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RESEARCH PAPER

Magnetic fields exhibit a positive impact on lipid and biomass yield during phototrophic cultivation of *Spirulina* sp.

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Abstract

This study aimed to investigate the effects of magnetic field (MF) application (1, 12 and 24 h day $^{-1}$) to *Spirulina* sp. LEB 18 in different photosynthesis cycles (dark and/or light) during short (15 days) and long periods (50 days) of cultivation. MF application was performed via two sources: ferrite magnets and solenoids. At the end of cultivation, the biomass was characterized in terms of lipids, proteins, and carbohydrates. In the 15 day cultures, the highest maximum biomass concentrations (2.06 g L^{-1} and 1.83 g L^{-1}) were observed when 30 mT was applied for 24 h day $^{-1}$ or 12 h day $^{-1}$ (on the light cycle), respectively. MF application throughout cultivation (24 h day $^{-1}$) for more than 30 days is not recommended. In all conditions, there was an increase in the lipid concentration (from 14 to 45%). The protein profile suggested important changes in photosystems I and II due to MF application. Cell morphology was not altered by MF application. In conclusion, the effects on the metabolism of *Spirulina* sp. are directly related to the photosynthesis cycle and time period in which the MF was applied.

Keywords Ferrite magnets · Cyanobacteria · Solenoid · Microalgae biomass

Introduction

Microalgae are of great importance in various trophic chains since they are photosynthetic microorganisms capable of converting solar energy and atmospheric carbon dioxide into various forms of energy, biomass and oxygen [1]. These organisms are widely studied for biotechnological application because of molecules they synthesize during development and of their high growth rates and high capacity for development in well-defined synthetic media [2].

Changes in culture conditions are an important strategy for modifying metabolic pathways to obtain high biomass yield and induce the synthesis of biomolecules of high commercial interest, thereby contributing to biomass valorization [3]. *Chlorella* and *Spirulina* microalgae are among the most studied biotechnologically microalgae genera and have GRAS (generally recognized as safe) certification [4], and the use of biomass as food is allowed and exhibits no health risks, as long as it is handled properly [5].

Magnetic field (MF) application in algae, microalgae and cyanobacteria may affect metabolism and influence the synthesis of biomass [6–8], carbohydrates, pigments [9], essential amino acids, enzymes, and O_2 release [10, 11]. MF application may be achieved via several sources; among them, the application of permanent magnets coupled directly to the photobioreactor or by cell recirculation is noteworthy [12]. Studies have exposed microalgae cultures to MF for a short time, for example, 1 h day $^{-1}$ during the light photosynthesis cycle [6, 7, 13–15] or during the entire cultivation (24 h day $^{-1}$ during the light and dark cycles) [6, 7, 13–16]. In addition, *Spirulina* cultures usually have cultivation times of 10-day [14, 17] or 15-day [7, 13, 16], but sometimes the cultures did not reach the stationary phase, so it is important to evaluate MF application to *Spirulina* cultures with longer cultivation times.

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Photosynthetic metabolism is driven by a series of reactions that vary according to the period of exposure to light. Thus, it is extremely important to assess the effects of exposure to MF during light and/or dark cycles to identify whether the electrostatic variations that occur during the two cycles due to the effect of the MF application can provide new metabolic responses. Therefore, this study aimed to investigate the effects of MF application to *Spirulina* sp. LEB 18 during the light and/or dark photosynthesis cycles and evaluated this effect during short and long periods of cultivation.

Material and methods

Microorganism and culture medium

Spirulina sp. LEB 18 was isolated from the Mangueira Lagoon located in Santa Vitória do Palmar, RS, Brazil (latitude 33°31′ 08'' S and longitude 53°22′ 05'' W) [18]. This strain is from the Culture Collection of the Laboratory of Biochemical Engineering at the Federal University of Rio Grande (Rio Grande, RS, Brazil). Assays were performed in Zarrouk medium, as described by Costa et al. [19].

Magnetic fields application to cultures

Cultures were carried out in a 2 L vertical tubular photobioreactor (VTP) with 90% of the volume filled [20] and an initial biomass concentration of 0.2 g L^{-1}. Cultures were maintained in a growth chamber at 30 °C, illuminance of 60 $\mu mol_{photons}$ m^{-2} s^{-1} and a photoperiod of 12 h of light and 12 h of dark for 15 or 50 days of cultivation. The evaporation of water in cultures was compensated for by the daily replacement of sterile distilled water.

MF application was performed via two sources: ferrite magnets and solenoids. The ferrite magnets were arranged 180° from each other on walls of the VTP and 150 mm from the base [7]. The permanent magnets, with an area of 750 mm^2 each, generated a nonhomogeneous MF, with an average intensity of 30 mT. To identify which photosynthesis cycle the MF would stimulate or inhibit of the responses evaluated, the assays with ferrite magnets were performed for 15 days under the conditions shown in Table 1: control culture (CC) without MF application, and cultures with MF application for 12 h day^{-1} in the light cycle (L12), 12 h day^{-1} in the dark cycle (D12), 1 h day^{-1} at the beginning of the light cycle (L1), 1 h day^{-1} at the beginning of the dark cycle (D1) and 24 h day^{-1} during the light and dark cycles (LD).

In addition, LD_{50} assays was performed, where the cultivation time was 50 days and ferrite magnets were applied to the culture with 24 h in the light and dark cycles and its respective control culture (CC50).

Table 1 Source and period of magnetic fields (MF) application and cultivation time for cultures of *Spirulina* sp. LEB 18

Assay	Source	Time period of application	Cultivation time (day)
CC	–	–	15
L12	Ferrite magnet	Light cycle (12 h day^{-1})	15
D12	Ferrite magnet	Dark cycle (12 h day^{-1})	15
LD	Ferrite magnet	Light and dark cycle (24 h day^{-1})	15
L1	Ferrite magnet	Light cycle (1 h day^{-1})	15
D1	Ferrite magnet	Dark cycle (1 h day^{-1})	15
S	Solenoid	Light cycle (1 h day^{-1})	15
CC50	–	–	50
LD50	Ferrite magnet	Light and dark cycle (24 h day^{-1})	50

Solenoid application (S assay, Table 1) was performed using a 100 turn enameled copper wire coupled around the outside of the VTP. The ends of the solenoid conductor wires were connected to the power supplies (Impac, IP1520A, Brazil). A uniform 6 mT was applied in the perpendicular direction to the straight section in the solenoid. MF intensity was measured by a teslameter (Global Mag, TLMP-HALL 05 k, Brazil). In this assay, *Spirulina* was exposed for 1 h day^{-1} in a light cycle.

CCs were made under the same culture conditions used in the assays with MF application, where the magnets or solenoids were replaced by inert material.

