



Effects of electric field on photosynthetic pigments in *Dunaliella salina*

Neha Chhipa*, Gajendra Pal Singh

Department of Botany, University of Rajasthan, JLN Marg, Jaipur, Rajasthan, India

Abstract

The current study aims to investigate the effect of different doses of electric field on photosynthetic parameters in the marine algae, *Dunaliella salina*, including total chlorophyll and carotenoid content over different time points. The results of the study show increase in alga 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 upsurge in carotenoid production in the algae while chlorophyll destroyed at higher voltage. 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 pigment production.

Keywords: *Dunaliella salina*, electric field, chlorophyll, carotenoids etc

Introduction

The last few years have witnessed an abrupt increase in demand for sustainable and renewable resources, making microalgae as a versatile platform in order to produce valuable biomass, pigments, as well as bioactive compounds. Out of the various microalgae species, *Dunaliella salina* has garnered significant attention owing to presence of high content of carotenoids, particularly beta-carotene, accompanied with its exceptional ability to thrive under extreme environmental conditions. The literature shows several examples pertaining to the fact that this alga has shown promising applications in the production of nutritional supplements, biofuels, as well as natural pigments. The last few years have demonstrated exceptional advancement in algal cultivation techniques showing ameliorative effect of various physical and chemical methods in order to augment algal growth and productivity (Goswami *et al.*, 2022; Singh *et al.*, 2016; Xu *et al.*, 2018) [4, 12, 14]. In this context, usage of electric field has emerged as a promising tool for optimization of algal cultures owing to its profound influence on algal cellular processes such as nutrient uptake, metabolic activity as well as stress responses, contributing to increased algal biomass and pigment production.

Dunaliella salina is a halophilic green alga renowned for its exceptional ability to aggregate high levels of carotenoids under stressful conditions. Carotenoids are valuable in the cosmetic and pharmaceutical industry for their excellent antioxidant properties as well as potential health benefits. However, the currently prevalent traditional cultivation methods are limited in their approach owing to several conditions such as nutrient availability, growth efficiency and environmental control. In this context, usage of electric fields offers an attractive non-invasive method in order to potentially overcome some of these limitations. Application of electric field aims to potentiate algal cultures with an aim to augment algal growth rates accompanied with improved nutrient uptake as well as stimulation of production of valuable pigments (Ahmed *et al.*, 2017; Wang *et al.*, 2021; Barbosa *et al.*, 2023) [1, 2, 13].

A number of previous studies have showcased the ameliorative effect of electric fields on positively influencing algal biomass and productivity as well pigment

content; however, the underlying mechanisms pertaining to different algal species as well as growth stages still remain as ongoing areas of investigation. Considering this, the current study has been drafted to evaluate the effect of electric fields on *Dunaliella salina*, focusing on photosynthetic pigments.

Understanding these effects would play an instrumental role in optimization of cultivation practices while also substantiating the economic viability of algal-based products.

Materials and methods

Determination of chlorophyll

Total chlorophyll were calculated by the Arnon method (1949). The selected species were taken, separately. One-gram fresh mass was weighed from control and each treatment, separately. The leaves were quickly grinded and homogenized with 40ml of 80% acetone with the help of mortar and pestle. Sample processing was carried out in dark condition (Holden, 1976) to avoid photobleaching and to prevent any pheophytin formation a pinch of NaHCO₃ was mixed with that. The homogenate was processed in centrifuge and upper parts were taken in separate tubes. This step was repeated thrice for the complete extraction of pigments. After that the volume of each supernatant was raised to 100 ml with 80% acetone. Obtained clear supernatant was studied for the total chlorophyll.

The OD at 645nm, 652nm and 663nm of each sample was taken by spectrophotometer against the blank (80% acetone) for the calculation of the total amount of chlorophyll. Three replicates were taken and the quantity of Chlorophyll a, b was calculated in mg/g of sample by using the equation derived by Arnon, (1949) as follows:

$$\text{Chlorophyll a} = \frac{11.3A_{663} - 0.96A_{645}}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll b} = \frac{18.3A_{645} - 3.9A_{663}}{a \times 1000 \times w}$$

(Where, A = Absorbance; α = Length of the light path in the cell (1 cm); V = Volume of each sample (ml); w = weight of the sample used in gm.)

