



Effects of electric field on growth in *Dunaliella salina*

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Abstract

The current study aims to investigate the effect of different doses of electric field on growth parameters in the marine algae, *Dunaliella salina*, including optical density and dry weight over different time points. The results of the study show increase in algal biomass on treatment with electric field doses (1kv/10, 1kv/20, 1kv/30, 1kv/40, 1kv/50 and 1kv/60) for a period of I, II, III, IV and V weeks. Exposure to electric field led to significant affect growth of the algae. The findings of the current study will enable optimization of electric field dynamics in order to harness potential benefits of the algal mechanics to increase production.

Keywords: *Dunaliella salina*, electric field, optical density, dry weight etc.

Introduction

Algae are a group of diverse photosynthetic micro-organisms that hold paramount significance in the maintenance of ecological homeostasis owing to their enormous potential to be utilized for food, medicine as well as energy generation in the industrial sector. Algae are used in the production of a number of commercially important substances used in the industrial sector, including, lipids, pigments, carbohydrates as well as vitamins (Kannan *et al.*, 2021) [7].

However, the growth and cultivation of algae has been hampered owing to a number of factors such as high manufacturing cost coupled with unsuccessful marketing strategies, inefficient practices for biomass conversion, contamination of algal cultures and high cost associated with cultivation of microalgae. In addition to these, other abiotic factors such as salinity and temperature further degrade the algal cultivation (Bošnjaković and Sinaga, 2020) [6].

Different types of mutagens lead to induction of different types of mutations in the microalgae. For instance, chemical mutagenesis using EMS involves alkylation of DNA bases (specifically guanine) and causes induction of point mutations.

Dunaliella salina, a halophilic microalga, has garnered significant attention due to its unique ability to thrive in extreme saline environments and its potential applications in biotechnology and aquaculture. This green microalga is known for its high carotenoid content, particularly beta-carotene, which has valuable antioxidant properties and is widely used in food, cosmetics, and pharmaceuticals. Recent studies have begun to explore the influence of external factors on the growth and metabolic processes of *D. salina*, with one intriguing area of investigation being the effect of electric fields on algal growth (Barbosa *et al.*, 2023) [8].

The application of electric fields in biological systems, known as electroculture, has been shown to enhance plant growth and improve nutrient uptake in various terrestrial species. These electric fields can induce physiological changes at the cellular level, influencing processes such as cell division, photosynthesis, and metabolic activity.

Understanding how electric fields impact *D. salina* can reveal new methods for optimizing algal cultivation, potentially leading to increased biomass production and carotenoid yield.

This study aims to investigate the effects of varying electric field strengths on the growth rate in *D. salina*, thereby providing insights into the practical applications of electroculture in microalgal biotechnology.

Materials and Methods

Treatment of electric field

Unialgal culture of *D. salina* was added to 250 ml conical flasks containing 100 ml artificial sea water medium (ASWM). Electric current was given having strength of 1 Kv and 5 Kv. Each voltage was given for different times i.e. 10 minutes., 20 minutes, 30 minutes, 40 minutes, 50minutes and 60 minutes.

Treated as well as control algae were sub-cultured further for 5 week and at every week, growth parameters were determined for each set every week upto 5 weeks.

Determination of growth of *D. salina*

Growth was followed through optical density and dry weight. Optical density was recorded with the help of Elico CL 160 photo colorimeter (India) at 670 nm. For dry weight and fresh weight, 50 ml of culture was centrifuged with the help of Sartorius 3-18K centrifuge (Germany). The pellet was weighed for fresh weight and it was dried at 37 C for overnight in oven for dry weight. Growth rate was calculated from dry weight by Guillard (1973) equation.

$$\mu \text{ (divisions/day)} = 3.322(\log DW_2 - \log DW_1) / (t_2 - t_1)$$

Dry weight

For dry weight measurement, a known volume from culture samples was filtered on pre-weighed whatman filter paper no. 4 of 10 cm diameter. The filter paper containing the algal biomass was rinsed with distilled water and weighed after drying for 24 h at 80°C in hot air oven.