At the end of culture, the biomass was separated by centrifugation (Hitachi Himac CR-GIII, Japan) at 16,000×*g* for 30 min at 10 °C, resuspended in distilled water and centrifuged again under the same conditions to improve nutrient removal. The recovered biomass was frozen for 48 h at − 80 °C and lyophilized (Labconco, USA) to be used in carbohydrate, protein, lipid and antioxidant activity determinations.

Analytical determinations

Biomass concentration

The biomass concentration (X g L^{-1}) was determined daily at 670 nm by a UV–Vis spectrophotometer (SHIMADZU UV MINI 1240, Japan), and the optical density was compared to a standard curve of *Spirulina* sp. LEB 18.

Determination of carbohydrate, protein and lipid concentrations

To determine the protein and carbohydrate concentrations, 10 mg of lyophilized biomass and 20 mL of distilled

water were mixed, and the proteins and carbohydrates were extracted using ultrasonic-assisted extraction (Cole–Parmer, CPX 130, USA) for 10 min in cycles of 59 s (59 s bound and 59 s off). Protein content was determined by the colorimetric method according to Lowry et al. [21] with a standard bovine serum albumin curve. Carbohydrate content was determined by the phenol–sulfuric method developed by DuBois et al. [22] with a standard glucose curve. Lipid content was determined by the method proposed by Folch et al. [23].

Determination of chlorophyll-a concentration and antioxidant activities

For chlorophyll-a determination, 1.5 mL of culture was centrifugated (2000×*g*, 10 min), and the precipitated was resuspended in methyl alcohol, vortexed for 30 s, and ultrasonicated for 20 min. The samples were maintained in the dark at 4 °C for 24 h. Afterwards, the absorbance was measured at 652.4 and 665.2 nm according to Lichthenthale [24], and the chlorophyll-a concentration was calculated applying Eq. 1:

$$\text{Chlorophyll - a (mg L}^{-1}) = 16.72\ (\text{Abs}_{665.2}) - 9.16\ (\text{Abs}_{652.4}). \quad (1)$$

The antioxidant activities were evaluated by reducing power, DPPH (2,2–diphenyl–1–picrylhydrazyl) and ABTS (2,2′–azino–bis(3–ethylbenzothiazoline–6–sulfonic acid) diammonium salt) methods using methanolic extract composed of 25 mg of lyophilized biomass and 10 mL of methyl alcohol. The methanolic extract was maintained at − 4 °C for 24 h.

The 0.25 mL of methanolic extract (2.5 mg mL $^{-1}$), 2 mL of sodium phosphate buffer (0.2 mol L $^{-1}$, pH 6.6) and 2 mL of potassium ferrocyanide ($C_6N_6FeK_4$, 1% w v $^{-1}$) were homogenized to determine the reducing power. The mixture was incubated at 50 °C (water bath) for 20 min, and 2 mL of trichloroacetic acid (10% w v $^{-1}$) was added. Samples of 2 mL were taken and mixed with 2 mL of distilled water and 0.4 mL of ferric chloride ($FeCl_3$, 0.1% m v $^{-1}$). After 10 min, the absorbance was measured at 700 nm in a spectrophotometer [25].

DPPH was quantified according to Rufino et al. [26] with modifications. Then, 0.1 mL aliquots of methanolic extract (2.5 mg mL $^{-1}$) and 3.9 mL of DPPH (0.06 mmol L $^{-1}$) in methyl alcohol were maintained in the dark for 1 h. The control solution contained 0.1 mL of methanol (CH_3OH, 99% m v $^{-1}$) replacing the methanolic extract. Absorbances were measured at 517 nm in a spectrophotometer [27]. The percentage of inhibition to the DPPH radical was determined according to Eq. 2:

$$\text{Inhbition}\,(\%) = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100. \quad (2)$$

The capability of the ABTS free radical was quantified according to Rufino [28] with modifications. A stock solution of ABTS (7 mmol L $^{-1}$) was prepared. Radical cations ($ABTS^+$) were prepared from the reaction of 5 mL of the stock solution of ABTS with 88 µL of potassium persulfate solution ($K_2S_2O_8$, 140 mmol L $^{-1}$), and the mixture was allowed to stand in the dark at room temperature for 16 h. A sample of this mixture (1 mL) was diluted in ethanol until absorbance of 0.7 at 734 nm. In the dark environment, 30 µL of sample (2.5 mg mL $^{-1}$) was transferred into test tubes with 3.0 mL of the $ABTS^+$ radical. Absorbances were determined in a spectrophotometer after 6 min of reaction. Equation 2 was used to calculate the antioxidant activity.

Electrophoresis

A sample of 0.01 g of lyophilized biomass was resuspended in buffer [1 mol L $^{-1}$ tris/HCl, pH 6.8, 8% m v $^{-1}$ sodium dodecyl sulfate (SDS), 30% (v v $^{-1}$) glycerol, 1% (w v $^{-1}$) purple M-cresol and 0.4 mol L^{-1} 2–mercaptoethanol] and heated at 100 °C for 5 min for electrophoresis. The liquid protein extract was then used to run electrophoresis after centrifugation at 10,000×*g* for 1 min. The gel was formulated according to Laemmli [29] with 5% acrylamide gel and 12.5% acrylamide separation gel. The wells of the gel were loaded with 100 µL of protein extract and then subjected to discontinuous SDS-PAGE.

Kinetic parameters

Kinetic parameters evaluated were maximum biomass concentration (X_{max} g L $^{-1}$), maximum biomass productivity ($P_{max} = (X - X_0)/(t - t_0)$ g L $^{-1}$ day $^{-1}$) and maximum specific growth rate (μ_{max}, day $^{-1}$) obtained by exponential regression applied to the logarithmic growth cycle (g L^{-1}) at time t (day), and X_0 is the biomass concentration (g L^{-1}) at time t_0 (day). The doubling time (tg) was determined in the exponential growth cycle according to the equation ($tg = \ln(2)/\mu_{max}$ day).

The efficiency of MF application ($\eta = (C_{MF} - C_{cc})/C_{cc} \times 100$) corresponds to the difference between responses with MF application (C_{MF}) and control cultures (C_{cc}) [6].

Cell morphology

To identify possible morphological changes caused by the action of MF, samples of cells from different experiments were submitted to scanning electron microscopy under a microscope (Jeol, JSM-6610LV, Japan).

Statistical analysis

The responses from cultures with MF application and control cultivation were evaluated using analysis of variance (ANOVA) followed by Tukey's test, with a confidence interval of 95% ($p < 0.05$).

Results and discussion

Biomass concentration

Microalgae remained in the lag phase until the third day of cultivation in CC, L12 (light cycle, 12 h day $^{-1}$) and D12 (dark cycle, 12 h day $^{-1}$) (Fig. 1a). This fact can be directly related to the stress caused by the MF application because microalgae were adapted only to the culture medium. However, in the LD assay (light and dark cycle, 24 h day $^{-1}$), *Spirulina* sp. was exposed to the same MF intensity and had no lag phase. Therefore, microalgae are easily adapted to continuous MF exposure. The trend lines in Fig. 1 represent the fourth-order polynomial curve fittings for each treatment.

