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Electric field assisted improvement of growth and photosynthetic efficiency of *Chlorella sorokiniana*

Pinky Dotaniya[1], GP Singh[2]
[1] Department of Botany, University of Rajasthan, Jaipur, India
[2] Professor, Department of Botany, University of Rajasthan, Jaipur, India

Abstract
This study investigated the influence of electric field (EF) exposure on growth and photosynthetic pigment content *in Chlorella sorokiniana* over a five-week incubation period. Microalgal cultures were subjected to EF treatment at 2kV for 25, 50, 75, 100, and 125 minutes, and changes in dry cell weight (DCW) and chlorophyll content (Chl a, Chl b, and total chlorophyll) were evaluated. DCW increased in all treatments with time, reaching the highest improvement in cultures treated for 50 min, which exhibited up to 4.9% higher biomass than control by 2nd week. However, exposure beyond 75 min resulted in a decline, with 5-week DCW decreasing by ~2%–5% compared to control. Photosynthetic pigments exhibited a similar trend: Chl a content increased 2.8 folds to 3.9 folds across treatments, with the highest enhancement (up to 130.4% over control) in 50-min treated cells. Chl b and total chlorophyll followed a comparable pattern, showing maximum increases of 4.2-folds and ~116% over control, respectively, after 50-min EF exposure. Prolonged exposure (≥75 min) caused decline in pigment accumulation, though values remained higher than control. These findings demonstrate that moderate EF exposure (50 min) enhances biomass and photosynthetic efficiency in *C. sorokiniana*, while prolonged exposure may impose stress, reducing growth and pigment synthesis.

Keywords: Microalgae, physical stress, dry cell weight, chlorophyll content

Introduction

Increasing consumer preference for natural products over synthetic products have led to a growing interest of both industrial and scientific community in the diverse applications of microalgae bioproducts. As a consequence, microalgae cultivation is increasingly recognized as they have been lately recognised to have significant potential to be utilized as a source of energy, food, feed, cosmetics, and pharmaceuticals due to their efficient photosynthesis, high biomass production, high capacity for creating beneficial biologically active substances like carotenoids, chlorophylls, phycobilins, fatty acids, vitamins, sterols & other industrially important compounds, and rapid development compared to other energy crops (Pulz and Gross 2004 [17]; Widjaja *et al*. 2009 [23]; Hannon *et al*. 2010 [9]; Skrede *et al*. 2011 [21]; Draaisma *et al*. 2013) [3]. However, only a few numbers of microalgae species have been successfully scaled up for production, mostly because of the high costs and complex downstream processes involved.

Chlorella sorokiniana is the sole species of *Chlorella* identified as able to thrive in elevated temperatures ranging from 38°C to 42°C. It is a stationary and single-celled freshwater microalga (Morita *et al*. 2000) [15]. This species is renowned for its ability to amass substantial amounts of protein and lipid, making it a famous figure in the realm of microalgae due to its multifaceted responsibilities (Wan *et al*. 2011) [22]. This specific type of *Chlorella* is highly appreciated because to its remarkable growth rate, high lipid content, and flexibility. This plant's quick growth makes it an ideal choice for biofuel production due to its renewable and sustainable energy source. Furthermore, it has a notable ability to remove contaminants from water, aiding in bioremediation and environmental preservation. Its excellent nutritional value, with a significant amount of proteins and important elements, makes it a promising option for creating functional foods and natural dietary supplements. This specific species of *Chlorella* has a broad range of uses, from environmental remediation to human nutrition.

Recent advancements in molecular biology and modification of growth conditions such as temperature, pH, light, carbon supply, salinity, and nutrients have enhanced the production of larger yields of microalgae-specific bioproducts (Gao *et al*. 2013 [5]; Jeon *et al*. 2013 [10]; Gassel *et al*. 2014) [6]. The capacity to operate large photobioreactors efficiently to manage biomass and metabolites at high levels, along with technological advancements, are crucial for the economic feasibility of commercially utilizing various products derived from microalgae (Luengo *et al*. 2014) [14]. Currently, there are still challenges in completely utilizing microalgae that produce bioproducts, particularly in properly extracting these molecules from the cell biomass.

