### **REVIEW**

# **The efect of magnetic feld treatment on the cultivation of microalgae: An overview of involved mechanisms**

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#### **Abstract**

Several studies have demonstrated the benefts of magnetic treatment on the productivity of secondary metabolites, growth, and the state of microalgae cultures. This technology offers advantages such as low operating costs, absence of toxic effects, absence of secondary pollution, a wide range of applications, and a long useful life. Despite these advantages, its use in industrial microalgae culture systems has been limited due to the lack of consensus on the mechanisms that explain the observed efects on microalgae. In this paper, the efect of magnetic feld treatments on microalgae and cyanobacterial cultures and the possible mechanisms are critically reviewed. However, it is still necessary to conduct more studies directly relating the experimental effects observed with one or several proposed mechanisms.

**Keywords** Microalgae · Magnetic felds · Cultivation · Mechanism of action

# **Introduction**

Microalgae are a group of microscopic photosynthetic organisms (Osanai et al. [2017](#page-11-0)). These include eukaryotic and prokaryotic microorganisms, which can be found in all terrestrial and aquatic habitats (Siqueira et al. [2018](#page-11-1)). In microalgae, typically, plant metabolic properties are combined with characteristics of microbial cells, such as the capacity for rapid growth in liquid culture, simplicity of nutritional requirements, metabolic plasticity, tolerance to extreme conditions, ability to synthesize and secrete some metabolites, and the potential to develop genetically engineered strains in the interest of biotechnology industry (Khavari et al. [2021](#page-10-0)).

These characteristics have made these microorganisms a promising strategy for wastewater treatment. They accumulate and metabolize nutrients in liquid wastewater (Molazadeh et al. [2019\)](#page-11-2). In addition, the biomass obtained

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is a source of biomolecules of industrial interest, containing lipids, proteins, and carbohydrates, highly valued as a source of raw materials for diferent industries such as the pharmaceutical sectors (Yan et al. [2016\)](#page-11-3), aquaculture (Shah et al. [2018](#page-11-4)), food (Gateau et al. [2017](#page-10-1)), cosmetics (Ariede et al. [2017](#page-10-2)) or bioenergy (Su et al. [2017](#page-11-5)). Despite all the applications microalgae cultivation provides, large-scale production still needs to be improved. This is because cultivation systems are not yet efficient enough (Garrido-Cardenas et al. [2018\)](#page-10-3). Therefore, new cultivation methods and strategies are being explored and developed to reduce large-scale production costs.

These production methods often focus on the establishment of microalgae inoculum isolated from natural sources with specific and highly efficient growth characteristics (Jiang et al. [2018](#page-10-4)), efective conditions of culture management, and strategies to avoid or detect predation to maximize biomass productivity and the concentration of some biomolecules of interest (Markou et al. [2014](#page-11-6); Joe et al. [2018\)](#page-10-5), the development of methods for the efficient separation of biomass from the culture medium and for metabolite extraction (Vandamme et al. [2013;](#page-11-7) Sathasivam et al. [2019](#page-11-8)). A relatively novel alternative method for productivity improvement of microalgae cultures with wastewater treatment is applying magnetic feld treatment. This technology has the advantages of low capital and operating cost, has no proven toxic efects, and does not produce secondary contamination (Zhang et al. [2017](#page-11-9)).



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Research carried out in this area has repeatedly reported that with the application of magnetic feld treatment, the productivity of the algal biomass and intracellular biomolecule concentration improved (Deamici et al. [2016;](#page-10-6) Bauer et al. [2017](#page-10-7); Huo et al. [2020\)](#page-10-8). Moreover, it has also been observed that  $CO<sub>2</sub>$  fixation of microalgae increased (Deamici et al. [2019b](#page-10-9)). It also improved the rate of nutrient uptake, heavy metals absorption from wastewater (Shao et al. [2018\)](#page-11-10), and the rate of photosynthetic oxygen production (Tu et al. [2015](#page-11-11)).

However, implementing this technology presents limitations that impede its development since the theoretical foundations that support it still need to be understood more. Hence, this review provides an overview of the current understanding of the action mechanisms reported.