In all assays (Fig. 1a), the maximum biomass concentration (X_{max}) was obtained at 15 days of culture, i.e., LD (2.06 ± 0.03 g L^{-1}) and L12 (1.83 ± 0.04 g L^{-1}) (Table 2). According to Duarte et al. [30], Rosa et al. [31] and Vaz et al. [32], X_{max} of *Spirulina* sp. LEB 18 varies from 0.89 to 1.32 g L^{-1}.

MF influenced biomass production more effectively when applied to the light photosynthesis cycle (L12) than when MF was applied to the dark photosynthesis cycle (D12) (Table 2, Fig. 1a). According to Chibowski et al. [33], MF effects remain even after the end of MF application. This fact was evidenced in L12, in which MF was applied only in the light cycle, and biomass concentration was not significantly different from that of LD (Table 2), in which MF application

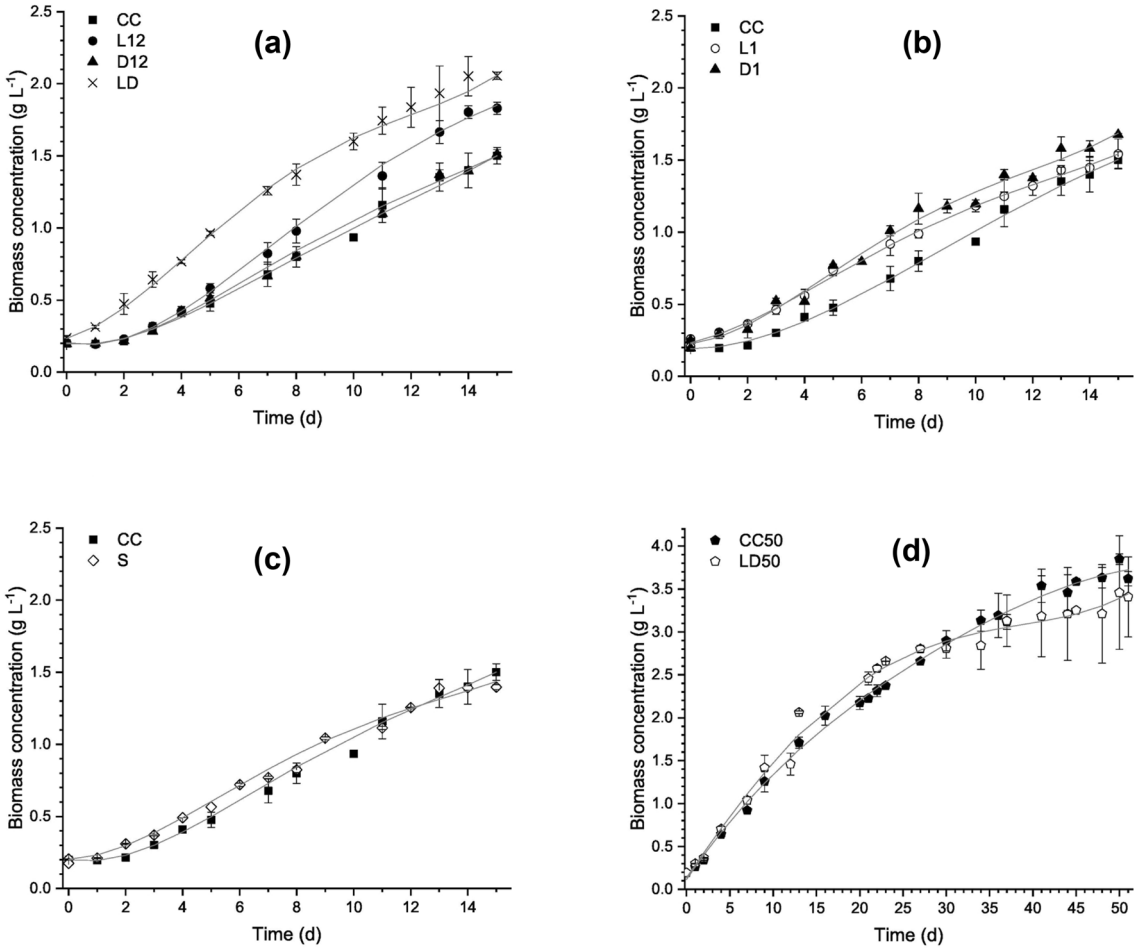

Fig. 1 Biomass concentration of *Spirulina* sp. LEB 18. **a** CC (control culture); L12 (light cycle, 12 h day^{-1}); D12 (dark cycle, 12 h day^{-1}) and LD (light and dark cycle, 24 h day^{-1}). **b** CC (control culture); L1 (light cycle, 1 h day^{-1}) and D1 (dark cycle, 1 h day^{-1}); **c** CC (control culture) and S (MF application with solenoid for 1 h day^{-1} in the light cycle); **d** CC50 (control culture) and LD50 (cultures for 50 day at 30 mT for 24 h day^{-1})

Table 2 Kinetic parameters of growth of *Spirulina* sp. LEB 18

Assays	X_{max} (g L^{-1})	P_{max} (g L^{-1} day^{-1})	μ_{max} (day^{-1})	t_g (day)
CC	1.50 ± 0.06^{c}	0.09 ± 0.01^{a}	0.13 ± 0.01^{c}	5.33 ± 0.52^{ab}
L12	1.83 ± 0.04^{ab}	0.09 ± 0.02^{a}	$0.25 \leq 0.01^{a}$	$2.82 \leq 0.01^{c}$
D12	1.51 ± 0.03^{c}	$0.09 \leq 0.01^{a}$	$0.12 \leq 0.01^{c}$	5.81 ± 0.16^{ab}
LD	2.06 ± 0.03^{a}	0.12 ± 0.03^{a}	$0.16 \leq 0.01^{bc}$	4.32 ± 0.03^{abc}
L1	1.55 ± 0.11^{c}	0.10 ± 0.01^{a}	0.14 ± 0.01^{c}	4.94 ± 0.41^{abc}
D1	1.68 ± 0.01^{b}	0.11 ± 0.04^{a}	0.11 ± 0.02^{c}	6.20 ± 0.82^{a}
S	1.40 ± 0.02^{c}	$0.13 \leq 0.01^{a}$	$0.20 \leq 0.01^{b}$	3.54 ± 0.02^{ab}
CC50	3.90 ± 0.09^{A}	$0.13 \leq 0.01^{A}$	0.15 ± 0.01^{A}	4.67 ± 0.22^{A}
LD50	3.64 ± 0.25^{A}	$0.15 \leq 0.01^{A}$	$0.15 \leq 0.01^{A}$	4.70 ± 0.08^{A}

