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Magnetic Fields as Inducers of Phycobiliprotein Production by *Synechococcus elongatus* PCC 7942

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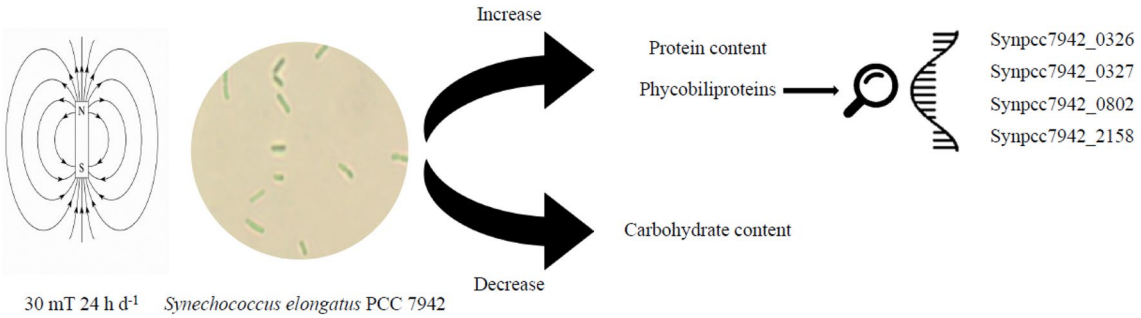
Abstract

This study aimed to analyze the effect of magnetic field (MF) application on the metabolism of *Synechococcus elongatus* PCC 7942. Concentrations of biomass, carbohydrate, protein, lipid, and photosynthetic pigments (chlorophyll-a, C-phycocyanin, allophycocyanin and phycoerythrin) were determined. In cultures with MF application (30 mT for 24 h d^{-1}), there were increases of 47.5% in total protein content, 87.4% in C-phycocyanin, and 332.8% in allophycocyanin contents, by comparison with the control. Allophycocyanin is the most affected pigment by MF application. Therefore, its biosynthetic route was investigated, and four genes related to its synthesis were found. However, the analysis of the gene expression showed no statistical differences from the control culture, which suggests that induction of such genes may occur soon after MF application with consequent stabilization over time. MF application may be a cost-effective alternative to increase production of compounds of commercial interest by cyanobacteria.

Graphical Abstract



Introduction

Microalgae are photosynthetic microorganisms cultivated in marine, hypersaline, brackish, fresh, and waste waters to produce high value-added compounds [1]. They grow fast and form the basis of the food chain of aquatic systems [2]. One of the microorganisms, *Synechococcus elongatus,* is considered a model by studies of gene manipulation due to its small genome and easy manipulation in laboratories [3]. In addition, the cyanobacterium has high growth rates and high tolerance for light and other stressors, a fact which makes it attractive to biotechnological applications [4].

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Many studies have shown that magnetic field (MF) application to microalga cultures may positively influence their growth and enhance production of compounds of interest. Deamici et al. [5] have just shown that combining outdoor conditions with MFs is advantageous, since high biomass concentration may be achieved at low-energy expenditure. Costa et al. [6] showed that MF application with ferrite magnets was efficient to stimulate lipid synthesis. Silva et al. [7] applied 30 mT and reached increase of 59.4% in carotenoid production and 99.3% in carotenoid productivity. Furthermore, according to Repacholi and Greenebaum [8], MFs exert forces that move ions in solution (electrolytes, for example), which originate induced electric fields and currents, and may produce torques in certain molecules and some ferromagnetic material, thus, changing their three-dimensional structures. They may also change energy levels and electron spin orientation with biological consequences.

Phycobiliproteins and carotenoids are some of the main pigment groups in microalgae and cyanobacteria. The former accumulates in cells as phycobilisomes and binds to thylakoid membranes of chloroplasts, which plays the main role of antennas in the process of collecting light energy [9]. In pharmaceutical and nutraceutical industries, phycobiliproteins exert antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties. Regarding cyanobacterial cells, tasks of phycobiliproteins are inhibition of reactive oxygen species (ROS) and activity as alkoxyl scavengers in the stressful environment (nutrient level, light, salinity, or pH value during cultivation) [10, 11]. Thus, this study aimed to analyze the effect of MF application on the metabolism of *S. elongatus* PCC 7942.

Material and Methods

Microorganism and Cultivation Conditions

Synechococcus elongatus PCC 7942 used by this study is originally from the Pasteur Collection Cells (PCC, Paris, France) and was provided by the Molecular Biology Laboratory that belongs to the Institute of Biological Sciences at the Federal University of Rio Grande (FURG), located in Rio Grande, RS, Brazil.

Cultures were grown in BG-11 medium [12] composed of (g L^{-1}): $NaNO_3$ (1.5); $K_2HPO_4.7H_2O$ (0.04); $MgSO_4.7H_2O$ (0.075); $CaCl_2.\ 2H_2O$ (0.036); $C_6H_{11}FeNO_7$ (0.006); $C_{10}H_{14}N_2Na_2O_8.2H_2O$ (0.001); Na_2CO_3 (0.02); $C_6H_8O_7$ (0.006); and A^{5+}Co solution (1 mL L^{-1}). A^{5+}Co solution contains (g L^{-1}): H_3BO_3 (2.86), $MnCl_2.4H_2O$ (1.81), $ZnSO_4.7H_2O$ (0.222), $NaMoO_4$ (0.015), $CuSO_4.5H_2O$ (0.079), and $Co(NO_3)0.6H_2O$ (0.0494).