The difference between the final and initial weight was the dry weight of algal biomass and calculated in terms of g/l (Richmond and Grobbelaar, 1986)

Results

Effect of electric field on growth of *Dunaliella salina* (OD)

The results showed effect of different doses of electric field (1 KV and 5 kV) on optical density (OD) of the algae *Dunaliella salina* over a period of I, II, III, IV and V weeks. The results show a consistent increase in OD of the algae due to exposure to increasing dose of electric field till 40 minutes time point (for 1 kV dose) and till 20 minutes time point (in 5 kV dose). Thereafter, the OD of the algae declines in 50 minute and 60-minute time points (in 1 kV dose) and 30, 40, 50 and 60-minute time point (in 5kV dose).

The initial OD of the algae was found to be 0.02 (units) at the initial time point, which increased significantly, reaching a maximum of 0.19 (18.26%) at the 40-minute time point in the 1 KV condition during the first week. In complete contrast to this, the 5-kV condition also showed an increase,

however, it peaks at a lower OD of 0.19 (18.26%) at the 20-minute mark and then declines to 0.11 (-31.54%) by the end of the first week. Similarly, In the second week, the 1 kV condition achieves an OD of 0.35 (34.10%) at the 40-minute time point, followed by a gradual decrease, while the 5 kV condition peaks at 0.35 (34.10%) at the 20-minute mark, followed by a decrease to 0.21 (-19.54%) in the 60-minute time point. Similar trend was observed in the third and fourth week, where too, the 1 kV condition reaches an OD of 0.89 (31.33%) and 0.99 (17.21%) at the 40-minute point. The 5-kV condition, however, shows lesser increases, peaking at 0.79 (16.58%) and 0.99 (17.21%) but experiencing slight declines thereafter.

In the fifth week, the 1 kV condition peaks at an OD of 1.51 (22.50%). On the contrary, the 5-kV condition, however, stabilizes at 1.18 (-4.27%) by the end of the week, highlighting a significant difference in response to the electric field between the two different voltage conditions.

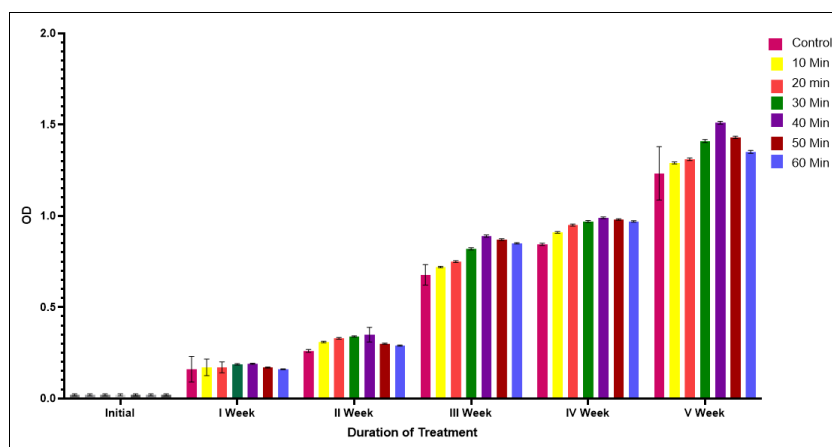


Fig 1: Effect of low dose of electric field on optical density

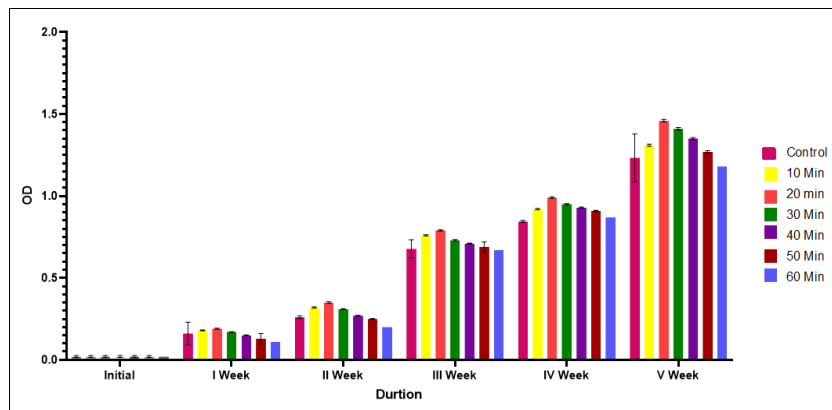


Fig 2: Effect of high dose of electric field on OD

Effect of electric field on growth of *Dunaliella salina* (OD)