CC control culture, assays with ferrite magnet (30 mT) for 15 day; *L12* 12 h day^{-1}, light phase, *D12* 12 h day^{-1}, dark phase, *LD* 24 h day^{-1}, light and dark phase, *L1* 1 h day^{-1}, light phase, *D1* 1 h day^{-1}, dark phase; assays with solenoid (6 mT) for 15 day: S (1 h day^{-1}, in light phase); *CC50* culture control for 50 day; and assay with ferrite magnet 30 mT for 50 day: *LD50* 24 h day^{-1}, light and dark phase

Equal lowercase letters indicate that there are no significant differences on a column at 95% confidence by Tukey's test ($p > 0.05$)

Equal capital letters indicate that there are no significant differences on a column at 95% confidence interval by t test ($p > 0.05$)

was performed throughout cultivation (in the two photosynthesis cycles). According to Zhiyong et al. [34], adequate magnetic forces may alter the absorption of nutrients and cause changes in the rate of photosynthesis reactions. Thus, the increase in biomass yield may be correlated with the possible increase in nutrient absorption, mainly in media with metallic elements.

The μ_{max} at L12 (0.25 day^{-1}) was 92% higher than that at CC (0.13 day^{-1}) (Table 2). Hirano et al. [8] applied MF from 0.05 to 70 mT for 24 h day^{-1} on *Spirulina platensis* IAM M-135 autotrophic cultures. The increase in MF from 0.05 to 10 mT increased μ_{max} from 0.14 to 0.22 day^{-1}. These authors suggested that MF can accelerate the light excitation of chlorophyll, production of radical pairs, reactions of electron transfer and the conversion of light energy to chemical energy.

The effect of MF application for short periods was evaluated in L1 and D1, in which the microalga was exposed for 1 h day^{-1} in the light and dark cycles, respectively (Fig. 1b). The biomass concentration was higher than that of the control since the first day, and unlike what occurred with exposure to MF for 12 h day^{-1}, high microalgal growth occurred when exposed to the dark photosynthesis cycle (Fig. 1b, Table 2). The maximum biomass concentration (1.68 g L^{-1}) in D1 was significantly different ($p < 0.05$) from those in CC and L1. The other parameters evaluated did not differ statistically among these conditions (Table 2). Exposure to 30 mT for 1 h day^{-1} (L1) started in the first hour of the light cycle; therefore, there were still traces of the dark cycle. In this phase, cells alter their metabolic pathways, electron transport chain systems and some coenzyme-dependent metal cofactors are being produced again to keep the metabolism functioning in this new condition (light cycle). This fact influences the interaction between the MF and object, especially when the phenomenon occurs by ferromagnetic induction. In D1 at 30 mT applied for 1 h day^{-1} in the first hour of the dark cycle, the same behavior was not observed, since all previously mentioned molecules are theoretically already available, allowing the direct interaction of MF.

Different MF intensities were investigated by Li et al. [35] in a 3.5 L air-lift photobioreactor used for *Spirulina* sp. cultivation. The authors concluded that in 15 day assays, intensities up to 40 mT stimulated growth, but when intensities above 40 mT were evaluated, there was inhibition in microalgal growth. Similar behavior was obtained at 30 mT for 12 h day^{-1} or 24 h day^{-1} assays, i.e., all kinetic parameters were higher than those of the control.

Unlike what was shown in this study, Deamici et al. [7] exposed *Spirulina* sp. LEB 18 to 30 mT for 1 h day^{-1} in the light cycle, and an increase in X_{max} (2.37 g L^{-1}) was observed when compared with control culture (X_{max} of 1.22 g L^{-1}); however, when it was exposed for 24 h day^{-1}, the microalgae growth (X_{max} of 1.67 g L^{-1}) was statically equal to that of the control culture. In this way, the MF effect is not linear, and in addition to the factors of time, intensity and frequency, the cellular condition of the strain used must be considered, as already evidenced by Hirano et al. [8], Small et al. [10] and Tu et al. [36].

Exposure to MF by solenoid with an average intensity of 6 mT applied to 1 h day^{-1} (S assay, Fig. 1c) was evaluated. Under this condition, an increase in the biomass concentration was reached until the 12th day, after which MF started to inhibit microalgal growth. Microalgae respond differently due to different MF intensities, application strategies (magnet and solenoid) and physiological states of the strain. Deamici et al. [7] with the same strain of *Spirulina* observed an increase in growth when 5 mT was applied to 1 h day^{-1}, i.e., 1.47 g L^{-1} with MF (solenoid) and 1.22 g L^{-1} in the control.

The increase in biomass concentration when the microalga was exposed to 30 mT may have occurred due to the increase in photosynthetic efficiency. Small et al. [10] observed that all photosynthetic parameters measured increased with 10 mT. Net photosynthetic capacity and respiratory rate were 2.1 and 3.1 higher with MF applications. Deamici et al. [14] demonstrated that 30 mT applied to *Arthrospira platensis* increased photosystem II activity. The highest biomass concentrations were obtained when microalgae were exposed to 30 mT for 24 h day^{-1} (2.06 g L^{-1}) and 30 mT for 12 h day^{-1} in the light cycle (1.83 g L^{-1}). Thus, the application of 30 mT for 24 h day^{-1} was chosen to evaluate the effects on the cultures due to MF action

for long periods (50 day) (Fig. 1d). The X_{max} values were 3.90 ± 0.09 g L^{-1} and 3.64 ± 0.25 g L^{-1} for the CC50 and LD50, respectively. The kinetic parameters of growth were not significantly different between the MF application and its control cultivation. MF application until the 30th day stimulated biomass concentration and after this period led to an inhibitory effect on the growth of *Spirulina* sp. LEB 18 (Fig. 1d). Therefore, the exposure of the microalga to MF for more than 30 days is not recommended. According to Laramee et al. [37], exposure time and MF intensity are important factors when organisms are subjected to MF application.

The difference between the effects observed in the LD and LD_{50} assays may be related to the window effect. According to Panagopoulos and Margaritis [38], the "windows" represent a still unexplored area of the biological effects of the MF, where increased bioactivity appears within certain physical parameters of the field, not meaning that the intensity and lower times or higher caused a linear effect.

In three out of six conditions evaluated, exposure to MF provided an increase in microalgal growth. This biomass stimulation varied from 12% (D1) to 37.3% (LD). In the other three conditions, a null effect was observed (D12, L1 and S), since X_{max} was not different from the control. These results agree with the effects observed by Luna et al. [39], who evaluated the effect of 30 mT with *Chlorella vulgaris*, and an increase in cellular growth was reached with application for 24 h day^{-1} and 1 h day^{-1}.