Cultures were grown in photobioreactors (1 L culture flasks) with useful volume of 0.8 L, maintained at 30 °C, 12 h light/dark, 0.2 vvm (volume of air sparged per unit volume of growth medium per minute) [13], inoculum concentration of 0.26 g L^{-1}, and illuminance of 50 µmol m^{-2} s^{-1} for 15 days. In cultures with MF application, ferrite magnets (80 × 80 × 10 mm) were positioned in the center of the outer base of the photobioreactors throughout cultivation. Intensity of MFs (30 mT) was measured by a teslameter (Global Mag, TLMP-HALL 05 k, Brazil) at several points in the photobioreactors.

The control culture was carried out under the same culture conditions but without any MF application except terrestrial MFs (approximately 0.005 mT). All cultures were grown in triplicate. Water evaporation during cultivations was controlled by maintaining the volume with daily replacement of sterile-distilled water.

Analytical Determinations

Determination of Biomass Concentration and pH

Biomass concentration was determined by daily readings of optical densities of cultures by a spectrophotometer (Quimis, Q798DRM, Brazil) at 750 nm. The measurement is related to dry biomass of microalga standard curve.

The pH was measured by direct reading carried out by a digital pHmeter (KASVI, K39-2014B, China).

Determination of Carbohydrate, Protein and Lipid Concentrations

Uncleared extracts were prepared with 5 mg lyophilized biomass in 10 mL-distilled water to determine carbohydrate and protein contents in biomass. The solution was sonicated by an ultrasonic probe for 10 cycles of 59 s (59 s on and 59 s off). Carbohydrate concentration in extracts was determined with the use of a standard glucose, as proposed by Dubois et al. [14]. Protein concentration in the biomass extract was determined with the use of a standard bovine serum albumin curve, in agreement with Lowry et al. [15]. Lipids were extracted and quantified by the procedure proposed by Marsh and Weinstein [16] with a standard tripalmitin curve.

Extraction and Quantification of Photosynthetic Pigments

Extraction of chlorophyll-*a* was conducted in agreement with the procedure described by Lichtenthaler [17], which consisted of resuspension of cells in methyl alcohol 99.8 (v v $^{-1}$) and reading by a spectrophotometer at 665.2 nm and 652.4 nm. The concentration is determined by Eq. 1.

$$\text{Chlorophyll} - a(mg \cdot L^{-1}) = 16.72\,A_{665.2} - 9.16\,A_{652.4} \quad (1)$$

Extraction of phycobiliproteins was carried out in agreement with Ores et al. [18]. Lyophilized biomass was solubilized in a 10 mM pH 8.3 Tris-HCl buffer at concentration of 5 g L^{-1}. Cells were ruptured by an ultrasonic probe (COLE PARMER, CPX 130, USA) at a frequency of 20 kHz for 10 min in an ice bath. The extract was centrifuged (HITACHI, Himac CR-22GIII, Japan) at 6.000 g for 10 min, and the supernatant was centrifuged again under the same conditions.

Concentrations (g L^{-1}) of C- phycocyanin (C-PC), allophycocyanin (APC), and phycoerythrin (PE) were calculated as described by Bennett and Bogorad [19] using Eqs. 2, 3, and 4, with modifications in wavelength. Absorbance readings at 619, 677, and 536 nm represent maximum absorbances of C- PC, APC, and PE, respectively. They were carried out by a UV–vis scanning spectrophotometer (SHIMADZU, UV-2550, Japan).

$$\text{C-PC} = \frac{A_{619} - 0.474 \times A_{677}}{5.34}, \quad (2)$$

$$\text{APC} = \frac{A_{677} - 0.208 \times A_{619}}{5.09}, \quad (3)$$

$$\text{PE} = \frac{A_{536} - 2.41 \times (\text{C-PC}) - 0.849 \times (\text{APC})}{9.62}. \quad (4)$$

Evaluation of Kinetic Parameters of Growth

Maximum biomass productivity (P_{max}), specific growth speed (μ_{max}), biomass concentration (X_{max}), and generation time (t_g) were determined. Productivities (P, g L^{-1} d^{-1}) were calculated by Equation P=(X–Xi)/t. P_{max} value was the highest value while X (g L^{-1}) was biomass concentration at time t, Xi was initial biomass concentration (g L^{-1}), and t was time.

Maximum specific growth speed (μ_{max}) resulted from exponential regression in the logarithmic phase of cell multiplication. Generation time (t_g) was determined in the exponential growth phase of every assay by Equation $t_g = \ln(2)/\mu_{max}$ [20].