# **Defnition and classifcation of magnetic felds**

All life forms on earth are under the action of a natural and stable geomagnetic feld of around 0.5 G. Although this intensity is considered relatively large concerning most manufactured sources, this does not prevent the evolution of living beings subjected to this geomagnetic field. This phenomenon is probably one of the reasons why various researchers worldwide have been interested in studying the biological efects and possible applications of magnetic felds on biological systems (Kataria et al. [2017](#page-10-10); Mohammadi et al. [2018;](#page-11-12) Radhakrishnan [2019;](#page-11-13) da Silva et al. [2020](#page-10-11); Sarraf et al. [2020;](#page-11-14) Chen et al. [2021](#page-10-12); Lew et al. [2021](#page-11-15)).

An electromagnetic feld (EMF) is produced by accelerating electric charges. It can be viewed as the combination of an electric feld and a magnetic feld (Zhang et al. [2017](#page-11-9)). These EMFs are divided along the electromagnetic spectrum based on frequency. They can be classifed as nonionizing (low-level radiation generally perceived as harmless to humans) or ionizing (high-level radiation potentially damaging cells and DNA).

### **Classifcation according to its variation over time**

Magnetic felds (MFs) can be classifed according to their variation in time: static or dynamic. When the intensity of the magnetic feld does not vary in time, it is referred to as a static magnetic feld (SMF). On the other hand, when the intensity of the magnetic feld changes over time, they are referred to as a dynamic magnetic feld (DMF) (Zhang et al. [2017\)](#page-11-9). One of the most studied DMFs is pulsed magnetic felds (PMF), generated by a strong electromagnet passing an electric current through its windings, generating a brief but strong magnetic feld pulse (Shneerson et al. [2014](#page-11-16)). The efect of PMF on biological systems has been reported in several studies, for example, bacteria, cancer cell tissues, as

well as plant cells and microalgae cells (Schmiedchen et al. [2018](#page-11-17); Sengupta and Balla [2018;](#page-11-18) Bodewein et al. [2019;](#page-10-13) Lin et al. [2019;](#page-11-19) Akaberi et al. [2020\)](#page-9-0). Studies carried out with these MFs have shown various cellular responses depending on the intensity of the applied EMF and the frequency. In studies carried out by Baldev et al. [\(2021](#page-10-14)) with cultures of *Chlorella vulgaris* and a PMF in a magnetic fux density range of 600–900 mG, it was observed that there was no linear relationship between the magnetic fux density and the average dry cell weight of the cultures. However, research with these PMFs has taken a back seat in recent decades. Specifcally, in microalgae cultivation, there are fewer and fewer research reports on PMFs. This is because the variability of parameters such as frequency and intensity make it difficult to understand the mechanisms of action of these magnetic felds on biological systems.

In this context SMFs have re-emerged as a viable option for studies of the biological mechanisms afected by the action of this physical agent. In contrast to DMFs, SMFs have fewer variables in their configuration. The devices most used for these studies are built from permanent magnets, allowing more accessible experimental facilities to be developed (Chu et al. [2020](#page-10-15); Deamici et al. [2021](#page-10-16)). In addition, the results obtained with this type of magnetic feld are more accessible to scale due to its simple confguration.

### **Classifcation according to the intensity of SMFs**

In biological systems studies, the intensity of the applied SMF can be classified as weak  $(< 1$  mT), moderate  $(1 \text{ mT})$ to 1 T), high (1 T to 20 T), and very high  $(> 20$  T). The magnetic feld density is a vector B, expressed in Tesla (T) according to the International System of Units (SI). However, to be compatible with biological investigations, it can also be expressed in Gauss (G), where:

#### 1 T (Tesla) = 10000 G (Gauss)

Various authors have widely studied the intensity of SMF on organisms (Chu et al. [2020;](#page-10-15) Chen et al. [2021](#page-10-12)). As with other characteristics, the intensity of the SMF does not generate the same efects from one organism to another. Furthermore, there is not always a linear behavior about the increase or decrease of the intensities of the SMF studied (Chen et al. [2021](#page-10-12); Hassanpour and Pourhabibian [2022;](#page-10-17) Khorshidi et al. [2022](#page-10-18)). In the research carried out by the Khorshidi research group (Khorshidi et al. [2022\)](#page-10-18), diferent intensities of SMF (0, 2, 4 and 8 mT) were applied to the suspension culture of *Haematococcus lacustris* cells. This study showed that applying 8 mT SMF at the beginning of the logarithmic phase of growth increased the maximum cell concentration. In comparison, the maximum accumulation of astaxanthin was obtained by applying 4 mT SMF.

