Harvesting of microalgae *Nannochloropsis oculata* by electroflocculation

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Abstract

Harvesting of biomass from microalgal culture needs high energy inputs, as small algal cells need to be separated from a large volume of surrounding media. The biomass harvest of *Nannochloropsis oculata* was evaluated using electroflocculation. Different electrodes like Aluminum, Zinc, Copper, Brass and Iron were used as both anode and cathode. The electrodes were connected to DC supply and the flocculation was performed at different voltages viz; 20,40,60,80 and 100 V at constant power (90mA) and time (30 minutes). From the above study, it was concluded that Zinc electrodes performed better with 80% harvesting efficiency at 40V. The flocculated cells by Zinc electrode were 80% viable which was confirmed by inoculating the flocculated cells.

***Keywords**: Electroflocculation, Nannochloropsis oculata, zinc electrodes*

Introduction

The microalgae, *Nannochloropsis oculata* plays an important role in marine finfish larval rearing as major feed for zooplankton like rotifers as well as in green water rearing systems used to maintain the water quality during the hatchery phases. Mass culture of microalgae on a commercial scale is essential to satisfy its huge requirement in the hatchery as well as for biomass production for application as functional food and nutraceuticals (Hu, 2014). Preserved microalgal concentrates are used during the summer months as an alternate source of feed for rotifer culture as well as inoculum to support marine finfish and shell fish hatcheries (Biji *et al.,* 2018).

Nannochloropsis being a temperate species, mass cultures at outdoor can be performed better during the winter months. After cultivation of microalgae on a large scale, there is a need to reduce the volume for concentrating the microalgal cells. Standardizing proper harvesting techniques is of paramount importance and commonly practiced harvesting methods include centrifugation, sedimentation, filtration, flotation and flocculation (Milledge and Heaven, 2013). Among these, flocculation is considered superior and during the process the cells are made to coagulate with the addition of flocculants by which larger particles are produced with higher settling velocity. Chemical flocculation involves using metal salts or polyelectrolytes, pH induced flocculation or bioflocculation with the intervention of bacteria or filamentous fungi are effective to concentrate the microalgae. In electroflocculation, with the use of aluminium electrodes and iron elecrodes, the metal ions released from the sacrificial electrode plays the role of a floculant (Vandamme *et al*., 2011). It is based on the principle that the surface of microalgae is negatively charged and behaves as colloidal particles which can move in an electric field. Once they are attracted towards the anode, they are neutralised and form algal aggregates (flocs), which can be easily be collected. During electrolysis of water, H_2 and O_2 gas in the form of bubbles are produced in the electrodes and this will rise to the surface taking with them the algal aggregates (flocs) and forms a layer of microalgal cells. In this study harvest of *Nannochloropsis* by electroflocculation with various metals at different voltages was tried and evaluated.

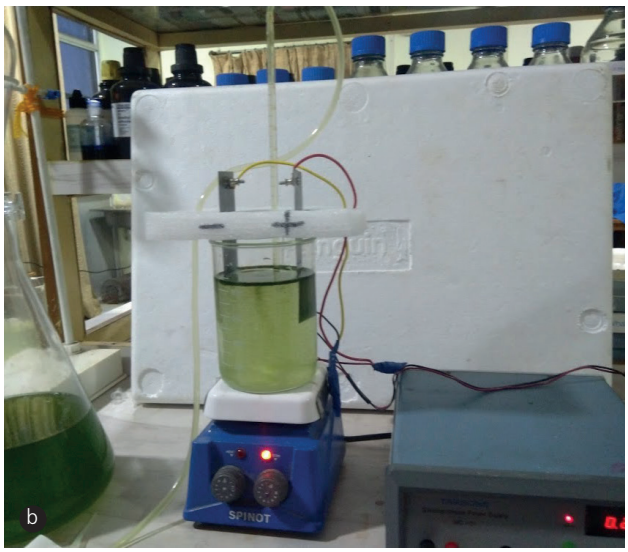

Fig.1. The electroflocculation process for *N.oculata* culture. a. Before electroflocullation and b. After electroflocullation

Microalgae electroflocculation

Marine microalgae, *Nannochloropsis oculata* culture maintained for larviculture of commercially important marine finfishes was used. was used as the culture medium. The algae were cultured in Conway medium at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25ppt and light intensity of 2000lux and culture in its exponential phase was used for the electroflocculation study. Five metals electrodes viz., comprising Zinc (Zn), Aluminium (Al), Copper (Cu), Brass and Iron (Fe) were used individually as both cathode and anode. The anode and cathode were placed at a distance of 7cm with depth of immersion of the plat es as 5cm. The experiment was performed in 1000ml capacity graduated beaker using 900ml of microalgae culture. The beaker was placed on a stirrer and the culture was gently stirred with a magnetic stirrer. The electrodes were connected to a DC power supply and the voltage was adjusted to 20, 40, 60, 80 and 100V with the current kept constant at 90mA. After 30 minutes the stirrer was stopped and the flocculated cells pulled up with the current formed a layer (Fig.1). The supernatant solution was siphoned out and the flocculated cell layer was collected by centrifugation, weighed and kept for further study. The flocculated cells collected with different metals as electrodes at different voltage were checked for its viability and were inoculated again for its reproducibility by estimating their cell counts.