Determination of Gene Expression

Based on the significant change in allophycocyanin production with MF application, its metabolic and biosynthetic routes were investigated. Free genomic data on cyanobacteria available on the Cyanobase platform were used to find genes related to the synthesis of the biomolecule. Specific primers were designed by Primer Express 3.0 software (Applied Biosystems) for the following genes: (i) allophycocyanin subunit beta (*Synpcc7942_0326*), (ii) allophycocyanin alpha chain (*Synpcc7942_0327*), (iii) allophycocyanin alpha chain-like protein (*Synpcc7942_0802),* and (iv) allophycocyanin subunit beta (*Synpcc7942_2158*). Their sequences are shown in Table 1.

Determination of gene expression was analyzed by quantitative real-time PCR (qPCR). It was carried out by the 7500 Real-Time PCR system using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). RNA extraction was performed with 50 mL aliquots of every assay in triplicate with Trizol (Invitrogen). Samples were frozen at – 80 °C for 16 h to perform cell lysis. The amount of RNA was measured by the Qubit fluorometer (Invitrogen) with the use of Quant-iT RNA BR Assay kit (Invitrogen) and analyzed by agarose gel electrophoresis 1%. Total RNA was treated with DNAse I (Invitrogen) to avoid contamination with the cyanobacterium genomic DNA. To conduct cDNA synthesis, 1 µg of total RNA and High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) were used. cDNA was also determined by the Qubit fluorometer and Quant-iT ssDNA Assay kit (Invitrogen).

Reactions of qPCR, in triplicate, with serial dilutions of cDNA were made to test efficiency of all primers. Conditions for qPCRs were 50 °C/2 min, 95 °C/2 min, followed by 40 cycles of 95 °C/15 s and 60 °C/30 s. Target gene expression was normalized by constitutive genes rnpB and rpoD [21]. All procedures followed the manufacturer's protocol, while data on qPCR were analyzed by the delta CT method [22].

Efficiency of the Application of Magnetic Fields

Efficiency of MF application was calculated for biomass characterization by Eqs284. 5 [23, 24], where C_{MF} are

Table 1 Sequence of primers used by this study

PRIMERS	Forward primer (5′-3′)	Reverse primer (5′-3′)
α- allophycocyanin		
Alpha- AlloPhyco- 1	GGGGGTTCCTAGCGATTTGT	ACCTGCGTCTTGTGACCTAC
Alpha- AlloPhyco- 2	GCCGTCATGTCTTCACCGTA	GCTGGCAACCAACTTTTCCA
β- allophycocyanin		
Beta- AlloPhyco- 1	ACATGGCATAGGTGGCGTAG	AGTGGCTAAATCGCTGCTCT
Beta- AlloPhyco- 2	CGGAGCTGATGTAGTCGAGG	CCTACAACAGTCTGGGCGTG

responses evaluated with MF application and $C_{control}$ are responses given by the control assay.

$$\text{Efficiency } (\eta)(\%) = \frac{C_{MF} - C_{Control}}{C_{Control}} \times 100. \quad (5)$$

Statistical Analysis

Responses given by cultures with MF application and the control were evaluated by the Student's t test at 95% confidence interval ($P < 0.05$).

Results

Biomass and pH Concentration

Synechococcus elongatus PCC 7942 grew similarly under both conditions (Figure 1). The control culture did not show any lag phase of growth and remained in exponential growth until the end of the experiment.

At the end of cultivation, biomass concentration with MF exposure to 30 mT for 24 h d^{-1} was statistically equal to the control ($P>0.05$). However, with MF application to the culture, cyanobacteria showed a lag phase up to 24 h of cultivation, exhibiting about 8 % less biomass by comparison with the control in this period.

Table 2 shows that, in relation to X_{max}, t_g, and μ_{max}, there was no statistical difference at 95 % confidence level ($P>0.05$) between both conditions under evaluation. X_{max} values were found on the last day of cultivation, both in the control and MF groups, indicating that cyanobacteria had not yet entered a steady state of growth and that nutrients were still available to be consumed in the medium.

P_{max} value was found 72 h after the beginning of cultivation with MF application. In the control, it was found only

Table 2 Kinetic parameters of growth, contents of proteins, carbohydrates and lipids on a dry basis and contents of photosynthetic pigments of *Synechococcus elongatus* PCC 7942

Parameters	Control	Magnetic field
X_{max} (g L^{-1})	0.74 ± 0.07^a	0.83 ± 0.05^a
P_{max} (g L^{-1} d^{-1})	$0.05 \pm \leq 0.01^a$	$0.05 \pm \leq 0.01^a$
μ_{max} (d^{-1})	0.09 ± 0.01^a	$0.08 \leq 0.01^a$
t_g (d)	9.30 ± 1.74^a	9.44 ± 0.27^a
Proteins (% $m_{protein}$ $m_{biomass}^{-1}$)	44.29 ± 2.77^a	65.31 ± 4.38^b
Carbohydrates (% $m_{carbohydrate}$ $m_{biomass}^{-1}$)	26.95 ± 6.97^a	9.63 ± 1.07^b
Lipids (% m_{lipid} $m_{biomass}^{-1}$)	13.37 ± 2.33^a	15.19 ± 2.86^a
Chlorophyll-*a* (mg L^{-1})	10.13 ± 2.24^a	11.19 ± 1.12^a
C-phycocyanin (mg mL^{-1})	1.34 ± 0.07^a	2.52 ± 0.12^b
Allophycocyanin (mg mL^{-1})	0.61 ± 0.05^a	2.64 ± 0.13^b