Thus, when the objective is the biomass production of *Spirulina* sp. LEB 18, MF must be applied to 12 h day^{-1} in the light cycle (L12) or for 24 h day^{-1} in the light and dark cycle (LD).

Effect of MF application on the chlorophyll-a concentration

The best chlorophyll-a concentration (Fig. 2a) was reached at 12 days in assays: CC (18.26 mg L^{-1}), L1 (17.50 mg L^{-1}) and D1 (16.33 mg L^{-1}). Elevated values of chlorophyll-a on the 12th day were also established for assays L12 (19.11 mg L^{-1}) and D12 (18.08 mg L^{-1}), for which the stimulation of chlorophyll synthesis was evident starting with day 6 of the cultivation cycle. On day 15, elevated chlorophyll values in the L12 (29.29 mg L^{-1}), D12 (25.02 mg L^{-1}) and S (19.58 mg L^{-1}) conditions occurred considering the background of the reduced chlorophyll in the control condition (14.75 mg L^{-1}).

The chlorophyll-a concentrations in L12 and D12 were 60.4% and 37% higher than those in the CC group, respectively. Stimulating effects of MF on chlorophyll-a synthesis were also found by other authors. Tu et al. [36] obtained an increase of 11.5% with *Scenedesmus obliquus* FACHB-276 when the microalga was exposed to 100 mT for 0.5 h day^{-1}. Li et al. [35] applied 250 mT for 24 h day^{-1} and verified an increase of 31.1% compared with the control. These results suggest that the increase in biomass concentration may be related to the increase in the synthesis of chlorophyll and other pigments.

The chlorophyll-a concentration in *Chlorella kessleri* LEB113 was 38.9% (8.87 mg L^{-1}) and 23% (7.85 mg L^{-1}) higher with the application of 60 and 30 mT for 1 h day^{-1}, respectively [9].

According to Nelson and Cox [40], in the photosynthetic cycle, metabolic reactions are directly correlated to the potential difference between membranes and cell structures. MF may act on the Mg, Cl, Ca, Fe and Cu ions that are essential in the reactions of electron transfer between photosystems. Chlorophyll is one of the molecules that has metallic ions in their structure, i.e., Mg ions that can undergo MF action by electromagnetic interactions. The chlorophyll-a concentration shown in Fig. 2a confirms this possible relation mainly when MF was applied to 12 h day^{-1}, since there was high chlorophyll-a production. Thus, MF applied to microalga cultivation has a high potential for application in the pharmaceutical and food industries. According to Koller et al. [41], chlorophyll can be used as a natural dye in natural foods.

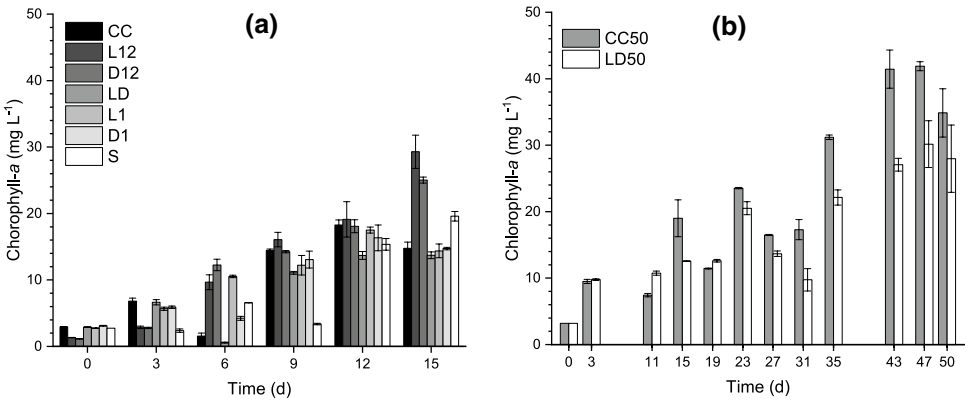

Fig. 2 Concentration of chlorophyll-a. **a** 15 day assays CC (control culture); L12 (light cycle, 12 h day^{-1}); D12 (dark cycle, 12 h day^{-1}), LD (light and dark cycle, 24 h day^{-1}), L1 (light cycle, 1 h day^{-1}), D1 (dark cycle, 1 h day^{-1}) and S (MF application with solenoid for 1 h day^{-1} in the light cycle), **b** 50 day assays at 30 mT for 24 h day^{-1} (LD50) and CC50 (control culture)

The chlorophyll-a concentration after 50 days of *Spirulina* cultivation was inhibited by MF (30 mT for 24 h day^{-1}) after the fifteenth day of cultivation (Fig. 2b). The highest chlorophyll-a concentration was found at 47 days, 41.9 mg L^{-1} and 30.2 mg L^{-1} for CC50 and LD_{50}, respectively.

Correlation analysis between biomass productivity and chlorophyll content was performed. Correlation coefficients (*R*) were 0.66 (CC), 0.86 (L12), 0.92 (D12), 0.78 (LD), 0.66 (L12), 0.54 (D12), 0.12 (S), 0.81 (CC50) and 0.76 (LD50). Only the D12 assay yielded a high *R* value (0.92) and showed a positive correlation between biomass productivity and chlorophyll content.

Effect of MF on protein, carbohydrate and lipid concentrations

The data shown in Table 3 show that exposure to MF caused an increase in lipid concentration in all 15 day cultures. However, the protein concentration was significantly different ($p < 0.05$) from that of the control cultures only when 30 mT was applied throughout the culture (LD). Under this condition, protein and lipid concentrations increased by 24.2 and 45.5% compared with the control, respectively.

These results differ from the effects observed by other authors, since in most of the studies, it was observed that MF inhibited lipid synthesis [7, 10, 39].

Deamici et al. [6] applied 30 mT *Chlorella fusca* LEB 111 for 24 h day^{-1} and increased 12.7% carbohydrate, 0.6% protein and 0.9% lipid synthesis. Cellular characteristics may explain these differences since *C. fusca* is a eukaryotic microalga and *Spirulina* is prokaryotic. Thus, these microalgae have physiological differences that lead to different responses of cells to the MF.

Small et al. [10] cultivated *C. kessleri* at 10 mT for 24 h day^{-1}, and carbohydrate and protein contents increased by 8.5% and 8.8%, respectively. Hirano et al. [8] studied the effect of MF exposure (0–70 mT) in *S. platensis* cultures and verified an increase in carbohydrate synthesis with 10 mT, and at other intensities, there was a reduction in synthesis.

MF application may change the incorporation and use of the available nitrate in the medium. According to Paredes [42], the assimilation of organic nitrogen has a great relation with light, and its intensity and quality may be used to modulate this nutrient assimilation. Thus, changes in protein synthesis may be expected, since any influence on nitrate uptake will directly influence the formation of amino acids and may have consequences on the expression of some genes.

