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Carotenoid Production by *Dunaliella salina* with Magnetic Field Application

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Abstract

The use of external triggers in microalgae cultivation has emerged as a promising approach to enhance biomass production and biochemical composition. For instance, magnetic fields (MFs) have had their potential to modulate cellular metabolism and physiological responses explored. This study investigated the effects of MF exposure on *Dunaliella salina* and evaluated its impact on biomass production, pigment synthesis and biochemical composition. The highest biomass concentration (0.59 g L^{-1}) was observed under continuous exposure to 60 mT (MF60-24 h); it represented a 51% increase in comparison with the control. A gradual rise in pH, which reached 10.83, was observed during cultivation. MF exposure also enhanced chlorophyll-a (118%) and carotenoid (95%) concentrations; thus, it improved photosynthetic efficiency and potential oxidative stress responses. The biochemical composition revealed a shift in metabolic pathways after prolonged MF exposure (24 h d^{-1}), decreasing carbohydrate content by 7%, while increasing lipid accumulation by 7%. Scanning electron microscopy (SEM) indicated structural modifications on the cell surface induced by the MF. Therefore, MF applications improve *D. salina* cultivation and enhance biomass composition for biotechnological applications.

Keywords: magnets; biomass; biorefinery; macromolecules

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1. Introduction

Carotenoids represent a growing market segment due to the increase in consumers' demand for healthier food, particularly those of natural origin. According to a recent report, the global carotenoid market is expected to grow from USD 2.0 billion in 2022 to USD 2.7 billion in 2027 [1]. Rise in demand has driven interest in biotechnological production of carotenoids by microalgae, which are natural sources of high-value bioactive compounds [2,3].

The green unicellular species *Dunaliella salina* has been widely recognized as a major natural source of β-carotene. Although carotenoids are the most extensively studied compounds in *D. salina*, some studies have also explored the production of other valuable biocompounds, such as glycerol [4], polyunsaturated fatty acids (PUFAs) [5], proteins [6] and carbohydrates [7]. *D. salina* is also able to produce chlorophyll and other pigments that offer a reliable and sustainable source of raw material in comparison with traditional methods that often rely on pigment extraction from marine organisms [8–10]. Several

studies have investigated alternative strategies to enhance carotenoid biosynthesis by influencing enzymes involved in this metabolic pathway. However, there is no definitive consensus on the exact regulatory mechanisms governing carotenoid production and its interplay with other metabolic processes [11–14].

Despite advances in biotechnological production, a significant proportion of carotenoids have been chemically synthesized due to the high cost associated with natural production. For instance, β-carotene derived from *D. salina* is priced between USD 300 and 3000/kg, and the cost of biomass production is estimated at approximately USD 17/kg of dry weight based on process modeling [15]. However, demand for natural colorants has arisen in the food industry driven by consumers' preferences for clean labels and sustainable sourcing [16]. Carotenoids produced by *D. salina*, such as β-carotene and lutein, not only provide vibrant colors but also offer health benefits, further enhancing their attractiveness to food manufacturers [17].

In the cosmetics industry, *D. salina*-derived carotenoids are incorporated into skincare and makeup products, in which they serve as antioxidants and provide protection against the damaging effects of UV radiation [18]. Consumers have increasingly favored cosmetics that are free from synthetic additives, making natural choices more appealing [19].

The pharmaceutical sector has also recognized the therapeutic potential of carotenoids and other pigments produced by this microalga [18,20]. Potent antioxidant properties of pigments produced by *D. salina* make them valuable for the development of health supplements and pharmaceutical products with potential applications to eye health, cancer prevention and anti-aging effects [21].

Therefore, to make biotechnological production of carotenoids and other biocompounds more viable, a promising approach involves magnetic field (MF) application, which has been shown to influence cellular metabolism and the synthesis of valuable biomolecules in microalgae [22–24]. MFs may biochemically influence the activation of specific enzyme systems and metabolic pathways and alter plasma membrane flux and gene transcription due to cellular defense mechanisms [23].

The literature reports that the regulation of cellular metabolism by gene transcription alters plasma membrane flux and enables the entry of Ca^{2+} into the cytoplasm (responsible for cell growth) [25,26]. Activation of metabolic and enzymatic systems to produce macromolecules of interest or secondary metabolites has shown that MF exposure leads to positive effects on microalga cultures [27]. MF exposure has been reported to enhance pigment production by *Chlorella kessleri* [28], proteins by *Limnospira indica* [29], lipids by *Chlorella homosphaera* [30] and carbohydrates by *Chlorella minutissima* [31]. Application of this emerging biotechnological strategy is considered non-toxic, cost-effective and easily applicable to photobioreactor systems; thus, it is viable in chemical, pharmaceutical and food industries [22,32].

However, the effects of MFs on *D. salina* culture remain unexplored. It is still unknown whether MF exposure may induce metabolic changes and lead to high β-carotene accumulation under stress conditions. Therefore, this study aimed to evaluate the effects of MFs at different intensities and exposure times on biomass and pigment production by *D. salina*.

2. Material and Methods

2.1. Maintenance and Inoculum Cultivation of the Microorganism

The microalga *D. salina* was isolated in São Pedro da Aldeia, RJ, Brazil, by the research group at the Laboratory of Microalgae Biotechnology, which belongs to the National Institute of Technology (INT). Stock cultures were replicated every 7 days with culture medium described by Shaish, Ben-amotz and Avron [33] and incubated at 21 °C and a constant photonic flux density (PFD) of 93 μmol photons $m^{-2}s^{-1}$.

Inoculum was performed in Erlenmeyer flasks (500 mL) with 300 mL Shaish medium, agitated in an orbital shaker at 150 rpm and 28 °C and illuminated by white LED at a PFD of 250 μmol photons $m^{-2}s^{-1}$ for 4 days.