Different letters on a line correspond to the statistical difference ($P<0.05$) found by the Student's t test

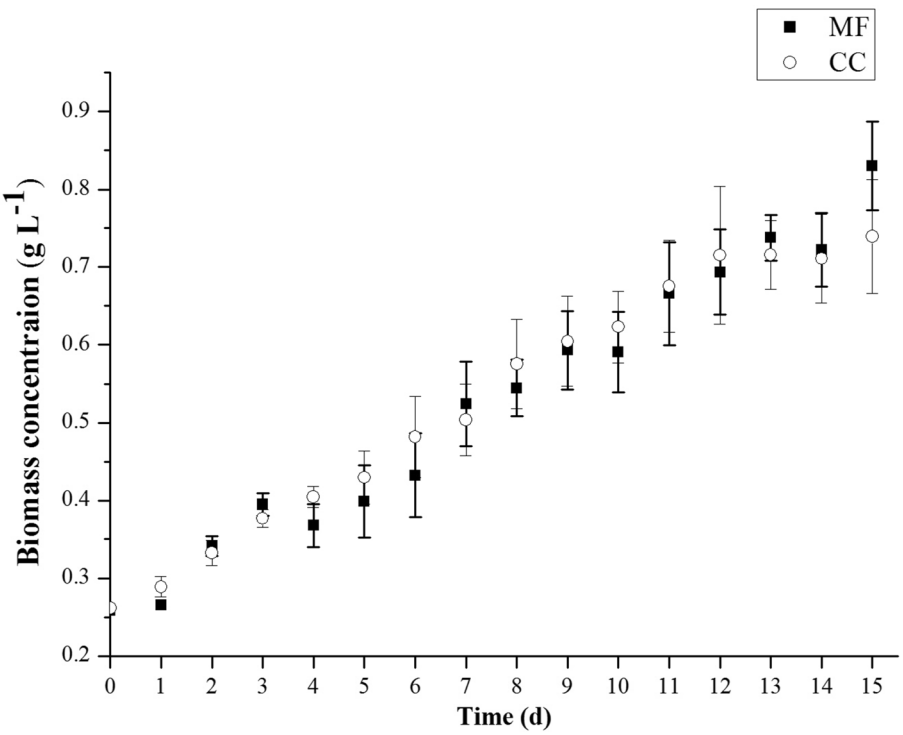

Fig. 1 Biomass concentration of *Synechococcus elongatus* PCC 7942 grown under MF application for 24 h d^{-1}(○) and control culture (■)

192 h after the beginning of cultivation. Nevertheless, these values did not differ statistically ($P > 0.05$).

Values of μ_{max} and t_g, with and without MF application, showed no statistical difference ($P > 0.05$).

Figure 2 shows that there was no statistical difference ($P > 0.05$) between pH values of cultures.

Protein, Carbohydrate, Lipid, and Photosynthetic Pigment Contents

Data in Table 2 show that total protein content with MF application throughout cultivation was 47.5% higher by comparison with the control. Regarding the carbohydrate content, there was decrease of 64.3% when cyanobacteria were exposed to 30 mT for 24 h d^{-1}, by comparison with the control ($P < 0.05$). The lipid content of cyanobacterium biomass with MF application did not show any statistical difference ($P > 0.05$). Data in Table 2 show that chlorophyll-*a* concentration did not show any statistical difference ($P > 0.05$) by comparison with the control.

According to Ducret et al. [25] and Sun and Wang [26], wavelength with the highest absorption of phycobiliproteins may vary, i.e., phycoerythrins with $\lambda_{max} = 490—570$ nm, phycocyanins with $\lambda_{max} = 590—652$ nm, and allophycocyanin with $\lambda_{max} = 650—665$. Maximum absorbance of extracts was determined by a scanning spectrophotometer to confirm wavelengths. Maximum absorbance values of C-phycocyanin, allophycocyanin, and phycoerythrin synthesized by cyanobacteria were 619, 677, and 536 nm, respectively. Values of C-phycocyanin and phycoerythrin remained within the range mentioned in the literature.

Production of both C-phycocyanin and allophycocyanin exhibited statistical difference ($P < 0.05$) between cultures. At the end of cultivation, C-phycocyanin and allophycocyanin concentrations were higher (87 and 332%, respectively) in cultures exposed to MFs. Extracted concentrations of C-phycocyanin and allophycocyanin were 1.34 mg g^{-1} and 0.61 mg g^{-1} in the control culture while in the culture with MF application, they were 2.52 mg g^{-1} and 2.64 mg g^{-1}, respectively.

Regarding quantification of phycoerythrin, the procedure was not efficient since values of C-phycocyanin and allophycocyanin versus their respective extinction coefficients were higher than the absorption of the solution at 536 nm, characterizing the matrix effect.

Determination of Gene Expression

Efficiency of MF application in relation to contents of carbohydrates, proteins, C-phycocyanin, and allophycocyanin showed that allophycocyanin was the most affected biomolecule, with increase of 332% by comparison with the control.