Use of both static and pulsed electric field treatment have been widely reported in bacterial biotechnology (Ohmura *et al*. 2002 [16]; Liu *et al*. 2015a) [12, 13] and improved extraction/recovery of microalgal biomolecules from *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Auxenochlorella protothecoides* (Goettel *et al*. 2013 [7]; Luengo *et al*. 2014 [14]; Rego *et al*. 2015 [18]; Silve *et al*. 2018 [20]; Han *et al*. 2019) [8]. Additionally, some studies have also indicated that electric fields (EF) can stimulate the growth and development of non-microalgae plant ells of *Arabidopsis thaliana* (Eing *et al*. 2009) [4]. However, there have been very few studies on use of electric field as pre-treatment to improve microalgal growth and recently Kim *et al*. (2018) [11] reported enhanced growth of *Haematococcus pluvialis* with electrical treatment.

Therefore, in this study effect of electric field pre-treatment on growth, and photosynthetic potential of *C. sorokiniana*

was evaluated with an aim to identify optimum time of electric field exposure for enhancing production of microalgal biomolecules.

Material and Methods

1. Microalgae culture conditions

Stock culture of *C. sorokiniana* (Accession Number) procured from Banaras Hindu University (BHU), India was grown on Blue Green – 11 (BG – 11) liquid medium (Allen 1968) [1] and the cultures were maintained at 25ºC and were illuminated with 20 watt tube lights having 2000 photon lux intensity for a 16 h photoperiod. The algal cultures were continuously agitated on rotary shaker at 100 rpm to ensure optimum growth.

2. Electric field treatment

10 ml portions of 20-days-old algal cultures were aseptically transferred into 6 separate sterile petri dishes and each of the plates was given electric shock at 2kV for different time intervals (0 min, 25 min, 50 min, 75 min, 100 min and 125 min) by placing the plates sequentially on the stage as illustrated in Figure 1. Algal culture treated for 0 min was used as control in the present study.

Fig 1: Equipment used for electric field treatment to algal cells

3. Experimental Design

3 flasks (n = 18) of algal growth medium (BG-11) were prepared for each treatment, with 150 ml of the medium being dispensed in 250 ml Erlenmeyer flasks. The growth medium was then autoclaved for 20 min at 121ºCelsius and 15 psi.

Algal cultures (2 ml) exposed to electric field were added as inoculum to the sterile growth medium and the inoculated flasks were incubated in a plant growth room for 5 wks. All the cultures were continually shaken at a speed of 100 rpm using a rotary shaker (Orbitek, India) to prevent the algal cells from aggregating. In addition, weekly aliquots were taken from each flask to assess the growth of the algae. The algal biomass of each treatment was extracted by centrifugation at 10,000 rpm for 20 min at 25ºC, followed by oven drying at 30ºC for lipid profiling content measurements as described in subsequent sections.

4. Determination of Microalgae Biomass

The microalgal biomass was quantified using the dry cell weight (DCW) method described by Liu *et al*. (2015b) [12, 13]. 1 ml of algal culture was measured for absorbance at 680 nm using a UV-2600 UV-Vis spectrophotometer from Shimadzu, Japan. Dry Cell Weight (DCW) was then estimated using the provided formulae:

DCW (g/L) = 0.2662 x A_{680} + 1.5796 (Liu *et al*. 2015b) [12, 13]

Determination of Chlorophyll Content

The chlorophyll pigments were extracted following the method described by Ritchie *et al*. (2015) [19] with minor adjustments. 1 ml of algal culture from each treatment was centrifuged at 10,000 rpm for 15 minutes at 25ºC and the algal cell pellet was suspended in chilled 90% (v/v) acetone (Rankem, India) and was incubated at -20ºC for overnight. The extract was centrifuged at 10,000 rpm for 10 min at 4ºC, the supernatant was collected in a separate vial, and the pellet was re-extracted with chilled 90% acetone three times to maximize pigment recovery. The supernatant obtained from each extraction was combined in a single vial for each treatment or sample. Using a UV-2600 UV-Vis spectrophotometer (Shimadzu, Japan), the absorbance of the resulting pigment extract was measured at 647 nm and 664 nm. The quantity of chl *a* and *b* was then estimated using the formulas given by Ritchie *et al*. (2015) [19].