2.2. Pigment Production with Magnetic Field (MF) Evaluation

Microalgae were cultivated in a saline medium described by Shaish, Ben-amotz and Avron [33]. It contains (g L^{-1}) the following: 4.20 $NaHCO_3$, 0.02 KH_2PO_4, 58.50 NaCl, 0.37 KCl, 0.71 Na_2SO_4, 0.42 $NaNO_3$, 1.01 $MgCl_26H_2O$ and 0.02 $CaCl_22H_2O$. One milliliter of a trace metal solution was added (g L^{-1}): 0.54 $FeCl_3$, 1.86 Na_2EDTA, 1.38 $MnCl_2$, 0.13 $CuCl_2$, 0.13 $ZnCl_2$, 1.23 $(NH_4)_6Mo_7O_{24}$ and 0.23 $CoCl_26H_2O$. Its pH was adjusted to 8.0 with HCl (1 mol L^{-1}) and NaOH (1 mol L^{-1}).

Cultivation was performed in a 500 mL flask with 270 mL medium and 30 mL inoculum (10% v v^{-1}), corresponding to the initial cell concentration of 1.0×10^5 cells mL^{-1}. The cultivation conditions were 28 °C, orbital agitation at 150 rpm and illuminated by white LED at a PFD of 250 μmol photons $m^{-2}s^{-1}$ for 5 days. To induce the MF, ferrite magnets ($80 \times 80 \times 10$ mm) were fixed at the base of Erlenmeyer flasks, with the positive poles facing up. The MF intensity was measured by a Teslameter (Tlmp-hall, GlobalMag, São Paulo, Brazil). The MF conditions under evaluation included different MF intensities of 30 and 60 millitesla (mT) and exposure times (1 h d^{-1} and 24 h d^{-1}). The cultures were designated as MF30-1 h, MF30-24 h, MF60-1 h and MF60-24 h. A control cultivation (Control) without any MF application was developed for comparison.

2.3. Determination of pH, Biomass and Cell Concentration

The pH was measured daily by a digital pH meter [34]. The biomass concentration (g L^{-1}) was determined by dry weight at the end of cultivation. Fifteen mL aliquots were vacuum filtered through glass fiber membranes (pore size 0.7–1.4 μm). Subsequently, 5 mL 0.5 mol L^{-1} ammonium bicarbonate was used for removing residual salts from the culture medium. The membranes were then dried at 60 °C until constant weight was achieved [35]. Daily cell counts were performed by a microscope.

At the end of every 5-day cultivation period, the biomass was harvested by centrifugation, frozen at −80 °C for 48 h and subsequently lyophilized by a lyophilizer (Liotop, L108, Liobras, São Paulo, Brazil).

2.4. Extraction and Determination of Carotenoid, Chlorophyll-a and Chlorophyll-b Concentrations

Ten mL aliquots were collected at the end of cultivation for carotenoid extraction. The cell suspension was filtered through glass fiber membranes (pore size 0.7–1.4 μm). The membranes were folded, and excess water was removed with a paper towel for subsequent freezing at −20 °C. The membranes were kept in the freezer until extraction. Five mL of acetone were added to the frozen membranes, which were crushed with a glass stick to disrupt the cells. The tube was kept in the freezer for 1 h. The homogenate was then centrifuged for 10 min at 3500 rpm [36].

Carotenogenic extracts were evaluated by a spectrophotometer at wavelengths of 470, 644.8 and 661.6 nm. The total carotenoids were quantified according to the method described by Lichtenthaler [37]. Equations (1)–(3) were used for calculating the concentrations of chlorophyll-a, chlorophyll-b and total carotenoids in the extract (mg L^{-1}), respectively. Equation (4) was applied to determine the volumetric carotenoid concentration (VCC) in the cultures. In the equations, c is the optical path of the cuvette (1 cm); [Chlorophyll-a] and [Chlorophyll-b] represent the concentrations of chlorophyll-a and chlorophyll-b in the extract (mg L^{-1}), respectively; total carotenoids refer to the total carotenoids in the extract; [VCC] is the volumetric carotenoid concentration in the culture (mg L^{-1}); $v_{acetone}$

is the volume of acetone used for extraction (mL); and $v_{filtrate}$ is the volume of filtered cell suspension (mL).

$$\text{Chlorophyll-a} = 11.24 \times A_{661.6} - 2.04 \times A_{644.8} \times c \quad (1)$$

$$\text{Chlorophyll-b} = 20.13 \times A_{644.8} - 4.19 \times A_{661.6} \times c \quad (2)$$

$$\text{Total carotenoids} = \frac{1000 \times A_{470} - [\text{Chlorophyll-a}] - 63.12 \times [\text{Chlorophyll-b}]}{214} \times c \quad (3)$$

$$\text{VCC} = \frac{1000 \times A_{470} - [\text{Chlorophyll-a}] - 63.12 \times [\text{Chlorophyll-b}]}{214} \times c \times \frac{v_{acetone}}{v_{filtered}} \quad (4)$$

2.5. Biochemical Composition of Biomass

Lyophilized biomass was used for determining the protein, carbohydrate and lipid contents [30]. Carbohydrates and proteins were determined in 20 mg freeze-dried biomass by alkaline hydrolysis with 0.8 mL NaOH 0.1 mol L^{-1} at 95 °C in a water bath. Then, 0.2 mL 0.4 mol L^{-1} HCl was added. In the hydrolyzed extract, protein was determined according to Lowry et al. [38], with the use of a standard curve of bovine serum albumin. The carbohydrate content was determined according to DuBois et al. [39], with the use of a glucose standard curve. The lipid content was extracted according to Marsh and Weinstein [40].

2.6. Scanning Electron Microscopy (SEM)

Samples of lyophilized biomass were examined by a scanning electron microscope (Jsm-6360LV, Jeol, Tokyo, Japan) with electron beams accelerated at 10 keV and 20 keV. The solid material was placed on double-sided carbon adhesive tape, fixed on stubs and then metallized with gold/palladium in a high-vacuum modular metallizer (Med 020, Baltec, Paffikon, Switzerland).

2.7. Statistical Analysis

The carotenoid, chlorophyll-a, chlorophyll-b, carbohydrate, protein and lipid concentrations were submitted to an analysis of variance (ANOVA), which was performed by the Statistica software program version 7 (Statsoft, Tulsa, OK, USA). All the experiments were conducted in triplicate, and the means were evaluated by Tukey's test at a 95% confidence level ($p < 0.05$).