The results showed effect of different doses of electric field (1 KV and 5 kV) on dry weight of the algae *Dunaliella salina* over a period of I, II, III, IV and V weeks. In the initial measurement, both the 1 kV and 5 kV conditions showed a constant value of 0.068, showcasing no immediate effect of the electric field exposure.

During the first week, the 1 kV exposure caused a slight increase in dry weight, reaching 0.083 (a 10.67% increase) at the 40-minute time point. In contrast, the 5-kV exposure exhibited peaking at 0.077 (2.67% increase) before declining to 0.060, showcasing a -20.00% decline by the

end of the first week. By the second week, the 1 kV group continued to show steady growth, attaining a maximum value of 0.108 (an 8.00% increase). In contrast to this, the 5-kV treatment exhibited minimal growth of just 2%, and its final value was 0.089, marking an 11.00% decrease. In the third week, the 1 kV group further increased to 0.235 (a 3.98% increase), while the 5-kV group showed a slight decline, finishing at 0.210, which represented a -7.08% decrease. The fourth week saw the 1 kV treatment peak at 0.292 (a 3.18% increase), whereas the 5-kV group initially increased slightly to 0.270 (a 1.06% rise at the 20-minute point) but later dropped to 0.270, showing a -4.59% decrease overall. In the final fifth week, the 1 kV group

experienced a minor decrease, reaching 0.335, which was a -0.59% change from the previous week. Meanwhile, the 5-

kV group continued its decline, finishing at 0.315, reflecting a -6.53% decrease.

