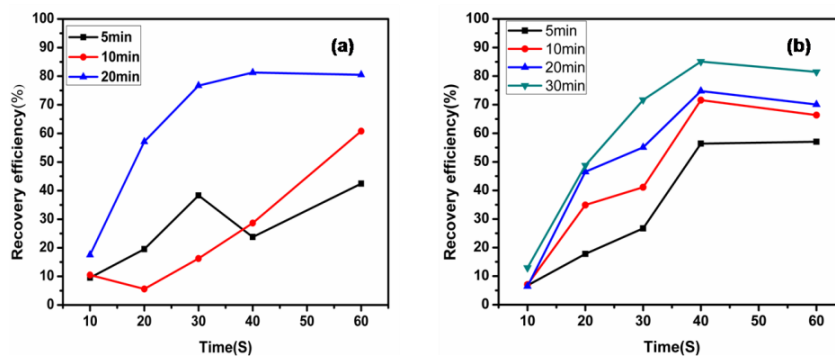


Figure 2: Biomass harvest efficiency determined at varying duration of 10A current supply for three indicated settling times after flocculation of microalgae a) *Dunaliella sp.*; b) *Nannochloropsis sp.*

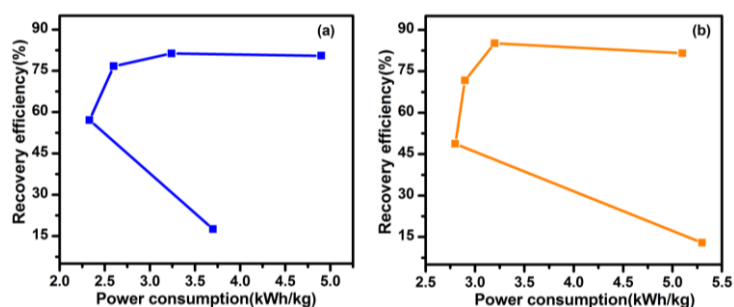


Figure 3: Power consumption determined for different biomass harvest efficiency for a) *Dunaliella sp* at 20 minutes settling time; b) *Nannochloropsis sp.* at 30 minutes settling time.

“Settling” or “holding” time has important role in time consumption when comes to efficient running of biofuel industry where harvesting and biofuel extraction process has to carry on in flow. When most of the time is consumed in settling process, a long gap between harvesting and extraction of biofuel will significantly affects the course of economy of microalgae industry. As seen in Fig. 4(a), 2 sets of flocculation with different electro flocculation time of 20s and 60s were conducted for *Dunaliella*. From the graph it is obvious that for flocculation time of 60s yield high recovery efficiency. In this case for settling time 10min and 20min, efficiency was 60%, and 80% respectively. It is observed that, 20 min holding time significantly increases the recovery efficiency. In the case of *Nannochloropsis* harvest, 47% and 49% recovery efficiency is obtained for settling time 20min and 30min at 20s of electro flocculation. 20min appears to be optimal settling time because holding above 20min render no significant increase in recovery efficiency. However, supplying current for 60s resulted 70% harvesting efficiency for 20min holding time, whereas for holding time 30 min, efficiency was 81% as shown in Fig. 4(b).

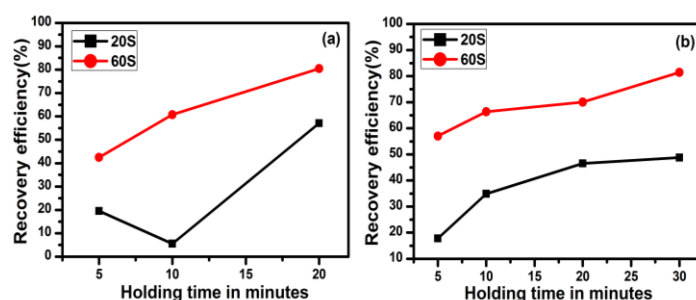


Figure 4: Effect of holding time on harvest efficiency of microalgae post-flocculation for 20s and 60s span of 10A current supply for a) *Dunaliella sp.* and b) *Nannochloropsis sp.*

The effect of electro flocculating time on harvesting efficiency was determined by measuring OD680 before and after electro. Using Equation (1), the harvesting efficiency was calculated for *Dunaliella*. The energy consumption in kWh/kg for each rated current were analysed using the equation mentioned above. From the optimal efficiency obtained (Fig. 2 & 3), it is seen that when 10A of current is supplied for just approximately 40s, energy consumed was 2.6 kWh/kg. Holding time respective to the highest harvesting efficiency was 30 min.

4. Conclusions

Electroflocculation is most efficient method for harvesting algal biomass. The optimal electro flocculation parameters for *Dunaliella sp.* were found to be 10 A current for 20 min holding time in terms power consumption reaching 77% harvesting efficiency using 2.6 kWh/kg. Maximum harvesting efficiency reached was 80% but at significantly higher power (3.2kWh/kg). Similarly for *Nannochloropsis sp.*, optimal harvesting efficiency obtained was 85% at 30 min settling time consuming 3.2 kWh/kg of power. This approach of optimising harvest efficiency and the data provided shall be useful in deciding on the choice of harvesting technology and the estimation of the cost of biomass production in biofuel industry. It must be stated that these optimal flocculation parameters for effective harvesting of microalgae may vary for different strains.