On the other hand, in studies developed with the bacterium *Clostridium pasteurianum* and the application of diferent intensities of SMF, the fermentation of glucose to obtain hydrogen was evaluated (Chen et al. [2021](#page-10-12)). The hydrogen production study demonstrates that SMF is a physical agent capable of improving the total glucose consumption and increasing the maximum rate of hydrogen production of the cultures, independent of the intensity of SMF applied compared to the control cultures. Furthermore, it was observed that there was no linear sequence of the effects of SMF observed concerning the intensity of the SMF applied.

### **Spatial distribution and exposure time of SMFs**

The spatial distribution of magnetic forces is also characteristic of SMFs, which can infuence biological systems. The observed effects of the spatial distribution of SMFs on biological systems depend on the interaction of SMFs with the magnetic moments of organisms. Binhi and Prato [\(2018\)](#page-10-19) have explained these efects through the Level Mixing Mechanism (LMM). This LMM expresses that, in some conditions, the precession of the magnetic moments that reside on rotating molecules can be slowed relative to the immediate biophysical structures. This means that variations in the quantum levels of magnetic moments can be observed. Therefore, a relationship is established between the biological efects in response to the inversion of the SMF vector. SMFs can be classifed according to their spatial distribution into homogeneous (when the feld strength is spatially constant) and non-homogeneous (when the feld strength varies spatially).

Another critical characteristic that changes the efects of MF on biological samples is the exposure time to MFs. In this sense, various changes found in biological systems depending on the time of exposure to MFs are still being investigated in depth. These changes are not always linear and can change profoundly from one biological system to another. This has been corroborated in diferent studies with diferent organisms. In the study developed with *Spirulina platensis* (current name *Arthrospira platen*sis) with diferent exposure times to SMF (0 min, 3 h, 6 h and 12 h), it was observed that this physical agent improves the biomass productivity of the microorganisms in two of the exposure schemes evaluated (Shao et al. [2018\)](#page-11-10). Applying the magnetic treatment for three and six hours was the best scheme compared with the control cultures. Nevertheless, applying SMF for 12 h inhibited biomass productivity concerning control cultures. In addition, with 1 h exposure to SMF, stimulator effects on cell growth have been observed in different microalgae such as *H. lacustris* (Khorshidi et al. [2022](#page-10-18)), and *Chlorella fusca* (current name *Desmodesmus abundans*) (Deamici et al. [2021](#page-10-16)). The results above constitute evidence of the variability of the efects of SMF depending on the type of organism to which it will be applied and the time of exposure to the MF.

# **Overview of reported general characteristics of microalgae and cyanobacteria cultures treated with MF**

Table [1](#page-3-0) summarizes some critical characteristic culture cultivation and treatment conditions and the main efects found in MF-treated microalgae and cyanobacteria culture studies. Research carried out in recent years has mainly used microalgae belonging to the genera *Chlorella* and *Spirulina* (*Arthrospira*) (Bauer et al. [2017;](#page-10-7) Shao et al. [2018;](#page-11-10) Deamici et al. [2019b;](#page-10-9) da Costa et al. [2020](#page-10-20); Deamici et al. [2021](#page-10-16)). These microalgae and cyanobacteria can be considered model microorganisms due to the great industrial interest and adaptability they present in biotechnology. However, research with the SMF has been extended to studying other species such as *Nannochloropsis, Tetraselmis*, and *Tribonema*. There is a wide range of MF intensities that have been studied. Not much variety is observed in terms of the material with which the devices for magnetic treatment are made; the most commonly used are ferrite magnets (Bauer et al. [2017](#page-10-7); Shao et al. [2018](#page-11-10); Deamici et al. [2019b](#page-10-9); Chu et al. [2020](#page-10-15); Huo et al. [2020;](#page-10-8) da Costa et al. [2020](#page-10-20); Deamici et al. [2021](#page-10-16)).