Determination of carotenoids

The OD at 480nm, 645nm and 663nm of each sample was taken by spectrophotometer against the blank (80% acetone) for the calculation of the total amount of Carotenoid. Total carotenoid content (mg/g) was calculated by the equation of Jenson's (1965) as follow:

$$\Delta E_{Car480} = [E_{480} + (0.114 \Delta E_{663}) - (0.638 \Delta E_{645})]$$

(Where, Car ΔE_{480} = increasing in absorbance at 480nm due to presence of carotenoids, ΔE_{480} = extinction at 480nm, ΔE_{645} = extinction at 645nm, ΔE_{663} = extinction at 663nm)

Results

Effect of electric field on total chlorophyll content of *D. Salina*

The results showed effect of different doses of electric field (1 KV and 5 kV) on total chlorophyll content of the algae *Dunaliella salina* over a period of I, II, III, IV and V weeks. At the beginning of the experiment, the initial total chlorophyll content for both the 1 kV and 5 kV conditions is recorded as 0.000057225, with no changes observed at any of the early time points.

Thereafter, In the first week, the 1 kV condition shows a significant increase in chlorophyll content, reaching 0.002218 (24.99%) at the 30-minute mark before slightly declining to 0.001508 (-15%) by the 60-minute time point. In contrast to this, the 5-kV condition initially increases to 0.0019336 (7.88%) at the 20-minute mark but thereafter

experiences a significant decline to 0.00115133 (-35.7%) by the end of the week.

In the second week, the 1 kV condition continues to showcase impressive growth, with chlorophyll content peaking at 0.183735 (97.60%) at the 30-minute mark, before gradually declining to 0.1232 (32.49%) by the 60-minute mark. The 5-kV condition, however, peaks at 0.12378 (33.20%) at the 20-minute mark, followed by a dramatic fall to 0.018 (-80.6%) by the end of the week.

In the third week, the 1 kV condition maintains its positive response, reaching a peak of 0.167815 (51.32%) at the 30-minute mark, before declining to 0.087 (-21.5%) at the 60-minute point. The 5-kV condition also shows modest increases, with a peak of 0.118561 (6.93%) at the 20-minute mark, but it gradually decreases to 0.085 (-23.3%) by the end of the week.

During the fourth week, the 1 kV condition continues to show substantial increases, reaching 0.017785 (106.35%) at the 30-minute mark, but starts to decline to 0.0079 (-8.34%) by the 60-minute time point. The 5-kV condition, although showing some initial improvement, particularly at the 20-minute mark (0.011747, 36.23%), begins to decrease significantly, dropping to 0.005 (-42.01%) by the end of the week.

In the fifth week, the 1 kV condition reaches a remarkable peak of 1.03411 (140.04%) at the 50-minute mark, before decreasing to 0.42 (-2.5%) by the 60-minute mark. In comparison, the 5-kV condition also shows an increase in chlorophyll content, peaking at 0.590457 (37.05%) at the 30-minute mark, but its levels decline to 0.4 (-7.15%) by the end of the week.