The positive effect on the production of macromolecules, such as proteins and lipids, adds value to the *Spirulina* biomass. According to Mata et al. [43], this increase in biomolecules increases the added value of biomass, which can be consumed in nature or used in the formulation of food to humans or animals.

Exposure of the strain to 30 mT for 24 h^{-1} for 50 day (LD_{50}) inhibited protein synthesis (38.9%) but stimulated carbohydrate synthesis (63.5%) because the microalga was exposed to long periods of stress. Therefore, this effect may be linked to damage to DNA expression. According to Albuquerque et al. [44], MF may alter the phosphorylated and dephosphorylated states of the proteins, which may change the protein amount produced and type expressed during synthesis.

Table 3 Protein, carbohydrate and lipid concentration of *Spirulina* sp. LEB 18 with application of 6 mT and 30 mT at different times

Assays	Protein (%)	Carbohydrate (%)	Lipid (%)
CC	42.2 ± 1.4^{b}	10.3 ± 1.3^{a}	10.1 ± 0.3^{d}
L12	46.2 ± 2.2^{ab}	10.2 ± 0.9^{a}	11.6 ± 0.3^{c}
D12	46.3 ± 1.5^{ab}	9.6 ± 0.1^{a}	13.8 ± 0.4^{ab}
LD	52.4 ± 1.0^{a}	10.1 ± 0.5^{a}	14.7 ± 0.01^{a}
L1	43.3 ± 0.6^{b}	10.3 ± 0.3^{a}	13.8 ± 0.1^{ab}
D1	49.2 ± 1.1^{ab}	10.3 ± 1.0^{a}	12.7 ± 0.1^{bc}
S	46.4 ± 1.0^{ab}	11.8 ± 0.3^{a}	14.7 ± 0.04^{a}
CC50	42.2 ± 1.2^{A}	8.5 ± 0.6^{B}	13.7 ± 1.2^{A}
LD_{50}	25.8 ± 1.6^{B}	13.9 ± 1.1^{A}	15.9 ± 1.0^{A}

CC control culture; assays with 30 mT for 15 day: *L12* 12 h day^{-1}, light phase, *D12* 12 h day^{-1}, dark phase, *LD* 24 h day^{-1}, light and dark phase, *L1* 1 h day^{-1}, light phase, *D1* 1 h day^{-1}, dark phase; assay with solenoid, 6 mT for 15 day: S (1 h day^{-1}, light phase); *CC50* culture control for 50 day; assay with 30 mT for 50 day: *LD50* 24 h day^{-1}, light and dark phase

Equal lowercase letters indicate that there are no significant differences in the column at 95% confidence ($p > 0.05$)

Equal capital letters indicate that there are no significant differences in the column at the 95% confidence interval ($p > 0.05$)

Antioxidant activity

The oxidative activity of the methanolic extracts determined by the $ABTS^{+}$ method was not significantly different ($p < 0.05$) in all cultures performed for 15 days (Table 4). For the DPPH method, the highest inhibition of radicals was observed in assays L12 (light cycle, 12 h day^{-1}) and S (solenoid for 1 h day^{-1} in the light cycle), and the highest inhibition by the power reducing method was observed in assays CC (control culture), L12 (light cycle, 12 h day^{-1}) and D12 (dark cycle, 12 h day^{-1}). The defense system and the production of antioxidant compounds are activated when the cell is exposed to a factor that causes stress. MF application in the 15 day cultures, except assay S, did not increase antioxidative effects on *Spirulina* sp. LEB 18 when compared with control culture.

However, when the microalga was exposed to the same magnetic intensity of 30 mT (24 h day^{-1}) for 50 day (LD_{50}), there was a difference in the oxidant activity of the

Table 4 Antioxidant activities of *Spirulina* sp. LEB 18 extract by ABTS, DPPH and power reducing methods

Assays	ABTS (% inhibition)	DPPH (% inhibition)	Power reducing (Abs at 700 nm)
CC	9.7 ± 1.6^{a}	2.87 ± 1.0^{bc}	$0.05 \pm < 0.01^{ab}$
L12	10.0 ± 1.4^{a}	3.93 ± 0.2^{ab}	$0.07 \pm < 0.01^{a}$
D12	9.3 ± 0.2^{a}	2.66 ± 0.4^{bc}	0.08 ± 0.03^{a}
L1	11.9 ± 0.9^{a}	1.33 ± 0.0^{c}	$0.02 \pm < 0.01^{bc}$
D1	9.4 ± 0.8^{a}	2.24 ± 1.0^{bc}	0.01 ± 0.01^{c}
LD	11.5 ± 0.7^{a}	1.53 ± 0.4^{c}	$0.02 \pm < 0.01^{bc}$
S	11.3 ± 1.0^{a}	5.95 ± 1.5^{a}	$0.02 \pm < 0.01^{bc}$
CC50	13.2 ± 1.3^{A}	9.7 ± 7.2^{B}	$0.02 \pm < 0.01^{A}$
LD50	8.2 ± 0.5^{B}	30.0 ± 1.1^{A}	$0.02 \pm < 0.01^{A}$

CC control culture; assays with 30 mT for 15 day: *L12* 12 h day^{-1}, light phase, *D12* 12 h day^{-1}, dark phase, *LD* 24 h day^{-1}, light and dark phase, *L1* 1 h day^{-1}, light phase, *D1* 1 h day^{-1}, dark phase; assay with solenoid, 6 mT for 15 day: S (1 h day^{-1}, light phase); *CC50* culture control for 50 day; assay with 30 mT for 50 day: *LD50* 24 h day^{-1}, light and dark phase

Equal lowercase letters indicate that there are no significant differences in the column at 95% confidence ($p > 0.05$)

Equal capital letters indicate that there are no significant differences in the column at the 95% confidence interval ($p > 0.05$)

methanolic extracts by the DPPH and ABTS methods. There is a relation between the exposure period and free radical generation. Thus, this extract had a greater ability to interact with H^+ donor compounds and was able to interrupt oxidative chain reactions.

According to Barnes [45] and Timmel et al. [46], MF application may exert oxidative stress in biological systems due to the high production of triplet states in the electrons, which can dissociate these electrons in the form of free radicals in cells. This phenomenon was not observed in the 15 day cultures with 6 mT and 30 mT.

However, in the study by Wang et al. [47], with *C. vulgaris*, an increase in hydroxyl radical production (·OH) was observed that was proportional to the increase in MF intensity in the culture when exposed to 5–50 mT. The application between 10 and 35 mT stimulated cell growth and had a higher incidence of reactive oxygen species (ROS) than the control culture. However, ROS concentrations were high in cultures exposed to 45 and 50 mT, reaching concentrations close to CC.