Figure 3 shows relative gene expression of *Synpcc7942_0326, Synpcc7942_0327, Synpcc7942_0802,* and *Synpcc7942_2158* genes. There was no statistical difference between expression of biomass genes under MF application and the control culture of every gene ($P > 0.05$).

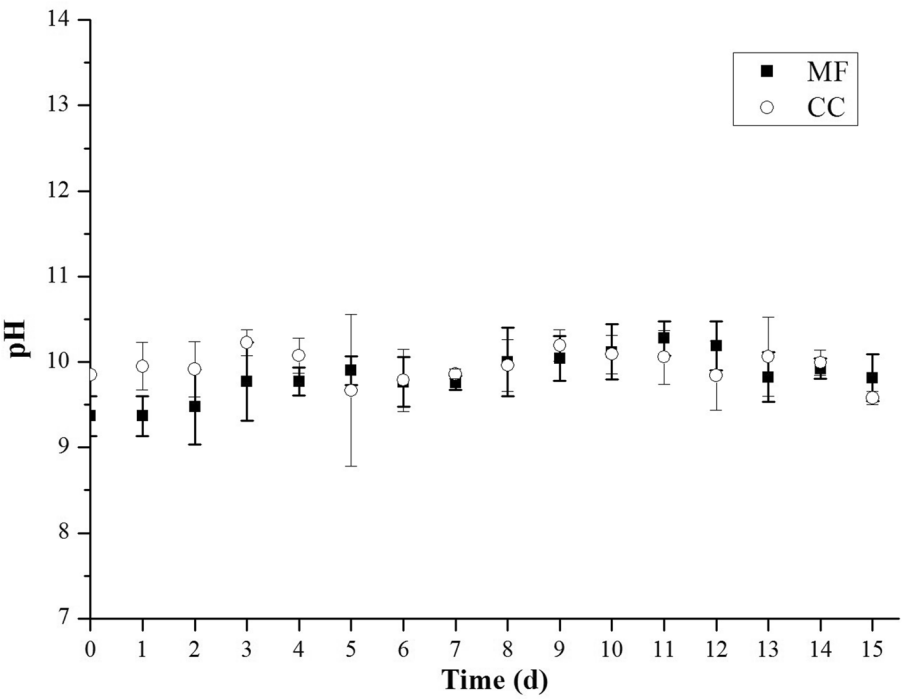

Fig. 2 Cultivation pH under MF application for 24 h $d.^{-1}$ (○) and control culture (■)

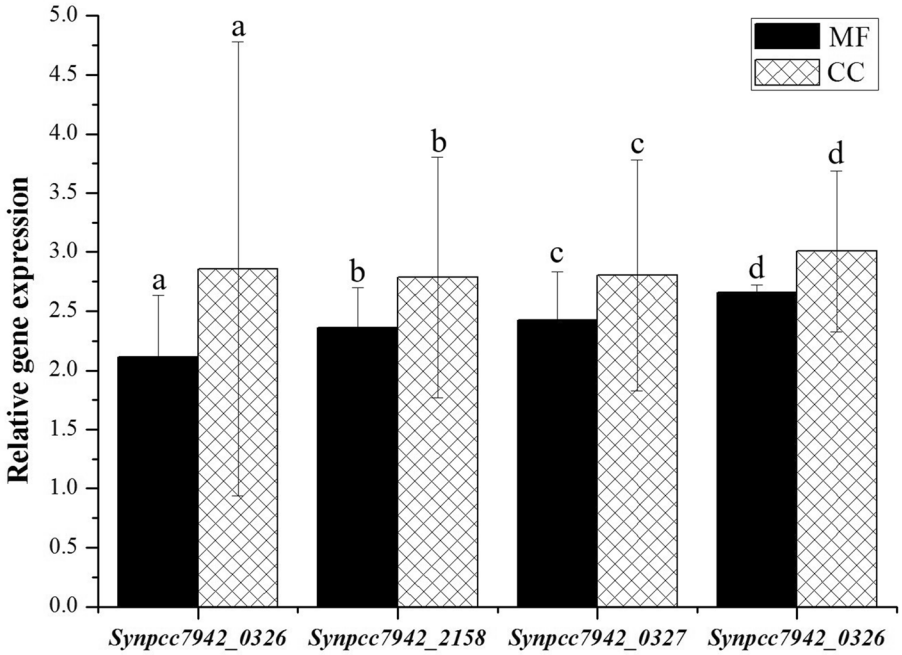

Fig. 3 Relative gene expression of allophycocyanin-forming monomers by *Synechococcus elongatus* PCC 7942. Equal letters in a gene column mean that there is no statistical difference ($P < 0.05$) by the Student's t test

Discussion

Biomass and pH Concentration

The lag phase up to 24 h of cultivation observed in the culture with MF exposure occurred because the inoculum of *S. elongatus* was not previously adapted to MFs; it had only been adapted to cultivation conditions. The lag phase was also observed by Deamici et al. [27] in cultures of *Chlorella fusca* LEB 111 with CO_2 injection and application of 30 mT (1 h d^{-1} and 24 h d^{-1}) and 60 mT (1 h d^{-1} and 24 h d^{-1}).