Table 1: Effect of electric field on total chlorophyll content of *D. Salina*

	Control	10 min	20 min	30 min	40 min	50 min	60 min
Initial	0.0000572	0.0000572(0%)	0.0000572(0%)	0.0000572(0%)	0.0000572(0%)	0.0000572 (0%)	0.0000572 (0%)
I week	1 Kv	0.0017745	0.00208 ±0.00005 ** (17.22%)	0.002173±0.00005 ** (22.49%)	0.002218 ±0.00005 *** (24.99%)	0.001956±0.00004 * (10.26%)	0.00173 ±0.00004 (-1.97%)
	5 Kv	0.0017923	0.00182 ±0.00004 (1.96%)	0.001933±0.00005 * (7.88%)	0.0018487±0.00005 (3.15%)	0.001610 ±0.00004 * (-10.13%)	0.0017247±0.00004 (-3.77%)
II week	1 Kv	0.092982	0.16150±0.0007 *** (73.69%)	0.1615±0.0007 *** (73.69%)	0.1837 ±0.0008 *** (97.60%)	0.1476±0.0006 *** (58.80%)	0.1381±0.0006 *** (48.56%)
	5 Kv	0.092928	0.0986±0.0005 (6.18%)	0.1237±0.0006 *** (33.20%)	0.0822±0.0004 * (-11.53%)	0.0679±0.0004 ** (-26.90%)	0.0216±0.0003 *** (-76.74%)
III week	1 Kv	0.110901	0.1338±0.0006 ** (20.70%)	0.1565±0.0007 *** (41.13%)	0.1678±0.0008 *** (51.32%)	0.14267 ±0.0006 ** (28.65%)	0.09051 ±0.0005 ** (-18.39%)
	5 Kv	0.110881	0.115404 ±0.0005 (4.08%)	0.118561 ±0.0005 (6.93%)	0.109209 ±0.0004 (-1.51%)	0.099688 ±0.0004 * (-10.09%)	0.090299 ±0.0004 ** (-18.56%)
IV week	1 Kv	0.008619	0.01077 ±0.0003 ** (24.96%)	0.01596 ±0.0004 *** (85.17%)	0.017785 ±0.0004 *** (106.35%)	0.015298 ±0.0003 *** (77.49%)	0.00819 ±0.0002 (-4.98%)
	5 Kv	0.008623	0.008864 ±0.0003 (2.79%)	0.011747 ±0.0004 *** (36.23%)	0.011078 ±0.0003 ** (28.47%)	0.0098026 ±0.0003 * (13.68%)	0.006313 ±0.0002 ** (-26.79%)
V week	1 Kv	0.430815	0.498971 ±0.003 * (15.82%)	0.834764 ±0.004 *** (93.76%)	0.968017 ±0.005 *** (124.69%)	1.03411 ±0.006 *** (140.04%)	0.704779 ±0.005 *** (63.59%)
	5 Kv	0.43082	0.450181 ±0.002 (4.49%)	0.546794 ±0.003 ** (26.92%)	0.590457 ±0.004 *** (37.05%)	0.519758 ±0.003 ** (20.64%)	0.423955 ±0.003 (-1.59%)

Effect of electric field on carotenoid content of *D. Salina*

The results showed effect of different doses of electric field (1 KV and 5 kV) on carotenoid content of the algae *Dunaliella salina* over a period of I, II, III, IV and V weeks. The initial carotenoid content in *Dunaliella salina* is 0.9 (units) for both the 1 kV and 5 kV conditions, with no change observed at the beginning of the experiment across all time points.

In the first week, the 1 kV condition shows a steady increase in carotenoid content, rising to 2.7 (41.36%) at the 60-

minute mark. The growth is progressive, with a 9.95% increase at the 10-minute mark, followed by 20.42%, 25.65%, 30.89% and 36.13% at 20, 30, 40 and 50 minute mark respectively. Similarly, 5 Kv condition also shows a marked increase in carotenoid content, but at a more accelerated rate compared to the 1 kV condition. It peaks at 3.3 (72.77%) at the 60-minute mark, with increases observed across all time points, starting from a 15.18% increase at 10 minutes and reaching a 62.30% increase by 50 minutes. In the second week, the 1 kV condition

continues to increase gradually, reaching 3.67 (11.21%) at the 60-minute mark. The increase remains steady, with smaller gains over time, such as 3.03% at 10 minutes and 9.70% at 40 minutes. Similarly, the 5-kV condition continues to rise, with a 12.73% increase at 50 minutes and peaking at 3.89 (17.88%) by the 60-minute mark. In the third week, the carotenoid content in the 1 kV condition continues to increase, reaching 4.38 (12.31%) at the 60-minute mark, with a 5.13% increase at 10 minutes and a 7.95% increase at 30 minutes. The 5-kV condition also shows steady growth, with carotenoid content reaching 4.54 (16.41%) at the 60-minute point. By the fourth week, the 1 kV condition reaches 5.26 (11.91%) at the 60-minute mark,