3. Results and Discussion

3.1. Effects of Magnetic Field (MF) on Cell Concentration, Biomass and pH

D. salina cells change structurally when exposed to adverse stress conditions. Several studies have reported variations in cell size and volume during cultivation [41–43]. One of the most reliable methods of cell growth monitoring is direct cell counting. In this study, to evaluate the influence of MFs on *D. salina* growth and cellular behavior over time, cell counts were performed over a 96 h cultivation period (Figure 1).

After the first 48 h, there was considerable increase in the cell concentration under MF-treated conditions in comparison with the control, suggesting a cumulative biological effect that becomes evident in the later stages of cultivation. At 96 h, cultivations with the MF application showed similar concentrations with no significant differences among them ($p < 0.05$) (Table 1) and higher than the ones of the control cultivation. The lowest cell concentration was observed in the control assay (6.5×10^5 cells mL^{-1}).

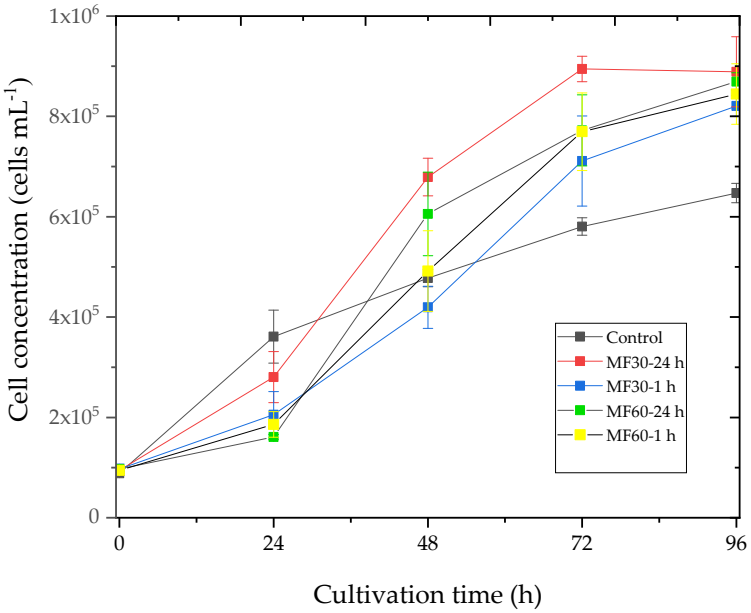


Figure 1. Cell growth of *D. salina* under different MF intensities (30 and 60 mT) and exposure times (1 h d^{-1} and 24 h d^{-1}).

Table 1. Biomass and cell concentrations in all cultivations after 96 h.

Conditions	Biomass Concentration (g L^{-1})	Cell Concentration (cells mL^{-1})
Control	0.39 ± 0.02^{b}	$6.47 \times 10^5 \pm 1.92 \times 10^{4\,b}$
MF30-1 h	0.50 ± 0.01^{ab}	$8.21 \times 10^5 \pm 2.08 \times 10^{4\,a}$
MF30-24 h	0.55 ± 0.01^{a}	$8.89 \times 10^5 \pm 6.99 \times 10^{4\,a}$
MF60-1 h	0.43 ± 0.07^{b}	$8.44 \times 10^5 \pm 6.43 \times 10^{4\,a}$
MF60-24 h	0.59 ± 0.04^{a}	$8.69 \times 10^5 \pm 1.73 \times 10^{4\,a}$

MF: Magnetic Field; Different letters (^{a,b}) in the same column indicate that the means were significantly different at a 95% confidence level ($p < 0.05$).

A similar pattern was found by Khorshidi et al. [27], who investigated the effects of MFs (0, 2, 4 and 8 mT) on *Haematococcus lacustris* IBRC-M 50,096 cultivation. Application of 8 mT at the beginning of the exponential growth phase significantly increased cell concentration by 50% (2.9×10^5 cell mL^{-1}) after 18 days of cultivation, suggesting that MF exposure may positively influence microalgal growth under specific conditions.

Regarding final biomass concentration, values ranged from 0.30 to 0.59 g L^{-1} (Table 1). The highest biomass concentrations were found in the MF30-24 h (0.55 g L^{-1}), MF60-24 h (0.59 g L^{-1}) and MF30-1 h (0.50 g L^{-1}) cultures. However, only MF30-24 h and MF60-24 h showed statistically significant differences ($p < 0.05$) in comparison with the control (0.39 g L^{-1}), representing increases of 41% and 51%, respectively.

Thus, MF exposure may stimulate *D. salina* growth. The results of cell counting and biomass concentration under MF exposure show higher values in comparison with the control. Regarding the final biomass concentration, continuous MF exposure yielded higher results than exposure for only 1 h per day. A plausible explanation for the divergence is that, according to Hristov [32], MF exposure may promote structural and metabolic changes in cells, such as high intracellular content (e.g., lipids, proteins and pigments) and modifications in cell size and volume. Consequently, biomass concentration may rise without any corresponding increase in cell concentration.

Similar studies have also shown that a moderate MF application may increase biomass production by microalgae. Deamici et al. [44] reported an increase in biomass concentration by *Chlorella fusca* under outdoor and indoor conditions. MF (25 mT) applied for 24 h d^{-1}

and 1 h d^{-1} under indoor conditions increased biomass concentration by 70 and 85%, in comparison with the control, respectively. Li et al. [45] also reported an increase in biomass concentration when they applied the same intensity as the one used in this study (30 mT) to two green microalgae (*Auxenochlorella pyrenoidosa* and *Tetradesmus obliquus*). Concerning *A. pyrenoidosa*, the final concentration was 2.1 g L^{-1}, in comparison with 1.6 g L^{-1}, without any MF. Regarding *T. obliquus*, the final concentration was 1.1 g L^{-1}, in comparison with 0.8 g L^{-1}, with no MF. However, these studies did not correlate the influence of MFs with cell count and biomass concentration, as demonstrated by this study.