Electrophoresis

The profile of intracellular proteins for the different exposure times and MF and EMF intensity was evaluated by the electrophoresis technique, as shown in Fig. 3. Most of the proteins corresponded to the 50 kDa and 18–15 kDa bands. The control culture (CC) (well 1) had a difference in protein expression compared with other assays, especially in assays

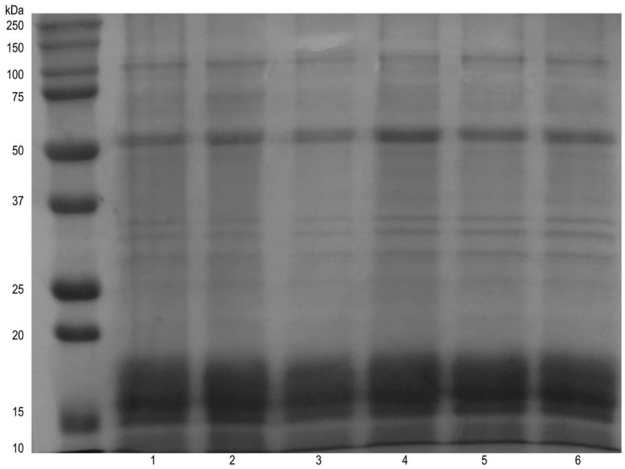

Fig. 3 Electrophoresis for the 15 day assays at 30 mT. Well 1: CC (control culture), well 2: L12 (light cycle 12 h day^{-1}), well 3: D12 (dark cycle 12 h day^{-1}), well 4: L1 (light cycle 1 h day^{-1}), well 5: D1 (dark cycle 1 h day^{-1}), well 6: LD (light and dark cycle 24 h day^{-1})

L12 (light cycle 12 h day^{-1}, well 2) and L1 (light cycle 1 h day^{-1}, well 4). The protein bands had the highest concentrations at approximately 100 kDa, 50 kDa and 15 kDa.

According to Esquível et al. [48] and Spreitzer and Salvucci [49], the enzyme ribulose 1,5–biphosphate carboxylase/oxygenase (Rubisco) has a molar mass of approximately 560 kDa and is divided into eight large subunits containing the active site and eight small subunits. The large subunits are encoded in the *rbcL* gene of the chloroplast genome and have approximately 55 kDa each. The small subunits are encoded in the *rbcS* nuclear gene, and their molar mass is approximately 15 kDa. Thus, exposure to MF under conditions in L12, L1 and LD may have induced the expression of this enzyme. Therefore, it influenced the photosynthetic system of *Spirulina*. This fact may explain the better cell growth in the L12 and LD, with an increase of 22% and 37% X_{max}, respectively.

In Figs. 3, 4, 5, the 100 kDa band had alterations in protein expression. According to Vierling and Alberte [50], proteins of the structure and functional center of photosystem I (PSI) are expressed in the 120 kDa range, such as the P700 chlrophyll-a protein. This protein is responsible for the oxidation of photons, and the oxidized form reacts with the reduction of ferrodoxin. Nelson and Cox [40] reported that this reaction is spontaneous and exergonic in the next step; ferredoxin is reduced to plastoquinone, and electrons are released passing to the cytochrome complex and transported along the electron chain until the cycle completes and return to the initial chlorophyll P700. The energy released during these reactions and the ATP and ADP power supply were used.

Unlike the electrophoretic profile of the CC and LD, the exposure of *Spirulina* sp. 18 to MF for long periods

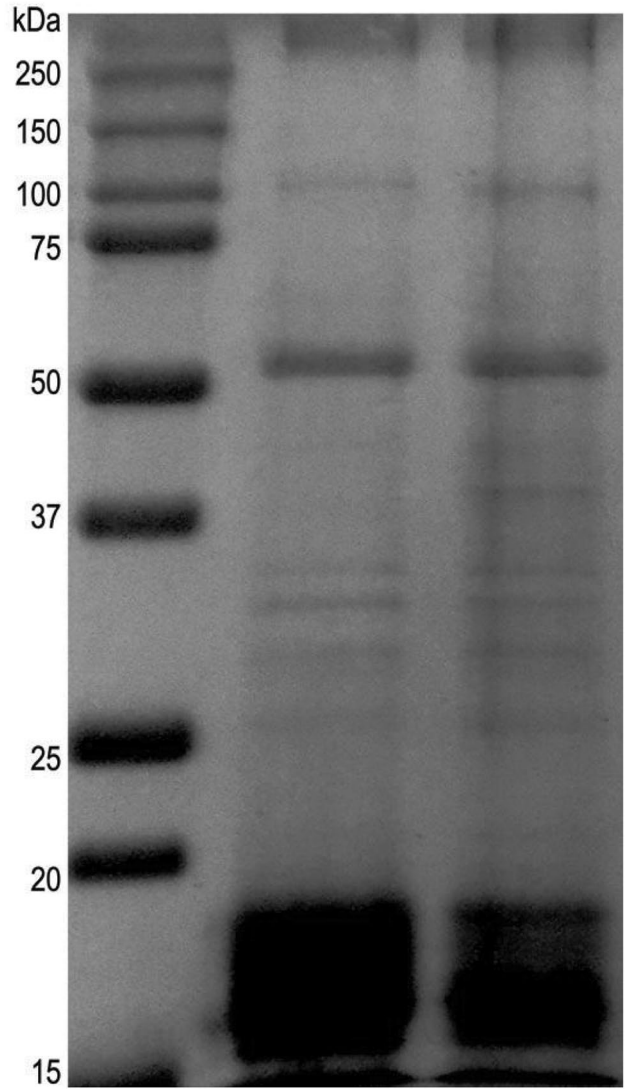

Fig. 4 Electrophoresis in 50 day assays. Well 1: CC50 (control culture) and well 2: LD50 (ligth and dark cycle 24 h day^{-1})