with a 2.13% increase at 10 minutes and a 6.60% increase at 30 minutes. Similarly, the 5-kV condition also shows substantial increases, peaking at 5.6 (19.15%) by the 60-minute mark, with an increase of 5.32% at 10 minutes and 12.98% at 30 minutes. In the fifth week, the 1 kV condition reaches its highest carotenoid content of 7.3 (8.96%) at the 60-minute mark, with steady increases across all time points, including a 2.99% increase at 10 minutes and a 7.46% increase at 50 minutes. The 5 kV condition peaks at 7.9 (17.91%) at the 60-minute mark, with significant increases across the time points, such as 7.61% at 20 minutes and 12.09% at 50 minutes.

Table 7: effect of electric field on carotenoid content of *D. Salina*

		Control	10 min	20 min	30 min	40 min	50 min	60 min
Initial	1 Kv	0.9	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)
	5 Kv	0.9	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)	0.9 (0%)
I week	1 Kv	1.91	2.1 ±0.1 *(9.95%)	2.3 ±0.1 ** (20.42%)	2.4 ±0.1 ** (25.65%)	2.5 ±0.1 ** (30.89%)	2.6 ±0.1 ** (36.13%)	2.7 ±0.1 *** (41.36%)
	5 Kv	1.91	2.2 ±0.1 ** (15.18%)	2.5 ±0.1 *** (30.89%)	2.7 ±0.14 *** (41.36%)	2.9 ±0.1 *** (51.83%)	3.1 ±0.13 *** (62.30%)	3.3 ±0.1 *** (72.77%)
II week	1 Kv	3.3	3.4 ±0.1 (3.03%)	3.5 ±0.1 (6.06%)	3.6 ±0.1* (9.09%)	3.62 ±0.1 *(9.70%)	3.64 ±0.1 *(10.30%)	3.67 ±0.1 * (11.21%)
	5 Kv	3.3	3.4 ±0.1 (3.03%)	3.47 ±0.1 (5.15%)	3.51 ±0.1 (6.36%)	3.59 ±0.09 *(8.79%)	3.72 ±0.1 *(12.73%)	3.89 ±0.1 ** (17.88%)
III week	1 Kv	3.9	4.1 ±0.1 (5.13%)	4.15 ±0.1 (6.41%)	4.21 ±0.1 *(7.95%)	4.26 ±0.1 *(9.23%)	4.31 ±0.1 *(10.51%)	4.38 ±0.1 * (12.31%)
	5 Kv	3.9	4.2 ±0.1 *(7.69%)	4.24 ±0.1 *(8.72%)	4.31 ±0.1 ** (10.51%)	4.39 ±0.1 ** (12.56%)	4.5 ±0.1 ** (15.38%)	4.54 ±0.1 ** (16.41%)
IV week	1 Kv	4.7	4.8 ±0.1 (2.13%)	4.9 ±0.1 (4.26%)	5.01 ±0.1 *(6.60%)	5.1 ±0.1 * (8.51%)	5.2 ±0.1 ** (10.64%)	5.26 ±0.1 ** (11.91%)
	5 Kv	4.7	4.95 ±0.1 (5.32%)	5.2 ±0.1 ** (10.64%)	5.31 ±0.1 ** (12.98%)	5.41 ±0.1 ** (15.11%)	5.45 ±0.1 ** (15.96%)	5.6 ±0.1 ** (19.15%)
V week	1 Kv	6.7	6.9 ±0.2 (2.99%)	6.99 ±0.2 (4.33%)	7.1 ±0.2 *(5.97%)	7.2 ±0.2 * (7.46%)	7.26 ±0.2 *(8.36%)	7.3 ±0.2 *(8.96%)
	5 Kv	6.7	6.965 ±0.2 (3.96%)	7.21 ±0.2 *(7.61%)	7.31 ±0.2 *(9.10%)	7.51 ±0.2 *(12.09%)	7.68 ±0.2 ** (14.63%)	7.9 ±0.2 ** (17.91%)

Discussion

The current study investigates the effect of different doses of electric field on various parameters of the algae *Dunaliella salina* by examining various algal parameters, such as total chlorophyll content, and carotenoid content over a period of I, II, III, IV and V weeks.