5. Statistical Analysis

The tests were run three times, and the mean values together with the standard error were determined using MS Excel. An ANOVA was conducted to assess different treatment methods, followed by a post-hoc test using IBM SPSS software.

Result and Discussion

1. Effect on growth parameters

Dry cell weight (DCW) increased in all treatments with increasing time (Figure 2) such that after 5 weeks of incubation, maximum % change (*w.r.t.* 1st week) in DCW was recorded in cells treated with electric field for 25 min (24.4%), followed by those treated for 50 min (23.5%), 0 min (23.3%), 75 min (19.8%), 100 min (19.02%) and 125 min (17.2%). However, maximum dry cell weight during entire incubation period was recorded in cells treated with electric field for 50 min which was ~1.8%, ~4.9%, ~3.7%, 4.1% and ~2.0% higher than control after 1st week, 2nd week, 3rd week, 4th week and 5th week of incubation (Table 1). When algal cells were exposed to electric field for ≥ 75 min, a declining pattern in algal biomass was recorded such that after 5 weeks the dry cell weight decreased (~2% - 5% less than the control) with increasing time of electric field exposure.

Table 1: Effect of electric field exposure for different time durations on microalgae biomass

EF treatment time (min)	Initial	1st Week	2nd Week	3rd Week	4th Week	5th Week
	DCW (g/L)					
C	1.58 ± 0.0	1.63 ± 0.01	1.65 ± 0.04	1.89 ± 0.01	1.94 ± 0.01	2.01 ± 0.00
25	1.58 ± 0.0	1.64 ± 0.02	1.71 ± 0.03	1.95 ± 0.00	1.98 ± 0.08	2.04 ± 0.02
50	1.58 ± 0.0	1.66 ± 0.01	1.73 ± 0.02	1.96 ± 0.01	2.02 ± 0.0	2.05 ± 0.01
75	1.58 ± 0.0	1.64 ± 0.02	1.69 ± 0.02	1.93 ± 0.04	1.94 ± 0.02	1.97 ± 0.01
100	1.58 ± 0.0	1.63 ± 0.01	1.68 ± 0.03	1.87 ± 0.01	1.90 ± 0.01	1.94 ± 0.11
125	1.58 ± 0.0	1.63 ± 0.01	1.71 ± 0.02	1.77 ± 0.01	1.82 ± 0.01	1.91 ± 0.04