Wang et al. [28] exposed *Chlorella vulgaris* to 45 and 50 mT and found that MF application did not alter cell growth by comparison with the control. However, even if biomass concentration does not change, MF application may alter compositions of biomass and other cellular constituents. Gao et al. [29] reported that growth of *Shewanella onedensis* MR-1 was not affected by 14.1 T; however, 21 genes were positively regulated whereas 44 genes were repressed.

MF application may result in stimulation, inhibition, or null effect on microalga growth. It depends on the species, time, and intensity of MF, physiological state of cells, and other factors. Wang et al. [28] described that, when constant, uniform and static MFs from 5 to 35 mT were used, there was growth stimulation of *Chlorella vulgaris*. Veiga et al. [30] also reported 37.2 % increase in biomass concentration when exposing *Spirulina* sp. LEB 18 to 60 mT for 24 h d^{-1} at 30 °C. Li et al. [31] exposed cultivation of *Spirulina platensis* to 0.25 T and its growth was 22 % higher than growth reached by the control. Bauer et al. [23] showed that application of 60 mT for 1 h d^{-1} to *Chlorella kessleri* cultures led to 83.2 % increase in biomass concentration.

Deamici et al. [32] reported higher P_{max} in cultures of *Spirulina* sp. LEB 18 with application of 30 mT for 24 h d^{-1}, 0.31 g L^{-1} d^{-1}, than the one found in the control culture, which was 0.19 g L^{-1} d^{-1}. The authors [27] also reported P_{max} that was 38 % higher in cultures of *Chlorella fusca* subject to 30 mT by comparison with the control.

Wang et al. [28] showed that *Chlorella vulgaris* cultivated with MF application between 10 and 35 mT exhibited μ_{max} between 0.09 and 0.11 d^{-1}, while the control one was 0.07 d^{-1}; thus, growth was higher throughout cultivation.

Duarte and Costa [33] cultivated *Synechococcus nidulans* LEB 115, a cyanobacterium that belongs to the same genus used by this study, in BG-11 medium, at 30 °C, 12 h light/dark photoperiod and 41.6 µmol photons $m^{-2} \cdot s^{-1}$ for 10 days (conditions were similar to the control). X_{max}, P_{max} and μ_{max} were 0.94 g L^{-1}, 0.09 g $L^{-1} \cdot d^{-1}$, and 0.11 d^{-1}, respectively. All of them were higher than parameters found by this study. It is worth mentioning that both studies employed different photobioreactors. Duarte and Costa [33] used tubular type reactors, while this study used Erlenmeyer, which has a smaller area of contact with light, by comparison with the tubular reactor. Another reason is the difference between species of cyanobacteria, a fact that allows different metabolic behaviors and, therefore, distinct results.

There was no statistical difference ($P > 0.05$) in pH values between cultures. It may have happened because the mechanism of bicarbonate consumption by cyanobacteria was not affected by MF application. According to Barsanti and Gualtieri [34], the ideal pH for most microalgae ranges between 7

and 9, although there are species that inhabit more acidic or basic environments, which may reach values above pH 9.5. Noaman et al. [35] reported that pH 8 was the optimum one to grow *Synechococcus leopoliensis*. Studies carried out by Miller and Colman [36] and Shiraiwa et al. [37] propose that variation in pH throughout the assay may be associated with the mechanism of assimilation of bicarbonate by microalgae and cyanobacteria. From two ions of bicarbonate incorporated by cells, one of them is consumed and internalized in the environment as carbon dioxide (CO_2), while the other is released as carbonate (CO_3), leading to change in pH in the environment.

Protein, Carbohydrate, Lipid and Photosynthetic Pigment Contents

Not only concentrations of biomass, but also results of protein concentrations differed from other studies of microalgae and cyanobacteria. Deamici et al. [27] also applied 30 mT for 24 h d^{-1} to cultivation of *Chlorella fusca*, and there was increase of 9.7% in protein content. The same authors [32] grew the cyanobacterium *Spirulina* sp. LEB 18 at 60 mT for 24 h d^{-1} and reached increase of 16.6% in the biomass protein content, by comparison with the control. However, when Deamici et al. [38] grew *Arthrospira platensis* SAG 21.9 at 30 mT for 24 h d^{-1}, they found 17.5% decrease in the protein content, by comparison with the control. Costa et al. [6] applied 30 mT for 24 h d^{-1} to *Chlorella homosphaera* and found 41.8% decrease in the protein content. Snoussi et al. [39] detected changes in the expression of 11 proteins from the external membrane of *Salmonella hadar* exposed to 200 mT for 9 h, i.e., seven were over-regulated while four were suppressed. These proteins are involved in the cell envelope (TolB, Pal), responses to oxidative stress (OmpW, dihydrolipoamide dehydrogenase, USPF), the state of oxidative stress (bacterioferritin), virulence (OmpX, Yfgl), and motility (FlgE and UspF).