Concerning pH, there was gradual increase throughout cultivation (Figure 2), starting at around 8.60 and reaching the maximum value of 10.83 (MF60-24 h). No significant differences in cultivation pH were observed in the conditions under evaluation. According to Zhang et al. [46], the rise in pH is likely associated with CO_2 consumption by microalgae during photosynthesis. Consequently, the concentration of carbonic acid ions decreases in the medium, which becomes more alkaline.

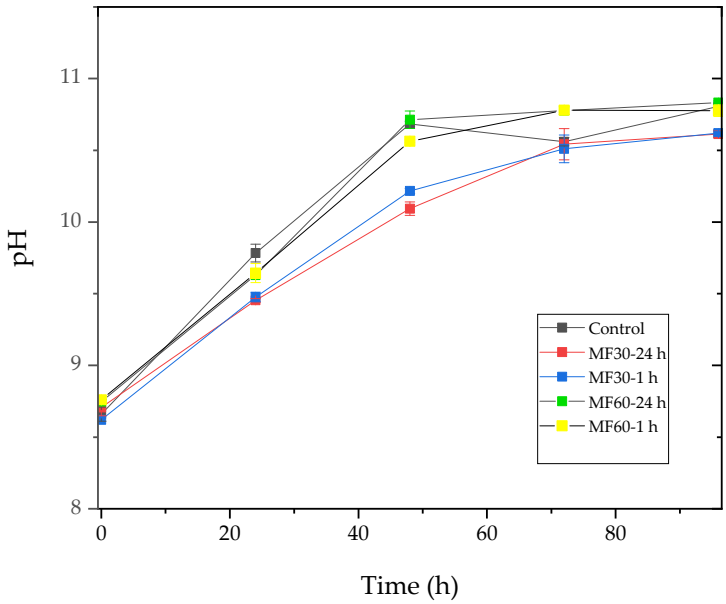


Figure 2. pH monitoring throughout cultivation of all assays.

Similar results were described by Ying et al. [47], who investigated the effect of CO_2 and pH on *D. salina* growth and demonstrated that microalgae promote an increase in pH by consuming CO_2 during photosynthesis. The maximum oxygen production rate was recorded at pH 7.2, but even under more alkaline initial pH conditions (8.5), *D. salina* maintained active growth. These results are consistent with those reached by this study, in which no significant drop in pH was observed throughout cultivation, suggesting that the progressive increase is related to active photosynthesis.

This behavior was also observed by Huang et al. [48], who evaluated the impact of CO_2 and pH on *D. salina* cultures. Under low CO_2 conditions (0.03%), the pH of the medium increased significantly due to the use of dissolved CO_2, which reduced the concentration of carbonic acid and increased pH throughout cultivation (maximum of 9.8). Thus, the behavior of the microalgae observed by this study was expected.

3.2. Influence of Magnetic Field (MF) on Pigment Production

Cultures exposed to MFs showed significant increases in the chlorophyll-a and carotenoid concentrations (Figure 3). The highest chlorophyll-a concentration (2.62 mg L^{-1}) was observed in MF30-24 h, reflecting an increase of 118% in comparison with the control

assay, which achieved 1.20 mg L^{-1}. On the other hand, the chlorophyll-b concentration did not show any significant variation in the experimental conditions ($p < 0.05$).

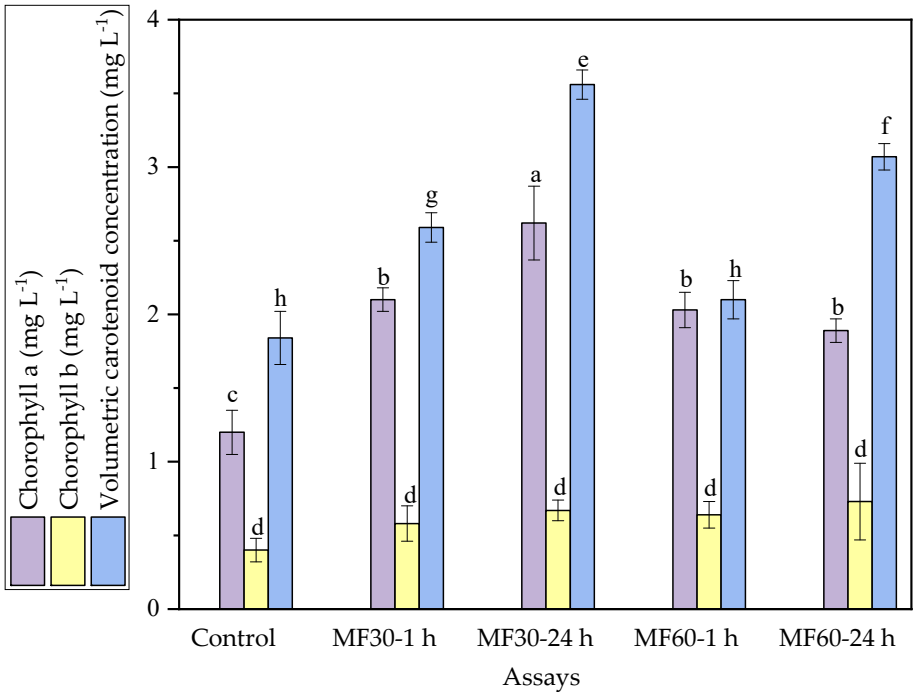


Figure 3. Pigment concentrations (mg L^{-1}) of all assays at different exposure times (1 h d^{-1} and 24 h d^{-1}) and MF intensities (30 mT and 60 mT) and control cultivation. Different letters indicate statistically significant differences within each pigment (Tukey's test, $p < 0.05$), with distinct letter groupings used per pigment (e.g., a–c for chlorophyll-a, d for chlorophyll-b, e–h for carotenoids).

Regarding carotenoid concentrations, the highest concentration (3.56 mg L^{-1}) was observed in MF30-24 h; it was 95% higher than the one of the control ($p < 0.05$). As mentioned in the previous section, this treatment also resulted in higher biomass production in comparison with the control, further supporting the effectiveness of this specific MF exposure in enhancing biomass yield enriched with carotenoids.