The exposure time of the MFs on the microalgae and cyanobacteria cultures has been one parameter of great importance in defning the best protocol for the magnetic treatment application. Most of the analyzed studies showed a wide range of exposure times to MF, ranging from 1 min to 24 h of exposure (Brailo et al. [2018](#page-10-21); Shao et al. [2018](#page-11-10); Deamici et al. [2021\)](#page-10-16). Although in almost all the cases studied for microalgae and cyanobacteria, positive efects were observed in terms of the increase in cell concentration, improvement of growth parameters, and increase in the concentration of metabolites of interest. The exposure (Bauer et al. [2017](#page-10-7); Deamici et al. [2019b](#page-10-9), [2021\)](#page-10-16).

The MF application protocols on cultures have been varied. In most of the analyzed research, the MF was applied during the whole cultivation period (Shao et al. [2018](#page-11-10); Deamici et al. [2019b](#page-10-9); Huo et al. [2020](#page-10-8); da Costa et al. [2020\)](#page-10-20). However, applying MF every day, but only during the light photoperiod of the microorganisms, increased the cell concentration and  $CO<sub>2</sub>$  biofixation of the microorganisms (Bauer et al. [2017](#page-10-7); Deamici et al. [2019b](#page-10-9)). These results open a window for a better understanding of the mechanism through which MF acts on photosynthetic microorganisms. It has been proven that this physical agent acts positively on photosystem II of microorganisms, increasing the quantum

<span id="page-3-0"></span>



yield and positively modifying the parameters related to the photochemistry fux ratios and specifc energy fuxes of the reaction centres (Deamici et al. [2019a\)](#page-10-22).

# **Mechanisms that explain the behaviour of MFs on biological systems**

The effect of the MFs on biological systems has been extensively studied in recent years. Positive and negative efects of this phenomenon have been observed. Despite the extensive literature, there still needs to be a complete and detailed understanding of the biological mechanisms and efects exerted by MF (Santos et al. [2017](#page-11-20); Zhang et al. [2017;](#page-11-9) Sarraf et al. [2020\)](#page-11-14). First, it is essential to note that all the proposed mechanisms are closely related to the magnetic properties of the atoms and molecules that make up biological systems. As is known, atoms and molecules can be classifed according to their magnetic properties (Zhang et al. [2017](#page-11-9)): *paramagnetic* (when exposed to an external MF, the substances are weakly magnetized in the same sense of the external feld)*, diamagnetic* (when exposed to an external MF the substances are weakly magnetized but in the opposite direction of the external feld) and *ferromagnetic* (these are the substances that when exposed to an external MF are strongly magnetized in the same sense of the external feld).

So far the results have followed one or more of the reported mechanisms that explain the effects of SMF found in biological systems. These mechanisms focus on three fundamental phenomena associated with MFs (Santos et al. [2017](#page-11-20)): (1) *magnetic induction*, (2) *the magneto-mechanical efect*, and (3) the *formation of radical pairs*. The mechanisms above are based on observations, reliable experimental data, and basic concepts of biophysical and biochemistry. However, the reproducibility of the results obtained is not always achieved, so currently the need to reach a consensus on this subject is one of the objectives of specialists in this area.

The frst mechanism, *magnetic induction*, is theoretically based on the "Hall effect" phenomenon. The Hall effect produces an electric feld *E* transverse across a conductor and perpendicular to an external magnetic feld *B* made perpendicular to the conduction direction (Parke [2020\)](#page-11-21). The electric field  $E = vB$  is generated by the Lorentz force acting to shift charges to one side as they move with speed *v* through the magnetic feld. The Hall efect has also been used to assess electron transfer between electrogenic bacteria and microbial fuel cell appliances (Cao et al. [2020](#page-10-23)). This study verifed through the measurement of the Hall efect, with the intensity of the magnetic feld B and the electric current of 0.63 T and 50 μA, respectively, that *Geobacter sulfurreducens* has the ability to transfer electrons to a surface without the use of electrical conductive pili. Currently, this knowledge about magnetic induction is the theoretical basis for developing equipment for continuous real-time monitoring of cerebral blood flow control for the treatment and prevention of cerebrovascular diseases (Zeng et al. [2022](#page-11-22)).