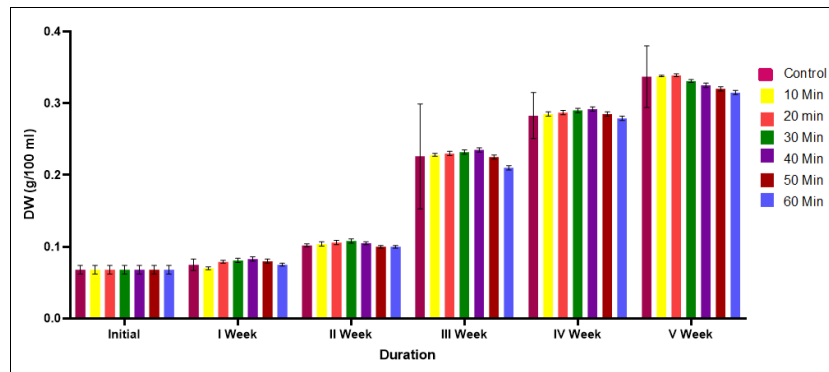


Fig 3: Effect of low dose of electric field on dry weight

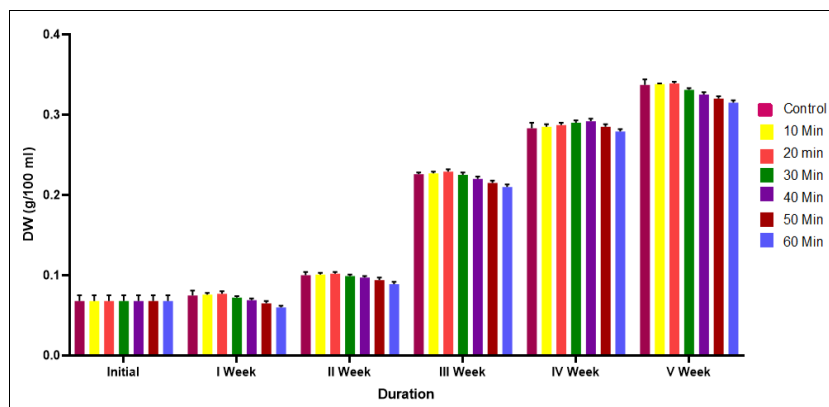


Fig 4: Effect of high dose of electric field on dry weight

Discussion

The effects of electric fields on the growth of *Dunaliella salina* have been investigated with a focus on varying strengths and exposure times. In this study, we observed that applying electric fields of 1 kV and 5 kV initially stimulated growth, as indicated by increased optical density and dry weight measurements. This aligns with previous findings that suggested low-intensity electric fields can enhance cell division and metabolic activity in microalgae (Reddy *et al.*, 2020) ^[1]. The initial growth promotion can be attributed to the electric field's ability to increase ion mobility and nutrient uptake, facilitating enhanced photosynthesis and cellular processes (Pacheco *et al.*, 2018) ^[2].

However, the results also revealed that prolonged exposure to electric currents led to a decline in growth rates after certain durations. Notably, higher electric field strengths and extended application times (beyond 30 minutes) resulted in slower growth. This phenomenon may be explained by the potential stress induced on the algal cells due to excessive electric fields. Prolonged exposure could lead to increased reactive oxygen species (ROS) production, which can cause oxidative stress and damage cellular components (Lee *et al.*, 2019) ^[3]. Moreover, higher electric fields may disrupt cellular membrane integrity, affecting nutrient absorption and overall metabolic activity (El-Badry *et al.*, 2021) ^[4].

The optimal exposure duration for *D. salina* appears to be crucial, as our results indicate that while brief exposure enhances growth, excessive duration at higher field strengths impairs it. This is consistent with the dual nature of electroculture, where mild electric stimulation can promote growth, but overstimulation can lead to adverse effects (Sinha *et al.*, 2020) ^[5]. These findings underscore the

importance of carefully optimizing electric field parameters to harness their potential benefits in algal cultivation.

Further studies are warranted to explore the underlying mechanisms by which electric fields influence *D. salina* growth and to identify optimal exposure conditions that maximize biomass production while minimizing stress. Additionally, examining the physiological and biochemical responses of *D. salina* under these conditions could provide valuable insights into improving cultivation strategies for this economically important microalga.

Conclusion

In conclusion, this study demonstrates that the application of electric fields of 1 kV and 5 kV can significantly influence the growth of *Dunaliella salina*, with initial exposure promoting growth as indicated by increases in optical density and dry weight. However, prolonged exposure to higher electric currents ultimately results in reduced growth rates due to potential oxidative stress and cellular damage. These findings highlight the need for careful optimization of electric field parameters to enhance the cultivation of *D. salina*, maximizing biomass production while minimizing adverse effects. Further research into the physiological responses of this microalga under varying electric field conditions could provide valuable insights for improving algal cultivation practices in biotechnological applications.

References

- Reddy TK, Kumar MA, Reddy KM. "Enhancement of Microalgal Biomass Production Using Electric Fields." *Renewable Energy Journal*, 2020; 135:150-159.

2. Pacheco MD, Vargas SM, González AJ. "Effect of Electric Field on Growth and Biochemical Composition of Microalgae." *Bioresource Technology*,2018:251:107-113.
3. Lee JH, Park HJ, Kim SY. "Oxidative Stress Response in Microalgae Under Electric Field Exposure." *Algal Research*,2019:41:101548.
4. El-Badry FFA, Hossain MM, Ali AS. "Impact of Electric Field on Microalgal Growth and Membrane Integrity." *Environmental Science and Technology*,2021:55(2):789-796.
5. Sinha BK, Bhattacharya A, Ghosh SK. "The Role of Electric Fields in Enhancing Plant and Algal Growth." *Journal of Applied Phycology*,2020:32(1);47-59.
6. Bošnjaković M, Sinaga N. The perspective of large-scale production of algae biodiesel. *Applied Sciences*,2020:10(22):8181.
7. Kannan N, Rao AS, Nair A. Microbial production of omega-3 fatty acids: an overview. *Journal of Applied Microbiology*,2021:131(5):2114-2130.
8. Barbosa M, Inácio LG, Afonso C, Maranhão P. The microalga *Dunaliella* and its applications: a review. *Applied Phycology*,2023:4(1):99-120.