Furthermore, when the analysis of pigment content was based on dry biomass (mg g^{-1}) (Table 2), the highest chlorophyll-a concentrations were observed in the MF30-24 h (4.77 mg g^{-1}), MF30-1 h (4.19 mg g^{-1}) and MF60-1 h (4.71 mg g^{-1}) assays, all significantly different from the ones of the control (3.06 mg g^{-1}). Although only MF30-24 h showed a significant increase in the chlorophyll-a concentration when expressed volumetrically (mg L^{-1}), the specific carotenoid concentration indicated that the other MF-treated cultures also promoted pigment accumulation in the biomass. Regarding carotenoids, MF30-24 h also resulted in the highest specific carotenoid concentration (6.47 mg g^{-1}), representing a 94% increase in comparison with the control (4.73 mg g^{-1}). This reinforces that continuous exposure to 30 mF (MF30-24 h) not only enhances biomass production but also stimulates metabolic pathways related to pigment biosynthesis.

Comparable enhancements in pigment content have been reported in the literature. Bauer et al. [28] reported that MFs may enhance pigment production by improving functionality of the photosynthetic apparatus. The authors evaluated the same conditions of intensity and exposure time that were used in this study (30 and 60 mT, 24 h d^{-1} and 1 h d^{-1}) in *Chlorella vulgaris*. Exposure to 30 mT for 1 h d^{-1} led to increases of 38.9% in chlorophyll-a, 59.1% in chlorophyll-b and 25% in total carotenoids. They suggested that MFs induce oxidative stress, which activates antioxidant pathways and signaling mechanisms, ultimately enhancing pigment synthesis.

Table 2. Specific carotenoid concentrations (mg g^{-1}) at the end of all assays at different exposure times (1 h d^{-1} and 24 h d^{-1}) and MF intensities (30 mT and 60 mT) and control cultivation.

Condition	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Carotenoids (mg g^{-1})
Control	3.06 ± 0.38^{b}	1.01 ± 0.21^{a}	4.73 ± 0.48^{b}
MF30-1 h	4.19 ± 0.17^{a}	1.16 ± 0.25^{a}	5.18 ± 0.21^{b}
MF30-24 h	4.77 ± 0.46^{a}	1.22 ± 0.13^{a}	6.47 ± 0.17^{a}
MF60-1 h	4.71 ± 0.28^{a}	1.50 ± 0.22^{a}	4.87 ± 0.30^{b}
MF60-24 h	3.21 ± 0.01^{b}	1.23 ± 0.44^{a}	5.21 ± 0.16^{b}

MF: Magnetic Field; Different letters (^{a,b}) in the same column correspond to significant differences among assays of all pigments under evaluation ($p < 0.05$) by the Tukey's test.

Therefore, the increase in chlorophyll-a concentration found in MF30-24 h may be associated with an increase in the efficiency of photosystem II and enhanced photochemical activity in chloroplasts induced by MFs. This hypothesis is supported by Small, Huner and Wan [49], who reported that MFs improved efficiency of photosystem II and electron transport rates in *Chlorella vulgaris*. As a result, it contributes to high pigment accumulation and biomass productivity.

No significant changes were observed in the chlorophyll-b concentrations in the assays, a fact that may indicate that the pathways specifically involved in chlorophyll-b synthesis are less sensitive to MF-induced changes. It is worth noting that chlorophyll-b synthesis is regulated differently from chlorophyll-a. According to Porra et al. [50], chlorophyll-a is synthesized directly through the main metabolic pathways, whereas chlorophyll-b may be reconverted into chlorophyll-a by specific enzymes, such as chlorophyllide-a oxygenase, enabling flexible adjustment of the chlorophyll a:b ratio in response to environmental conditions.

On the other hand, carotenoid biosynthesis by *D. salina* was more responsive to MF exposure, since carotenoids, especially β-carotene, protect cells against oxidative damage. The highest concentration was 3.56 mg L^{-1}, which was 95% higher than the one of the control (1.84 mg L^{-1}). Similar results have been reported by Serrano et al. [51], who investigated the effects of MF exposure (200 mT) on two microalgal species: *Scenedesmus obliquus* and *Nannochloropsis gaditana*. In the case of *S. obliquus*, MF exposure for 48 h led to a 40% increase in carotenoid concentration. According to the authors, MFs promoted a metabolic shift towards the biosynthesis of antioxidant pigments as part of the adaptive mechanisms of cells. Asundi et al. [52] also evaluated the impact of MF exposure (0.5 mT) on carotenoid production by *Chlorella vulgaris* and observed a 20.4% increase in β-carotene concentration after 15 days of cultivation.

Building upon these findings, it is important to examine not only metabolic distinctions between the biosynthesis of chlorophylls-a and b and carotenoids but also the potential effects of MFs on these processes. According to Wu et al. [53], chlorophyll-a and chlorophyll-b are synthesized by *D. salina* from common precursors through the aminolevulinic acid (ALA) biosynthetic pathway. ALA is converted into protoporphyrin IX, which then coordinates with magnesium ions (Mg^{2+}) to form chlorophyll. Carotenoid biosynthesis occurs via mevalonate and methylerythritol phosphate (MEP) pathways, which produce isoprenoid precursors that are essential for carotenoid formation. Despite these distinct biosynthetic routes, MF exposure appears to modulate both pathways, as indicated by the increase in chlorophyll-a and carotenoid concentrations found in MF30-24 h.

Previous reports have also shown that MFs may influence enzymatic activity and gene expression involved in pigment biosynthesis [23,54]. The hypothesis proposed by Yang et al. [23] suggests that MFs enhance photosynthetic electron transport by modulating ion mobility and altering electron spin states, thereby affecting the energy levels associated with photochemical reactions. These MF-induced changes may not only increase photosynthetic

efficiency but also activate regulatory mechanisms that govern the synthesis of chlorophylls and carotenoids.