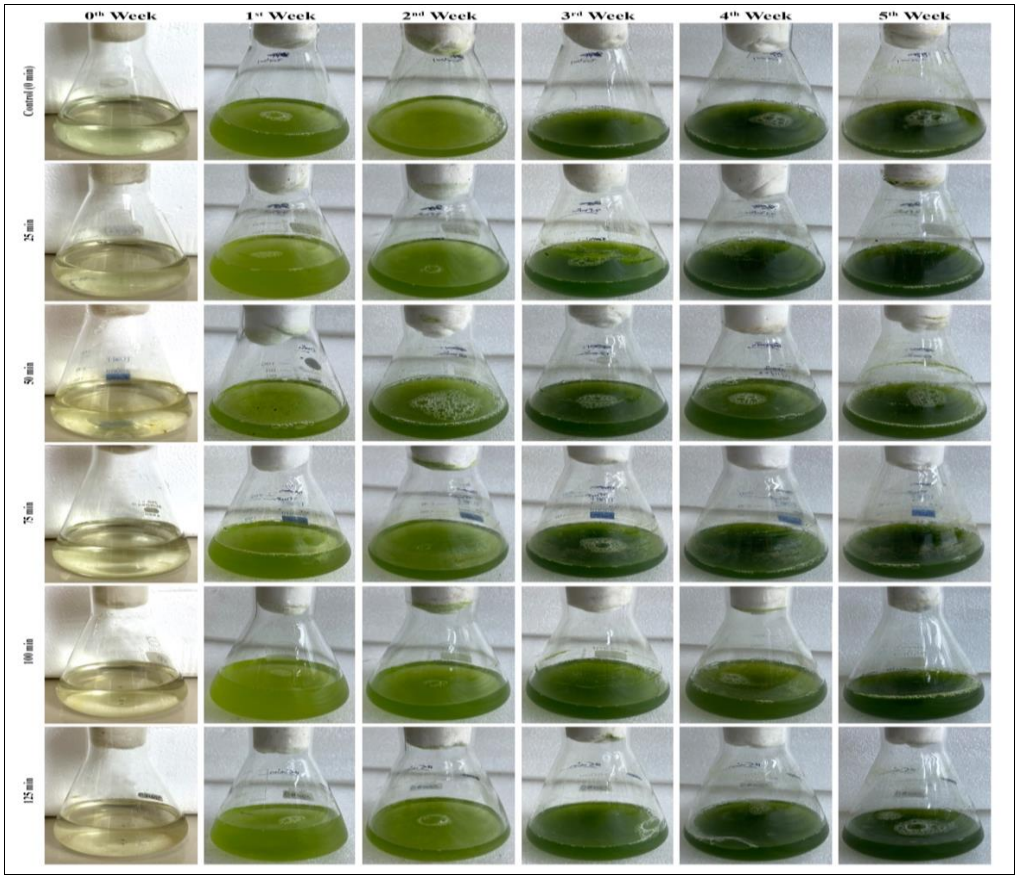

Fig 2: Growth pattern of *C. sorokiniana* across 5 weeks exposed to electric field for different time durations

2. Effect on chlorophyll content

In the present study, the abundance of Chl *a* and Chl *b* pigments in *C. sorokiniana* was utilized as a measure of the microalgae's photosynthetic efficiency due to their prevalence compared to other forms of chlorophylls.

The concentration of chlorophyll *a* (Chl *a*) increased over time in all treatments and after 5 weeks, the increase ranged from 2.8 to 3.9 folds, with the highest increase of around 3.9 folds in algal cells treated with electric field for 50 min (Figure 3). The content was higher than the control in algal cells exposed to electric field irrespective of the treatment duration, however maximum rise of 31.3% to 130.4% was recorded in cells exposed to electric field for 50 min (Figure 3). The content increased with increasing time of EF exposure upto 50 min and when the cells were treated more than equal to 75 min, though it was higher than control but a declining pattern was recorded during all 5 weeks (Figure 3).

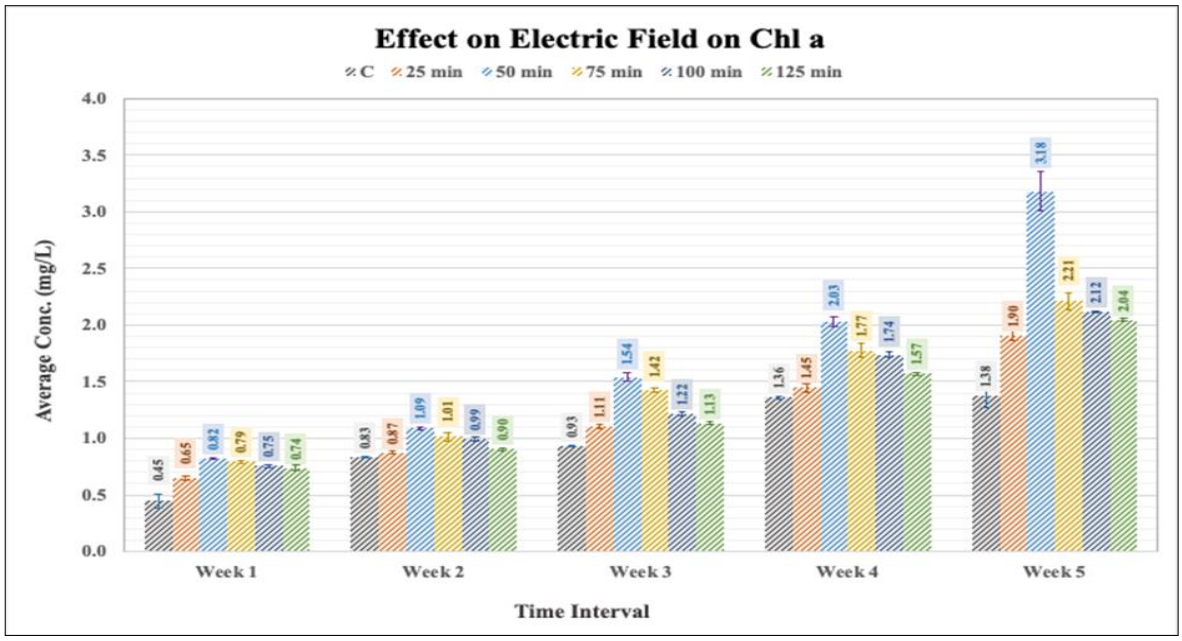

Fig 3: Week-wise chlorophyll *a* content of *C. sorokiniana* exposed to electric field for different time duration