Thereafter, the study showed increase in algal total chlorophyll content and carotenoid content of the algae *Dunaliella salina* on treatment with different doses of electric field (1kv/10, 1kv/20, 1kv/30, 1kv/40, 1kv/50, 1kv/60, 5kv/10, 5kv/20, 5kv/30, 5kv/40, 5kv/50 and 5kv/60) over a period of I, II, III, IV and V weeks. The % increase in algal total chlorophyll content as well as chlorophyll b was found to be the highest in lowest time point for all the doses (Week I) and declined thereafter in Week II, III, IV and V. However, at all the time points, the amount of total chlorophyll was found to be higher than the control. The increase in total chlorophyll content as well as chlorophyll b content of the algae on application of electric field may occur owing to the following reasons:

- Application of electric field stimulates photosynthetic activity, which in turn improves nutrient uptake as well as cellular metabolism, further potentiating increased biosynthesis of chlorophyll.
- Application of electric field ensures mass transfer and improved mixing in the growth medium, as a

consequence of which, favourable conditions are established for increased algal growth, accompanied by higher biomass and higher chlorophyll production.

- Induction of stress in the algae by electric field causes an increase in the biosynthesis of chlorophyll b in order to optimize light harvesting.
- Chlorophyll b aids in capturing light energy and transfers it to chlorophyll a, therefore, electric field induced boost in chlorophyll b biosynthesis is an attempt to improve absorption of light energy as well as its improved utilization by the algae (Benavente-Valdés *et al.*, 2016; Mirkovic *et al.*, 2017) ^[3, 9]

Similar results showing increase in total chlorophyll and chlorophyll b content of algae on exposure to electric field have also been shown in previous studies (Nezammahalleh *et al.*, 2016; Parniakov *et al.*, 2015; Luengo *et al.*, 2015) ^[10, 11, 16]

However, exposure to 5k/10 dose (the highest dose) led to a decline in total chlorophyll content of the algae, wherein the decrease was highest in Week I followed by Week II, III, IV and V. This may be attributed to the fact that exposure to high dose of electric field causes production of ROS, which can harm cellular components as well as other biomolecules like chlorophyll, hence low amount of both total chlorophyll and chlorophyll b. Also, high electric field causes

electroporation mediated physical damage to cellular membrane and thylakoid membrane, leading to leakage of chlorophyll as well as disruption of photosynthetic machinery. However, ROS induced damage and electric field damage starts to subside with increase in time duration owing to triggering of cellular repair machinery.

In the next section, the study showed increase in carotenoid content of the algae *Dunaliella salina* on treatment with different doses of electric field (1kv/10, 1kv/20, 1kv/30, 1kv/40, 1kv/50, 1kv/60, 5kv/10, 5kv/20, 5kv/30, 5kv/40, 5kv/50 and 5kv/60). The percentage increase in algal carotenoid content is the highest in 1kv/10 and declines thereafter till 1kv/60. Similarly, the percentage increase in algal carotenoid content is the highest in 5kv/10 and declines thereafter till 5kv/60. The increase in carotenoid content of algae under the effect of electric field is indicative of increase photosynthetic efficacy of algae, wherein, higher carotenoid production is a secondary effect of the treatment. Furthermore, exposure to electric field may lead to production of ROS in the algal cells, in consequence to which, the algal cells increase biosynthesis of carotenoids as antioxidant molecules to counter ROS mediated damage. Therefore, increased carotenoid accumulation is a cellular stress response and adaptation mechanism in order to cope with adverse circumstances. Also, high algal biomass in response to electric field boosts carotenoid biosynthesis. Similar results showing increase in carotenoid content of algae on exposure to electric field have also been shown in previous studies (Martínez *et al.*, 2019; Martínez *et al.*, 2018; Lafarga *et al.*, 2022) ^[5, 7, 8].