Moreover, MFs may alter plasma membrane permeability, as previously discussed, thereby facilitating absorption of essential nutrients for pigment formation, such as magnesium, a key component of chlorophyll. By increasing nutrient uptake, MFs may indirectly promote pigment synthesis by increasing the availability of precursors. As a result, regulatory pathways involved in pigment synthesis may be affected by MFs in different ways; certain metabolic pathways are preferentially activated depending on the type of pigment, a fact that may explain the changes found in the chlorophyll-a and carotenoid concentrations.

A study conducted by Geacintov et al. [54] showed that 14.5 mT may also affect the fluorescence of photosynthetic pigments in microalgae. The study highlights significant effects of MFs on the fluorescence of chlorophyll-a produced by *Chlorella pyrenoidosa.* An increase in fluorescence of 4–9% was observed when the excitation light beam was aligned parallel to MFs, while a decrease of 4–9% occurred when it was perpendicular. The authors associated this phenomenon with the reorientation of pigment molecules in response to MFs, which alter the dynamics of energy transfer within the photosynthetic apparatus.

Therefore, MFs may induce substantial changes in the photosynthetic process and influence the overall efficiency of energy capture and utilization in microalgae. These modifications, induced by MFs, might alter the metabolism of microalgae under study. Exposure to 30 mT (24 h d^{-1}) effectively increased the production of carotenoids and chlorophyll-a. The increase in pigment production is particularly relevant considering that *D. salina* has been widely cultivated for the commercialization of these valuable compounds. Thus, the ability of MFs to enhance the synthesis of photosynthetic pigments in microalgae represents a promising strategy for improving the industrial-scale production of bioactive compounds.

3.3. Influence of Magnetic Field (MF) on Biochemical Composition

MF exposure exhibited distinct effects on macromolecules (Table 3). The protein content ranged from 39.90% to 44.12%, with no statistically significant difference observed between the conditions ($p < 0.05$). This shows that the MF application conditions under evaluation did not alter protein synthesis. These findings are consistent with those reported by Sui et al. [6], who noted that, in the case of *D. salina,* the protein content in biomass may range from 40% to 80% of the ash-free dry weight.

Table 3. Biochemical composition of *D. salina* under different exposure times and MF intensities (30 mT and 60 mT) and control cultivation.

Assay	Carbohydrates (%, w w^{-1})	Protein (%, w w^{-1})	Lipids (%, w w^{-1})
Control	31.15 ± 0.59 ^{a}	44.12 ± 1.10 ^{a}	15.23 ± 1.01 ^{c}
MF30-1 h	23.73 ± 0.98 ^{b}	39.90 ± 2.60 ^{a}	22.19 ± 0.57 ^{a}
MF30-24 h	30.92 ± 1.10 ^{a}	43.65 ± 1.99 ^{a}	15.18 ± 0.88 ^{c}
MF60-1 h	25.80 ± 0.43 ^{b}	40.93 ± 1.71 ^{a}	18.77 ± 0.53 ^{b}
MF60-24 h	29.22 ± 1.04 ^{a}	40.41 ± 1.45 ^{a}	16.86 ± 1.07 ^{c}

MF: Magnetic Field; Different letters (^{a,b,c}) in the same column indicate that the means were significantly different at 95% confidence level ($p < 0.05$).

No significant changes were observed in the carbohydrate contents at 30 mT and 60 mT for 1 h d^{-1} (30.92% and 29.22%, respectively) in comparison with the control ($p > 0.05$). However, prolonged exposure (24 h d^{-1}) decreased the carbohydrate contents to 23.73% (30 mT) and 25.80% (60 mT). The decrease in the carbohydrate content under prolonged exposure may suggest enhancement in the utilization or conversion of carbohydrates into

other biochemical components, since these assays achieved lipid contents of 22.19% and 18.77%, respectively, and were significantly different from the control (15.23%).

Thus, prolonged MF exposure may promote lipid biosynthesis or alter lipid accumulation in *D. salina*. Moreover, the condition that resulted in the highest lipid concentration (MF30-24 h) also had the highest volumetric carotenoid concentration (3.56 mg L^{-1}) and biomass production (0.55 g L^{-1}). Therefore, this is the best condition to produce biomass enriched with biocompounds of industrial interest.

These results are in accordance with Chu et al. [55], who evaluated the effects of MF intensity on lipid production by *Nannochloropsis oculate* and observed that 20 mT applied for 24 h d^{-1} was sufficient to achieve the maximum lipid concentration of 216 mg L^{-1}. However, when MF intensity increased from 30 mT to 40 mT, the lipid concentration decreased, indicating that higher intensities inhibited lipid production.

Although there is evidence that MFs may alter biomass composition, their effect may depend on external factors. Hunt et al. [56] reported that the impact of MF exposure may vary, leading to stimulation, inhibition or null, depending on certain factors, such as intensity, frequency and duration of exposure to MFs. For example, Costa et al. [30] reported that MF stress may redirect the metabolism of microalgae to produce more lipids than carbohydrates. The authors investigated the influence of MF applications (15, 30 and 60 mT) for 1 h d^{-1} and 24 h d^{-1} on lipid synthesis by *Chlorella homosphaera*. The results were different from the ones of this study, since application of 30 mT for 1 h d^{-1} stimulated lipid production, with increases of up to 135.1% in lipid productivity (30 mT, 1 h d^{-1}) and biomass by 20.6% in comparison with the control culture.

Font et al. [57] explained how this phenomenon may be associated with the common adaptive response to the environment since, under adverse conditions, lipid production allows cells to create an efficient energy reserve, increasing their resistance to oxidative stress and preserving cellular integrity. This redirection involves changes in the metabolic pathway, where the activity of enzymes that promote carbohydrate synthesis, such as glucose-6-phosphate isomerase, may be reduced, while the fatty acid synthesis pathway is activated, promoting the conversion of metabolic precursors into lipids, rather than carbohydrates.