Among the different metals used, Zinc performed better with 80% harvesting efficiency at 40V (Table 1), followed by the same metal at 100 V (57%). Copper electrode

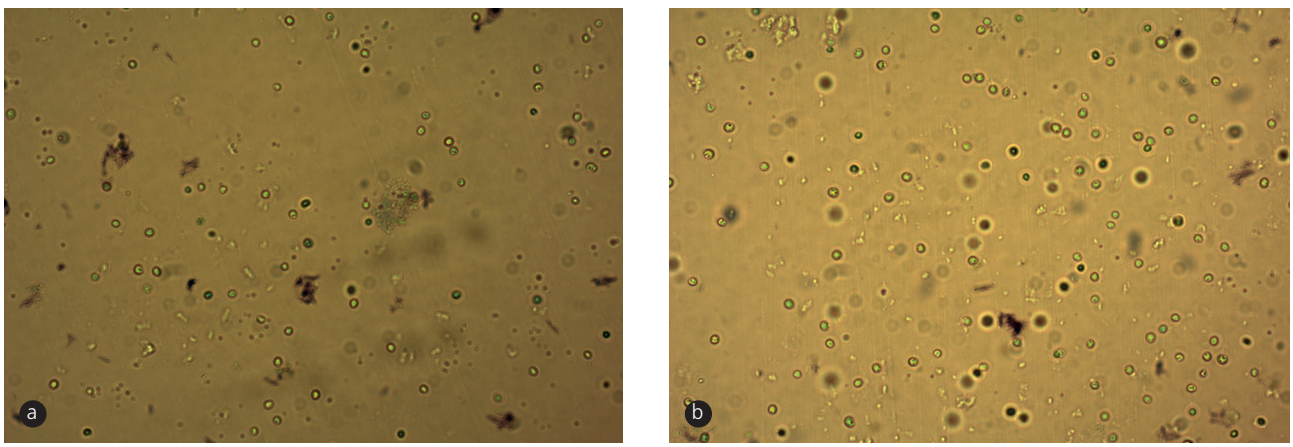

Fig 2. Flocculated *Nannocholoropsis* cells after Evan's blue staining following electroflocculation with Zn electode at 40 V (a) and 100 V (b)

Table 1. Cell count and harvesting efficiency before and after electroflocculation by electrode metals at different voltages

Metal	Voltage	Cell count before flocculation millions (10^6)/ml	Cell count of after flocculation billions (10^9/ml)	Harvesting efficiency (%)
Zn	20V	24	6.48	30
	40V	24	9.68	80
	60V	24	5.32	25
	80V	18	12.9	44
	100V	18	9.84	57
Cu	20V	32	8.32	29
	40V	32	6.16	21
	60V	32	5.12	18
	80V	28	10.8	37
	100V	28	9.36	32
Br	20V	40	4.2	12
	40V	40	6.24	17
	60V	40	7.3	20
	80V	40	9.6	27
	100V	40	9.0	25
Al	20V	32	6.8	24
	40V	32	9.6	33
	60V	32	9.52	33
	80V	32	8.36	29
	100V	32	4.0	14
Fe	20V	28	7.9	31
	40V	28	1.92	7.6
	60V	28	8.4	33
	80V	28	8.84	35
	100V	28	7.68	30

Note: cell count was estimated from 900ml of *Nannochloropsis* culture before and after flocculation.

showed maximum harvesting efficiency of 37% at 80V. Other metals like Brass and Fe, the flocculation efficiency was 27% and 35% respectively. When Cu, Brass and Fe were used, the coagulated cells settled at the bottom and were not lifted up. When Al was used, the algal cells flocculated and some were lifted up, but most settled at the bottom with a white precipitate. Aluminium electrode showed a maximum harvesting efficiency of 33% at 40V and 60V.

Cell viability test

Evan's Blue stain was used for testing the cell viability of the flocculated *Nannochloropis* cells. For staining, a 20 mL sample of each fresh or stored algal suspension was treated with 1 mL of 1% (w/v) stock solution of Evan's Blue. The samples were allowed to stand at room temperature for a minimum of thirty minutes before microscopic examination. A subsample of each stained suspension was then inspected at 250 X magnification

using an Improved Neubauer Haemocytometer (Superior Co., Berlin, Germany).

Zinc performed better with superior viability at different voltages compared to other metals. During the Evan's blue staining, most of the *Nannochloropsis* cells were greenish in colour and were hence not stained (80% viability). For other metals, the flocculated cells were with less percentage of viability (<5%).

Inoculation of flocculated *Nannochloropsis* culture

Pre-treated sea water passing through slow sand filter and UV filter and further treated with ozone was used. The flocculated microalgal cells, to be used as inoculum were diluted and mixed properly with the help of magnetic stirrer to ensure the uniform distribution of individual cells. The initial cell count was maintained at $5x10^5$ cells /ml. These were cultured at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25ppt and light intensity of 2000lux on Conway medium. Cell count was estimated after 7 days of inoculation. Among the various metal electrodes used, zinc electrode performed better with a maximum cell count of $8.5x10^6$/ml at 80V. Aluminium and iron electrodes could reach a maximum count of $5.0x10^6$/ml. The cell count in *Nannochloropsis* cells flocculated with copper and brass electrodes did not increase after inoculation of the flocculated cells. Further studies are required for its commercial application in fish hatcheries.

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