Concluding the results of the study in a nutshell, the current study showcases the constructive effect of electric field in total chlorophyll content and increased carotenoid content. However, high dose electric field has been shown to induce decreased total chlorophyll content, which is indicative of electric field mediated damage to algal cells. However, such damage to algae does not affect the algal biomass, on the contrary, carotenoid production in algae is seen to increase. This increased carotenoid biosynthesis is a stress response generated in algal cells to handle the electric field induce damage to algal cells. The current study underscores the ameliorative role of electric field in improvement of algal parameters to derive potential benefits for cosmetic and pharmaceutical industry. The findings of the current study will contribute to the development of sustainable practices in algal biotechnology and elucidate the role of electric fields in augmenting the productivity of *Dunaliella salina*, providing optimization of algal cultivation techniques enabling advancement and commercialization of algal-based products.

References

- Ahmed RA, He M, Aftab RA, Zheng S, Nagi M, Bakri R, *et al.* Bioenergy application of *Dunaliella salina* SA 134 grown at various salinity levels for lipid production. *Scientific reports*,2017;7(1):8118.
- Barbosa M, Inácio LG, Afonso C, Maranhão P. The microalga *Dunaliella* and its applications: a review. *Applied Phycology*,2023;4(1):99-120.
- Benavente-Valdés JR, Aguilar C, Contreras-Esquível JC, Méndez-Zavala A, Montañez J. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophyceae species. *Biotechnology Reports*,2016;10:117-25.
- Goswami RK, Agrawal K, Verma P. Microalgae *Dunaliella* as biofuel feedstock and β -carotene production: An influential step towards environmental sustainability. *Energy Conversion and Management*: X,2022;13:100154.
- Lafarga T, Aguiló-Aguayo I. Pulsed electric fields for the extraction of lipids, pigments, and polyphenols from cultured microalgae. In *Innovative and Emerging Technologies in the Bio-marine Food Sector*, Academic Press, 2022, 197-221.
- Luengo E, Martínez JM, Coustets M, Álvarez I, Teissié J, Rols MP, *et al.* A comparative study on the effects of millisecond-and microsecond-pulsed electric field treatments on the permeabilization and extraction of pigments from *Chlorella vulgaris*. *The Journal of membrane biology*,2015;248:883-91.
- Martínez JM, Delso C, Angulo J, Álvarez I, Raso J. Pulsed electric field-assisted extraction of carotenoids from fresh biomass of *Rhodotorula glutinis*. *Innovative Food Science & Emerging Technologies*,2018;47:421-7.
- Martínez JM, Gojkovic Z, Ferro L, Maza M, Álvarez I, Raso J, *et al.* Use of pulsed electric field permeabilization to extract astaxanthin from the *Nordic microalga* *Haematococcus pluvialis*. *Bioresource technology*,2019;289:121694.
- Mirkovic T, Ostroumov EE, Anna JM, Van Grondelle R, Govindjee, Scholes GD. Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. *Chemical reviews*,2017;117(2):249-93.
- Nezammahalleh H, Ghanati F, Adams II TA, Nosrati M, Shojaosadati SA. Effect of moderate static electric field on the growth and metabolism of *Chlorella vulgaris*. *Bioresource technology*,2016;218:700-11.
- Parniakov O, Barba FJ, Grimi N, Marchal L, Jubeau S, Lebovka N, *et al.* Pulsed electric field and pH assisted selective extraction of intracellular components from microalgae *Nannochloropsis*. *Algal Research*,2015;8:128-34.
- Singh P, Baranwal M, Reddy SM. Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress. *Pharmaceutical biology*,2016;54(10):2269-75.
- Wang X, Zhang MM, Liu SF, Xu RL, Mou JH, Qin ZH, *et al.* Synergistic bioconversion of lipids and carotenoids from food waste by *Dunaliella salina* with fulvic acid via a two-stage cultivation strategy. *Energy Conversion and Management*,2021;234:113908.
- Xu Y, Ibrahim IM, Wosu CI, Ben-Amotz A, Harvey PJ. Potential of new isolates of *Dunaliella salina* for natural β -carotene production. *Biology*,2018;7(1):14.