Regarding carbohydrates, Veiga et al. [30] applied 60 mT for 24 h d^{-1} to *Spirulina sp*. LEB 18 and there were also decrease of 69.1% in the biomass carbohydrate content. However, Menestrino et al. [40] added 2.50 mM of KNO_3 and applied 30 mT to cultures of *Chlorella minutissima* to find increase of 184.7% in the carbohydrate content. Costa et al. [6] applied 30 mT for 24 h d^{-1} to *Chlorella homosphaera* and reached decrease of 19.2% in the carbohydrate content. In assays carried out by Deamici et al. [27], who applied 30 mT for 24 h d^{-1} to cultures of *Chlorella fusca*, no statistical difference was observed in the biomass carbohydrate content in relation to the control. According to Deamici et al. [41], the decrease may be related to changes in metabolism of biological cells caused by electromagnetic force. Increase in the demand for protein metabolic routes may also have led to decrease in availability of nutrients to form carbohydrates.

Other authors, such as, Deamici et al. [24], who investigated *Chlorella fusca*, and Deamici et al. [32], who used *Spirulina* sp. LEB 18, observed the same behavior in lipid contents of cyanobacterium biomass with MF application. When Deamici et al. [27] grew *Chlorella fusca* and applied 30 mT throughout cultivation, there was decrease of 15% in the lipid content.

Concerning concentrations of chlorophyll-*a,* Deamici et al. [41] did not observe any change in the chlorophyll-*a* content when 30 mT was applied to *Spirulina* sp. LEB 18 cultures (indoor). However, in outdoor conditions, MFs led to increase of 137.7% in the chlorophyll-*a* content. Bauer et al. [23] also observed increase of 38.9% in the content of chlorophyll-*a* due to MF application to *Chlorella kessleri* cultures.

Repacholi and Greenebaum [8] stated that MF may cause oxidative stress to cells since they alter energy levels and orientation of electron rotation to increase activity, concentration, and life span of free radicals. According to Cherdkiatikul and Suwanwong [42], phycobiliproteins extracted from cyanobacteria exhibit several beneficial activities, such as antioxidant and antitumor activities and anti-inflammatory and neuroprotective effects. Therefore, increase in production of C-phycocyanin and allophycocyanin may have occurred due to their antioxidant character which acts against the oxidative stress caused by MFs. Deamici et al. [32] carried out a study of *Spirulina* sp. LEB18 and showed increase of 36% in phycocyanin content when subject to 30 mT for 24 h d^{-1}.

Effects described by this study–regarding decrease and increase in contents of biomolecules–are in line with findings reported by Bauer et al. [23] and Deamici et al. [24], who stated that MFs may either stimulate or inhibit cell growth and biomolecule production. According to Aarholt et al. [43], this variation may be related not only to microorganism used in experiments, but also to intensity and time of exposure.

MF application to microorganism cultures has been studied. A research group has recently published a review (Santos et al. [44]) showing the complexity of finding answers to changes and lack of linearity of results, when the same microorganisms exposed to MF are compared. Table 3 shows the compilation of some results found by several studies of different microorganisms when MF is applied. Thus, MF application is an issue that must be studied because each microorganism responds differently, depending on the state of the cell, components of the culture medium, type of reactor, cultivation conditions and type, time and intensity of MF application. The literature shows that production of biomass and biomolecules by microalgae may be stimulated by these process variables.

Table 3 Results of MF application to microalga cultures reported by different authors

Author	Microorganism	Magnetic field intensity and exposure time	Conclusion of MF application
This study	*Synechococcus elongatus* PCC 7942	30 mT for 24 h d^{-1}	Total protein (47.5%), C-phycocyanin (87.4%) and allophycocyanin (332.8%) increased. Expression of four genes with MF showed no differences in relation to the control culture
Deamici et al. [5]	*Chlorella fusca* LEB 111	25 mT for 24 h d^{-1} and 1 h d^{-1}	Outdoor conditions with MF are advantageous. The higher the biomass concentration, the lower the energy expenditure
Costa et al. [6]	*Chlorella homosphaera*	15, 30 and 60 mT for 24 h d^{-1} and 1 h d^{-1}	Lipid synthesis and biomass production was stimulated in all conditions under evaluation
Bauer et al. [23]	*Chlorella kessleri*	30 and 60 mT for 24 h d^{-1} and 1 h d^{-1}	Cell growth, lipid (13.7%), chlorophyll-a (38.9%), chlorophyll b (59.1%), total carotenoids (25%) and antioxidants (185.7%) were stimulated
Deamici et al. [24]	*Chlorella fusca* LEB 111	30 and 60 mT for 24 h d^{-1} and 1 h d^{-1}	Biomass (20.5%) and carbohydrate concentrations (24.8%) increased
Deamici et al. [27]	*Chlorella fusca* LEB 111	30 and 60 mT for 24 h d^{-1} and 1 h d^{-1}	Biomass production and CO_2 biofixation increased. MF application proved to be an inexpensive and nontoxic method
Wang et al. [28]	*Chlorella vulgaris*	10–50 mT for 12 h	10–35 mT promoted growth and regulated *C. vulgaris* antioxidant defense system to protect cells efficiently
Gao et al. [29]	*Shewanella oneidensis*	14.1 T for 1.5 and 12 h	Little effects on cell growth at log phase were observed. Changes at transcriptional levels were detected in some genes
Veiga et al. [30]	*Spirulina sp.* LEB 18	30 and 60 mT for 24 h d^{-1}	Biomass resulting from MF application is promising to be used in food and may be used as an ingredient in the manufacture of protein supplements
Li et al. [31]	*Spirulina platensis*	0–0.55 T for 24 h d^{-1}	Weak MF (e.g., 0.25 T) may enhance nutrition assimilation of C, N, P and minerals to stimulate *S. platensis* cultivation
Deamici et al. [32]	*Spirulina sp.* LEB 18	5, 30 and 60 mT for 24 h $d^{-1}$and 1 h d^{-1}	MF trigger a stimulating effect on *Spirulina* growth and may lead to twofold biomass concentration in equal cultivation time without MF
Deamini et al. [38]	*Arthrospira platensis* SAG 21.99	30 mT for 24 h d^{-1} and 1 h d^{-1}	Photossystem II was positively affected by MF application, since quantum yield increased under these conditions
Snoussi et al. [39]	*Salmonella enterica serovar Hadar*	200 mT for 9 h	There were alterations in the gene expression of 11 proteins involved in the integrity of the cell envelope and in response to oxidative stress
Menestrino et al. [40]	*Chlorella minutissima*	30 mT for 24 h d d^{-1}	Biomass and carbohydrate contents increased 30% and 163.1%, respectively
Deamici et al. [41]	*Spirulina sp.* LEB 18	25 mT for 24 h $d^{-1}$and 1 h d^{-1}	MF application for 24 h in outdoor conditions increased biomass concentration, chlorophyll-a content and altered protein profile