5. Acknowledgements

SA acknowledges UGC-RGNF for the financial support.

References:

1. Poelman, E., DePauw, N. and Jeurissen, B. 1997. Potential of electrolytic flocculation for recovery of microalgae, *Resources, Conservation and Recycling*. 119:1–10.
2. Xiong, Q., Pang, Q., Pan, X., Okonkwo Chika, A., Wang, L., Shi, J., Jia, L., Chen, C. and Gao, Y. 2015. Facile sand enhanced electro-flocculation for cost-efficient harvesting of *Dunaliella salina*, *Bioresource Technology*. 187:326-330.
3. Mohan, N., Hanumantha Rao, P., Ranjith Kumar, R., Sivasankaran, S. and Sivasubramanian, V. 2009. Studies on mass cultivation of *Chlorella vulgaris* and effective harvesting of bio-mass by low-cost methods, *J. Algal Biomass Utln.* **1(1)**: 29 – 39.
4. Uduman, N., Bourniquel, V., Danquah, M.K. and Hoadely, A.F. 2011. A parametric study of electro coagulation as a recovery process of marine microalgae for biodiesel production, *Chem. Eng. J.* **174(1)**: 249-257.
5. A. Ben-Amotz M. Avron. 1987 On the mechanism of osmoregulation in *Dunaliella*. In the energetic and structure of halophilic microorganism, Amsterdam: Elsevier/North-Holland: 529-541.
6. Hosseini Tafreshi, A. and Shariati, M. 2009. *Dunaliella* biotechnology: methods and applications, *J. Appl. Microbiol.* **107(1)**: 14-35.
7. Ma, X., Chen, T., Yang, B., Liu, J. and Chen, F. 2016. Lipid production from *Nannochloropsis*, *Mar. Drugs*. **14(61)**: 1-18.
8. Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. Microalgae for oil: Strain selection, indication of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, *Biotechnol. Bioeng.* **102(1)**: 100-112.
9. Radakovits, R., Jinkerson, R.E., Fuerstenberg, S.L., Tae, H., Settledge, R.E., Boore, J.L. and Posewitz, M.C. 2012. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*, *Nat. Commun.* **3(686)**: 1-10.
10. Vieler, A., Wu, G., Tsai, C.H., Bullard, B., Cornish, A.J., Harvey, C., Reca, I.B. Thornburg, C., Achawanntakun, R. and Buehl, C.J. 2012. Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779, *PLoS Genet.* **8(11)**: 323-335.
11. Shah, A. R., Ahmad, A., Srivastava, S. and Jaffar Ali., B.M. 2017. Reconstruction of a genome-scale metabolic model of *Nannochloropsis gaditana* and its application to evaluate flux changes under nitrogen limitation, *Algal Research*. **26**: 354-364.
12. Bleeke, F., Quante, G., Winkelmann, D. and Klöck, G. 2015. Effect of voltage and electrode material on electroflocculation of *Scenedesmus acuminatus*, *Bioresour. Bioprocess.* **2(36)**: 1-8.
13. Terry, K.L. and Raymond, L.P. 1985. System design for the autotrophic production of microalgae, *Enzyme. Microb. Technol.* **7(10)**: 474-487.
14. Kim, J., Ryu, B.G., Kim, B.K., Han, J.L. and Yang, J.W. 2012. Continuous microalgae recovery using electrolysis with polarity exchange, *Bioresour. Technol.* 123: 164-170.
15. Sasson, M.B., Calmano, W. and Adin, A. 2009. Iron-oxidation processes in an electroflocculation (electrocoagulation) cell, *J. Hazard. Mater.* **171(1-3)**:704–709.
16. Vandamme, D., Pontes, S.C., Goiris, K., Foubert, L., Pinoy, L.J. and Muylaert, K. 2011. Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater microalgae, *Biotechnol. Bioeng.* **108(10)**: 2320–2329.
17. Contreras, S., Pieber, M., Delrio, A., Soto, M.A., Toha, J. and Veloz, A. 1981. A highly efficient electrolytic method for microorganism flocculation from aqueous cultures, *Biotechnol. Bioeng.* **23**:1165–1168.
18. Mollah, M.Y.A., Schennach, R., Parga, J.R. and Cocke, D.L. 2001. Electrocoagulation (EC) – science and applications. *J. Hazard. Mater.* **84**:29-41.
19. Raut, N., Panwar, S., and Vaidya, R. 2015. Electroflocculation for harvesting microalgae, Lambert Academic Publishing 60.
20. Nobre, B.P., Villalobos, F., Barragan, B.E., Oliveira, A.C., Batista, A.P., Marques, P.A., Mendes, R.L., Sovova, H., Palavra, A.F. and Gouveia, L. 2013. A biorefinery from *Nannochloropsis* sp. Microalga—extraction of oils and pigments. Production of biohydrogen from the leftover biomass, *Bioresour. Technol.* **135**:128-136.
21. Lee, A.K., Lewis, D.M. and Ashman, P.J. 2013. Harvesting of marine microalgae by electroflocculation: the energetics, plant design, and economics, *Appl. Energy*. **108**: 45–53.
22. Salim, S., Bosma, R., Vermue, M.H. and Wijffelsalim, R, H. 2011. Harvesting of microalgae by bio-flocculation, *J. Appl. Phycol.* **23(5)**: 849-855.