Chlorophyll *b* content showed a consistent increase with longer incubation period such that after 5 weeks the chl *b* content increased upto 2.9 folds, 4.2 folds, 3.1 folds, 4.0 folds, 2.6 folds and 2.8 folds in algal cells exposed to electric field for 0 min, 25 min, 50 min, 75 min, 100 min and 125 min, respectively (Figure 4). Chl *b* content was 62.5%, 70.6%, 36.4% higher than control after 1st, 2nd and 4th week of incubation, and was ~30.4% and 100% higher than control after 3rd and 5th week of incubation, respectively (Figure 4).

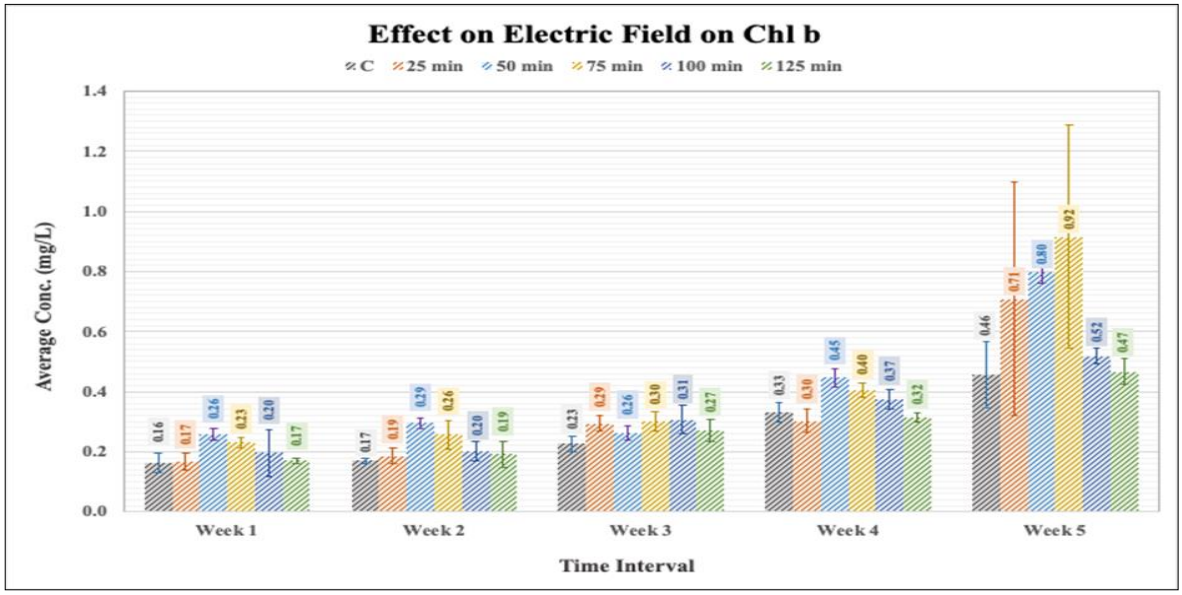

Fig 4: Week-wise chlorophyll *b* content of *C. sorokiniana* exposed to electric field for different time duration

Total chlorophyll also increased with increasing incubation time with an overall increase of ~2.8 folds to 3.7 folds after completion of incubation period (5 weeks) as evident from Figure 5. During entire incubation period, total chlorophyll content was around 34.4% to 77.05% (1st week), 6% to 38% (2nd week), 21.7% to 56.5% (3rd week), 11.8% to 46.1% (4th

week) and 37% to 116.3% (5th week) higher than control in cells exposed to electric field, irrespective of exposure time (Figure 5). Though the content was higher in all treated cells than untreated cells, yet maximum content was recorded in algal cells treated with electric field for 50 min which was around 38% to 116.3% higher than control, wherein cells exposed to electric field for durations equal to and/or longer than 75 min exhibited a slight decline in the content. Further, this pattern indicated that pattern of total chlorophyll content was followed similar pattern to that of chl *a* content.