Determination of Gene Expression

Allophycocyanin is a light-uptake pigment-protein complex found in the core of cyanobacteria and red alga phycobilisomes. It is a phycobiliprotein composed of three aggregates from two different subunits (α and β), which have a chromophore that collects solar energy for photosynthesis [45]. However, according to MacColl [46], its main function in photosynthesis is to channel the excitation energy absorbed by phycobilisome stem biliproteins, phycocyanin, phycoerythrin, and phycoerythrocyanin, to the chlorophyll of thylakoid membranes.

All phycobiliproteins are synthesized by linking both subunits α and β to form monomers (αβ) which are subsequently assembled in trimers $(\alpha\beta)_3$, in the case of allophycocyanin, or hexamers $(\alpha\beta)_6$, in phycocyanin and phycoerythrocyanin [47–49]. Therefore, in the synthesis of allophycocyanin, cells must first express genes that are responsible for producing molecules α and β monomers. Thus, in the analysis of allophycocyanin at the level of gene expression, primers were synthesized; targets were genes responsible for the synthesis of the monomers, namely: (i) *Synpcc7942_0326* (beta subunit), (ii) *Synpcc7942_0327* (alpha chain), (iii) *Synpcc7942_0802* (alpha chain like), and (iv) *Synpcc7942_2158* (beta subunit).

Increase of 332.8% in allophycocyanin concentration is certainly related to increase in genes that code for these proteins, but it does not occur at the same time. Gene expression is not concomitant with increase in molecule concentration. Genes are expressed as mRNA, which is read on ribosomes to make proteins. Therefore, gene expression precedes protein production. The fact that, in this study, genes under evaluation did not show the expected induction may be explained by two hypotheses. First, pigment synthesis may depend on a set of genes that was not tested by this study. For example, research on the genome of this cyanobacteria has shown that, in addition to the four genes previously described, there are five other sequences that may be related to the production of alpha and beta chains of allophycocyanin. They are: (i) *synpcc7942_0325, (ii) synpcc7942_0978, (iii) synpcc7942_1049, (iv) synpcc7942_1050,* and (v) *synpcc7942_1051*. These sequences, which are associated with the phycobilisome, have not been defined yet. The second hypothesis is associated with the moment in which samples for analysis of gene expression were collected. It is known that there is a time difference between gene expression and protein production. In general, after the stimulus, transcription of the gene begins and reaches its peak in a matter of hours or few days. After protein synthesis, gene transcription tends to gradually decrease, it only rises again when the protein is degraded or loses its function. Depending on the lifetime of the protein, transcription may increase again more quickly or more slowly.

In the case of allophycocyanin, McGregor et al. [50] attributed its high stability to the fact that they have many internal hydrogen bonds associated with hydrophobic interactions that decrease solvent activity. Thus, molecules with these characteristics tend to have a long half-life, which means that genes are first activated and remain at this level until protein concentration reaches the limit. Afterwards, the gene tends to return to a basal level of transcription until degraded proteins must be replaced. Considering that cultures lasted 15 days and that the material was collected only at the end of the experiment, genes may have reached their maximum level of expression in the first hours or days and, from the moment allophycocyanin increased its concentration, genes may have returned to the baseline level of transcription due to the high stability of the molecules.

Conclusion

Application of 30 mT to *Synechococcus elongatus* PCC 7942 cultures led to increase of 47.5% in total protein content, 87.4% in C-phycocyanin and 332.8% in allophycocyanin, besides decrease of 64.3% in carbohydrate content. MFs did not alter cell growth, lipid content, and chlorophyll-*a* concentration. This study showed that MF application may be used to trigger production of phycobiliproteins, mainly, since they are bioactive compounds with wide commercial application.

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