Therefore, high lipid content observed in MF30-24 h indicates a metabolic shift favoring lipid biosynthesis over carbohydrate accumulation, possibly as a cellular adaptation to environmental stress. Moreover, MFs have the potential to enhance biomass composition for biotechnological applications by promoting lipid and carotenoid production. Additionally, it may be explored as a strategy for producing lipids with carotenogenic properties.

3.4. Evaluation of Microalga Cell Morphology by Scanning Electron Microscopy (SEM)

Considering that MF30-24 h was the best of all the conditions under evaluation, Scanning Electron Microscopy (SEM) images of *D. salina* were taken to evaluate whether the MFs modified the cell structures (Figure 4). The control assay (Figure 4A,B) showed cells with more defined and uniform structures, potentially indicating a more stable state. The cell surface appears relatively homogeneous without many signs of damage or structural alterations. On the other hand, the MF assay (Figure 4C,D) shows structures with higher porosity and changes in the aggregate format (more irregular), which suggest changes in the structure or integrity of the cell surface.

Therefore, the effects of MFs were not limited to changes in metabolic processes, such as high production of key metabolites (carotenoids, chlorophyll-a and lipids), as previously discussed, but also extended to alterations in cell morphology, as shown by the SEM images. The higher porosity and irregularity of the cells observed in the MF assays may be related to a structural reorganization of the cell wall or plasma membrane, which could facilitate the transport of ions and metabolites, modifying internal metabolic processes.

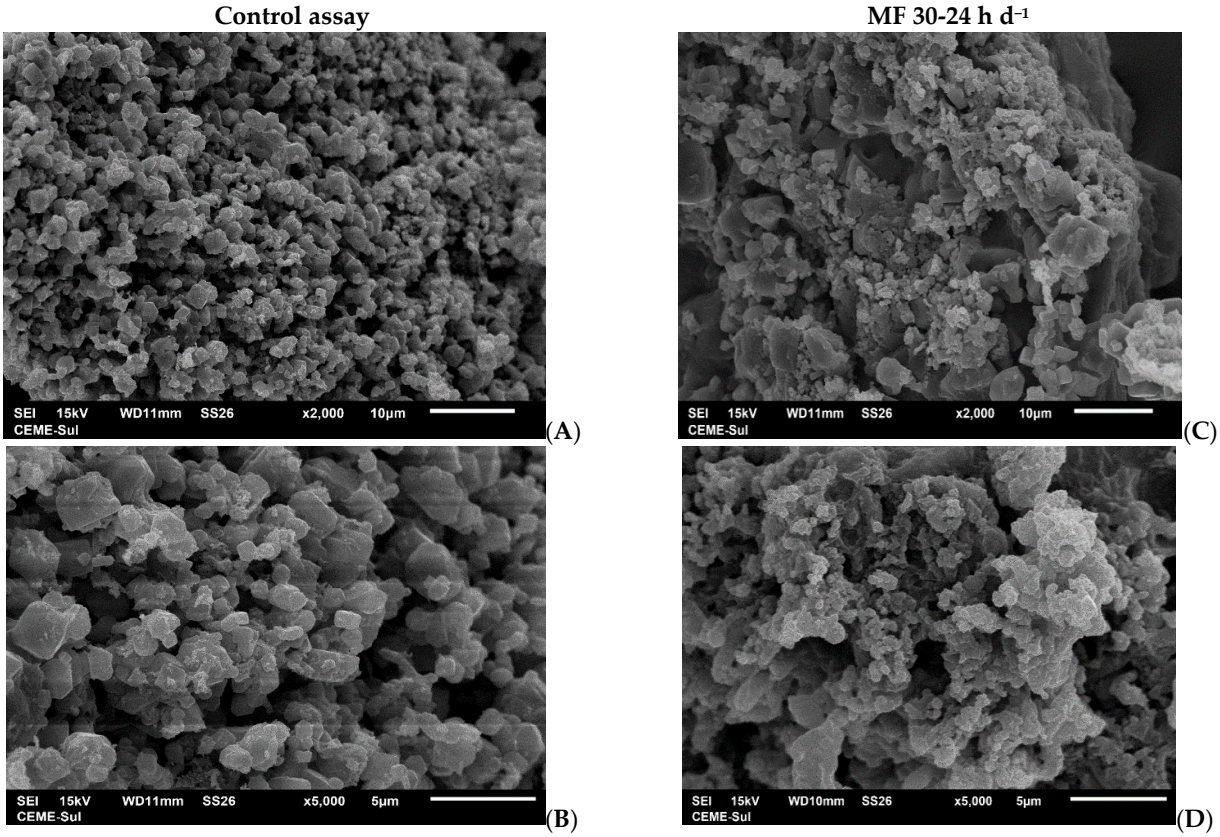


Figure 4. SEM images of *D. salina* in 2k and 5k magnitudes, where (**A**) is control assay 2k; (**B**) is control assay 5k; (**C**) is MF 30 (24 h d^{-1}) 2k; and (**D**) is MF 30 (24 h d^{-1}) 2k.

3.5. D. salina Exposed to Magnetic Field (MF): Applications of Biomass and Associated Technological Challenges

D. salina cultivation under MF exposure significantly changed the centesimal composition of the biomass. The results of this study indicate that both MF intensity and exposure time modulate distinct metabolic pathways in *D. salina*. Although no statistical differences were observed between MF30–24 h and MF60–24 h regarding the biomass, chlorophyll-b, proteins and carbohydrates, other compounds (carotenoids, chlorophyll-a and lipids) showed significant variation, depending on the intensity.

Thus, interaction between MF intensity and exposure time is a significant factor in the formation of the biochemical profile of biomass and, consequently, its suitability for biotechnological applications, since *D. salina* biomass may be integrated into a biorefinery approach, enabling extraction not only of carotenoids but also of a wide range of valuable biocompounds. The potential of pigment production under MF exposure exemplifies how blue biotechnology may contribute to circular economy [58]. This approach aligns sustainability principles by utilizing a non-toxic, emerging technology to generate high-value products while minimizing environmental impact [57].