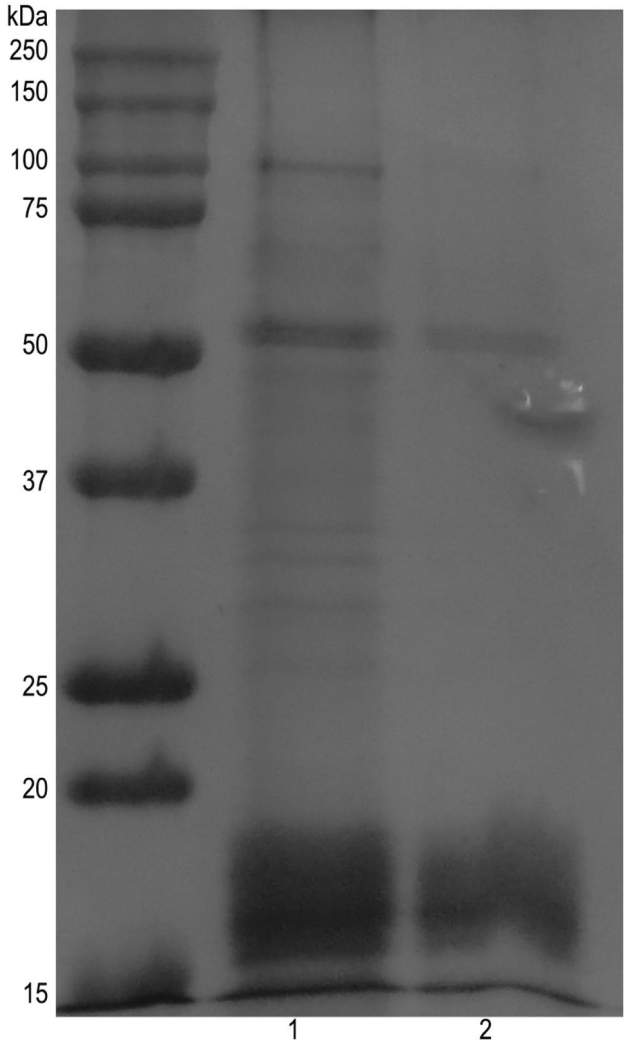

Fig. 5 Electrophoresis of MF assay with solenoid. Well 1: control culture (CC), well 2: 6 mT for 1 h day^{-1}

resulted in inhibition signals for molar mass proteins in the range of 15–18 kDa (Fig. 4, well 2). This decrease in protein expression was expected since this same condition led to protein inhibition (Table 3).

Exposure to an EMF of 6 mT for 1 h day^{-1} (Fig. 5) led to alterations in the electrophoretic profile in the 50 kDa bands and in the 15–20 kDa band (well 5, well 2). The bands evidenced for the different treatments in Figs. 3, 4, 5 may correspond to proteins of photosystems I and II. Among these, we note that the CP43 protein that has a molecular mass of 56 kDa and is related to the light capture system and OEC 16 protein that is encoded by the *psbQ* gene, which acts in stabilization in photosystem II.

Effect of MF on cellular morphology

The application of 6 mT and 30 mT did not alter the cellular morphology of the microalga *Spirulina* sp. LEB 18 (Fig. 6). According to Li et al. [35], MF may alter membrane permeability, leading to an increase in cell growth rates. This growth may be associated with an increase in the incorporation of nutrients, such as nitrogen and phosphorus, which in cyanobacteria are consumed rapidly due to the high growth rate.

Small et al. [10] observed changes in photosynthesis and cell structure of *C. kessleri* UTEX 398 when it was evaluated with transmission electron microscopy; furthermore, cell size decreased and there were changes in the organization of the chloroplast.

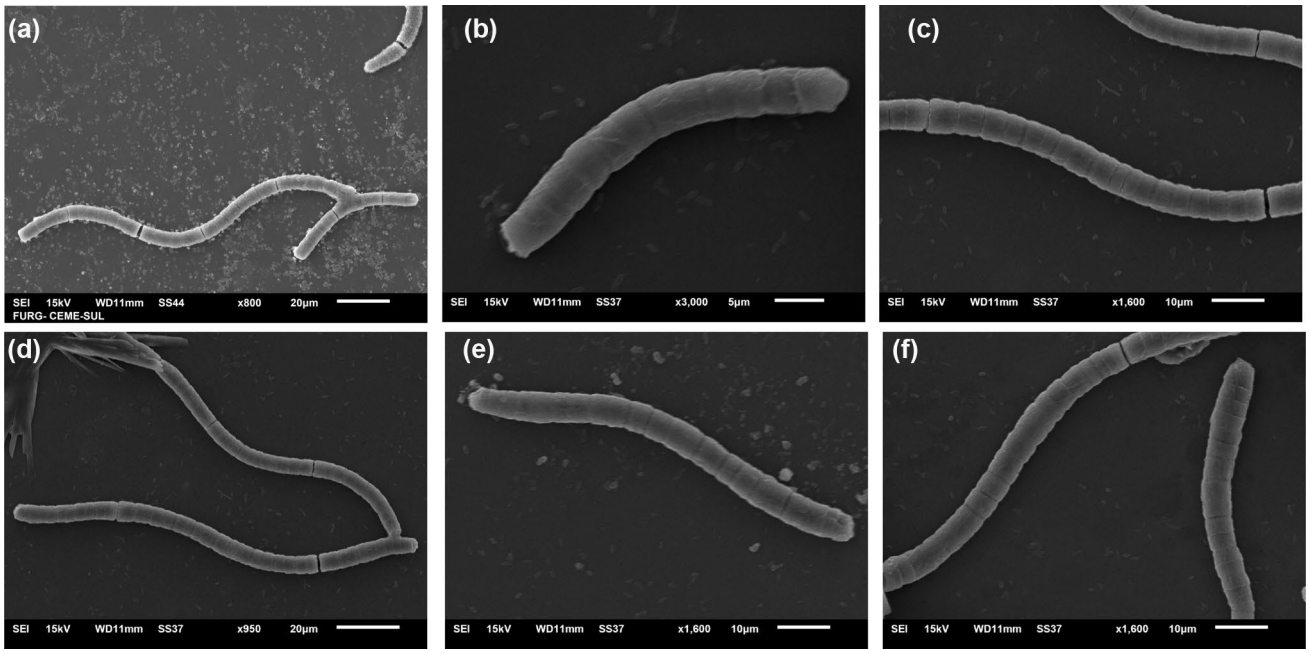

Fig. 6 Scanning electron microscopy (SEM) of **a** CC (control culture), **b** L15 (light cycle, 12 h day^{-1}), **c** D12 (dark cycle, 12 h day^{-1}), **d** LD (light and dark cycle, 24 h day^{-1}), **e** CC50 (control culture) and **f** LD50 (light and dark cycle, 24 h day^{-1})

Conclusion

Effects on the metabolism of *Spirulina* sp. LEB 18 is directly related to the photosynthesis cycle and time period in which MF was applied. In the 15 day cultures, there was an increase of 22% and 37.3% in biomass concentration when 30 mT was applied for 12 h day^{-1} (in the light cycle) and 24 h day^{-1} (in the light and dark cycles), respectively. Under all conditions, there was an increase in the lipid concentration. No morphological change was observed. However, long periods of exposure to MF (over 30 days) inhibited microalgal growth. The intracellular protein profile changed according to the exposure strategy, mainly protein bands of 50 and 15 kDa, and it is possible that photosystems I and II have underwent changes.

Authors contributions Material preparation, data collection, analysis, investigation, writing—original draft, review and editing and visualization were performed by BCM. Writing—review and editing was performed by LS. Supervision and writing—review and editing was performed by JAVC. Methodology, supervision and writing—review and editing were performed by JGB. Conceptualization, writing—review and editing, methodology, supervision, project administration and funding acquisition were performed by LOS. All authors read and approved the final manuscript.

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Declarations

Conflict of interests Not applicable.

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