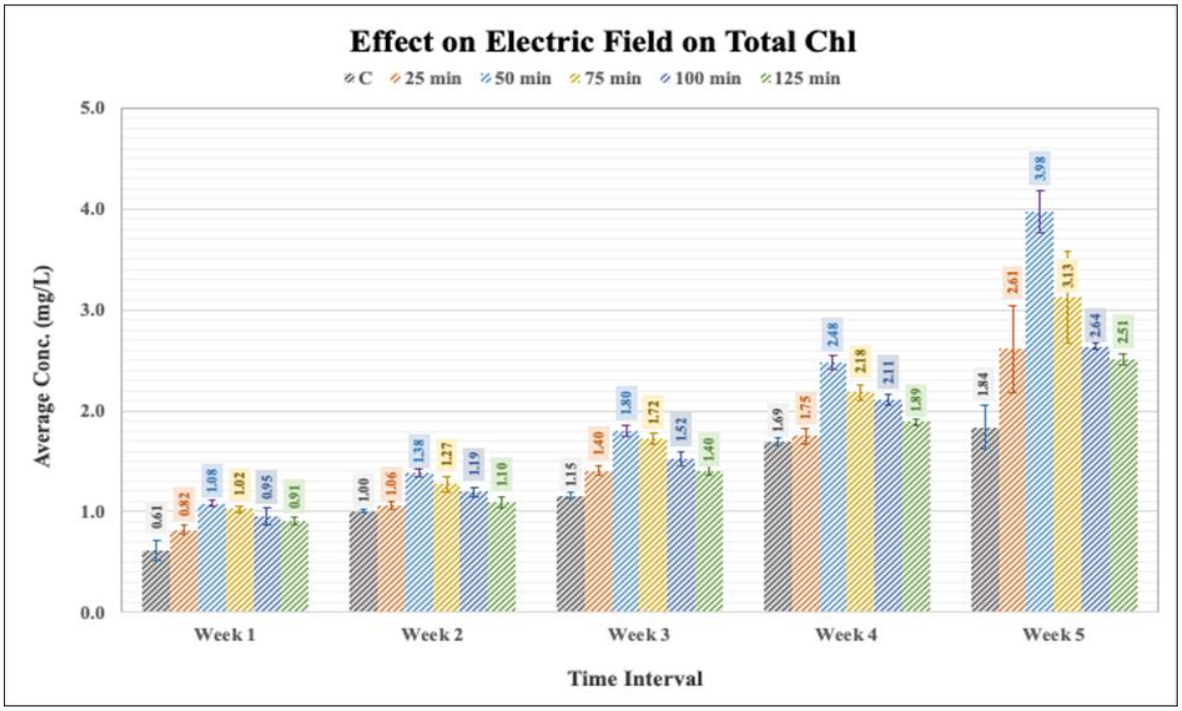

Fig 5: Week-wise total chlorophyll content of *C. sorokiniana* exposed to electric field for different time duration

Conclusion

The present study demonstrated that electric field (EF) exposure can significantly influence the growth and photosynthetic performance *of Chlorella sorokiniana*. Moderate EF treatment, particularly for 50 minutes, resulted in the highest biomass yield and pigment accumulation, with dry cell weight increasing up to 4.9% above the control and total chlorophyll content improving by as much as 116.3%. This enhancement suggested that EF acts as a stimulatory factor, potentially improving photosynthetic efficiency and overall productivity without chemical additives. However, exposure durations equal to or exceeding 75 minutes showed a gradual decline in growth and pigment synthesis, indicating that prolonged EF application may impose stress on algal cells. These findings underscored the importance of optimizing EF exposure time to harness its benefits effectively for large-scale algal cultivation and bioresource applications. Future research should focus on elucidating the underlying physiological and molecular mechanisms and assessing energy efficiency and scalability for industrial applications.

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