Although MF30 mT-24 h promoted significant increases in the production of carotenoids, chlorophyll-a and lipids, its application on an industrial scale still poses considerable technical and economic challenges. They include the need to integrate magnetic systems into photosynthetic bioreactors, ensure energy efficiency and guarantee scalability of the process [32]. Other technical limitations, according to Sincak et al. [59], are related to the fact that biological systems often exhibit nonlinear behavior. Small changes in specific parameters may cause disproportionately large impacts on the overall performance of the system, making it difficult to predict how the process will respond to changes

in MF exposure or other environmental conditions. It further increases the difficulty in maintaining uniform and stable MFs in large culture volumes.

Moreover, these challenges may increase the cost and complexity of adapting existing infrastructure and create potential problems related to durability and maintenance of permanent magnets. Despite significant advances in biological research, many aspects of the exact physiological and genetic responses of microalgae to MF exposure or other environmental variations remain unclear; it creates additional obstacles to the precise design and control of MF-assisted cultivation processes [32,59].

To date, no studies that directly quantify the technical and economic feasibility of applying MFs to microalgae production systems have been found. However, a recent study conducted by Xu et al. [60] performed a life cycle assessment and cost analysis of cultivation of three microalga species (*Chlorella protothecoides*, *Scenedesmus obliquus* and *Micractinium* sp.) with and without MF application (80 mT day^{-1} for 17 days). The authors reported not only the costs related to cultivation but also environmental impacts in 11 categories, such as global warming potential (GWP), ozone depletion potential (ODP) and acidification (AC). Concerning *S. obliquus*, all the indicators showed reductions under MF treatment, indicating a net environmental benefit. In contrast, C. *protothecoides* showed improvements in GWP, abiotic destruction (AD) and AC, but decrease in ODP, photochemical oxidation and terrestrial ecotoxicity scores, mainly due to limited productivity gains under MFs (from 0.090 to only 0.114 g L^{-1} day^{-1}). Thus, according to the authors, the higher the increase in productivity enabled by MFs, the lower its proportional environmental impact on an industrial scale.

Therefore, *D. salina* has become a promising potential source for MF-assisted cultivation, especially considering the high market value and wide applicability of its bioactive compounds. Since *Dunaliella* is one of the few microalgae with the Generally Recognized as Safe (GRAS) status from the Food and Drug Administration, it is essential to evaluate the potential applications of its biocompounds [61]. Its biomass may be used as an additive in food, cosmetics and pharmaceutical industries due to its lipophilic (carotenoids and α-tocopherol) and hydrophilic (glutathione and ascorbic acid) biomolecules. Since it is naturally red–orange, it has been widely used as a natural colorant, giving orange and yellow tones to products, such as juices, soups and sauces, and is often added to food and supplements as a nutrient [14,18,62]. It also has antioxidant properties that may help protect the skin from damage caused by free radicals [63]. For this reason, many types of tumors, including breast, cervical, ovarian and colorectal ones, have been treated with β-carotene [64,65]. These compounds contribute to the formulation of functional products that promote skin health and help prevent diseases related to cellular aging [66].

Lipids extracted from *D. salina* are highly valued in the production of biofuels, including biodiesel. They generate sustainable energy, help reduce dependence on fossil fuels and minimize environmental impacts. In addition, lipid extracts have antibacterial and anti-adherent properties and have been studied for the prevention of infections caused by pathogenic microorganisms [67].

Regarding the biochemical composition of *D. salina*, high levels of carbohydrates and proteins were observed. Although no statistically significant differences were observed in the conditions under investigation regarding protein content, these compounds, at high quantities (around 40%), still indicate their potential for biotechnological applications.

According to Darvish et al. [68], proteins in *D. salina* possess high nutritional value and have been widely explored by food supplement and nutraceutical industries. Proteins contain hydrophilic antioxidants, such as glutathione and ascorbic acid, which provide beneficial health properties. Consequently, these compounds are incorporated into products that aim at improving immunity and protecting against oxidative stress.

Carbohydrates also enhance the industrial value of *D. salina*. Arroussi et al. [69] reported that carbohydrates in *D. salina* biomass may be used for producing biogas, a renewable energy source that results from converting carbon into methane (CH_4). This process is particularly efficient due to the absence of a rigid cell wall, which enables anaerobic digestion of biomass.

Beyond the extractable biochemical fractions, the residual biomass—represented by the post-cultivation pellet—may retain significant levels of protein content and other nutrients, enabling it to be used by the feed industry. Repurposing this byproduct may reduce waste and enhance overall sustainability of the process. Thus, MF application to *D. salina* cultivation emerges as a promising strategy for optimizing biomass composition and guides its production toward high-value bioproducts. Establishing optimal MF exposure conditions may further maximize the synthesis of target compounds and reinforce this approach as a viable alternative for various biotechnological applications. This approach aligns sustainability principles by utilizing a non-toxic, emerging technology to generate high-value products while minimizing environmental impact.

4. Conclusions

MFs have significant impact on growth and biochemical composition of the microalga *D. salina*. Continuous exposure to 30 mT (MF30-24 h) was the condition that produced the best results, since it increased the biomass concentration by 41% (0.55 g L^{-1}), carotenoids by 95% (3.56 mg L^{-1}), chlorophyll-a by 118% (2.62 mg L^{-1}) and lipids (7%), in comparison with the control. SEM revealed distinct morphological changes under MF exposure. Cells cultivated under MF30-24 h showed high surface porosity and more irregular aggregation, suggesting modifications in the cell wall.

Therefore, MFs may influence microalgal physiology not only by modulating biochemical pathways but also by inducing morphological adaptations. It should also be highlighted that this study applied static MFs with fixed orientation and intensity. However, the biological response of microalgae to magnetic stimulation is known to be strongly dependent on the characteristics of the field.

From the biorefinery perspective, these results show the potential of MFs as a sustainable strategy for enhancing production of high-value compounds, such as pigments, lipids, proteins and carbohydrates. These compounds may be fractionated and applied to different industrial sectors, such as food, cosmetics, pharmaceuticals and bioenergy, thus supporting the development of integrated, circular and economically viable microalgal biorefineries.

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