The *magneto-mechanical efect* is another mechanism and is an intrinsic feature of ferromagnetism (Bulte et al. 2002). Specific molecules or ferromagnetic material, under the action of an external MF, will undergo mechanical movements to align their magnetic moments with the directions of the applied feld (Nikitin et al. [2022\)](#page-11-23). The development of this mechanism is based on the presence of magnetite in various organisms such as bacteria, sharks, pigeons, and phytoplankton (Lefèvre and Bazylinski [2013](#page-11-24); Nimpf et al. [2019](#page-11-25); Amor et al. [2020](#page-9-1)). Magnetite is an iron oxide (Fe<sub>3</sub>O<sub>4</sub>), which under the action of an external MF, acquires ferromagnetic properties by orienting its domains in the direction of the applied MF, becoming a magnet. The presence of this magnetoreceptor in various organisms has allowed them to orient themselves with the earth's magnetic feld and obtain additional biological responses. In the case of magnetotactic bacteria, the magnetite crystals are organized into magnetosomes. Magnetite crystals in bacteria are considered to be surrounded by a membrane, and this structure is connected to the cell wall through cytoskeleton flaments, providing a biochemical mechanism for force translation. Currently, this mechanism is studied due to its potential use in biomedical applications related to cell labelling, drug delivery, and biosensing, among others (Anik et al. [2021](#page-9-2); Baki et al. [2021\)](#page-10-24).

The last mechanism is related to the *formation of radical pairs*. It is frequently used to explain the variation of cell biological reactions and the infuence of the MF on the energy levels and spin orientation of electrons (Santos et al. [2017\)](#page-11-20). Various authors have extensively studied this mechanism (Chidsey et al. [1985](#page-10-25); Gilch et al. [1998;](#page-10-26) Hore and Mouritsen [2016](#page-10-27); Zadeh-Haghighi and Simon [2022\)](#page-11-26). However, it is still considered that several physical parameters are not considered related to magnetic moments (Binhi [2016\)](#page-10-28). The reorientation of the spins and the generation of radical pairs under the action of an MF is closely related to each atom's electronic configuration and, consequently, its intrinsic magnetic properties. This mechanism involves magnetically sensitive intermediate molecules due to the presence of radical pairs (Zadeh-Haghighi and Simon [2022\)](#page-11-26). So far, only two magnetoreceptor candidate molecules have been found for radical pair formation, i.e., cryptochrome and chlorophylls in photosynthetic organisms (Hore and Mouritsen [2016](#page-10-27)).

Sensitive magnetic reactions generally involve radicals. A radical pair is a short-lived reaction intermediate comprising two tandemly formed radicals whose unpaired electron spines can be antiparallel (a singlet state, S) or parallel (a triplet state, T) (Rodgers and Hore [2009](#page-11-27)). Under an external MF, a spin correlation is produced between two unpaired electrons, coming from a donor and an acceptor molecule (Wong et al. [2021](#page-11-28)). The external magnetic feld drives the conversion from state S to state T, or vice versa, which gives rise to diferent reaction products. Photoexcitation is another necessary characteristic for the development of radical pair reactions, which gives rise to forming radicals (Kattnig et al. [2016\)](#page-10-29). For example, in the case of cryptochrome, upon exposure to blue light, transfers an electron to the favin adenine dinucleotide (FAD), which results in both the protein and the favonoid having unpaired electrons, that is, the radical pair necessary for a radical pair reaction.

In the context of microalgae and cyanobacteria, considering the theoretical foundations of the mechanisms described above, it can be said that in the same cell culture, the efect of the three mechanisms could be observed simultaneously. This assumption is based on the fact that in the culture of microalgae and cyanobacteria, magnetite or other ferromagnetic molecules could be present intracellularly, giving rise to intracellular molecule rotations, which could open or close channels in cell membranes. However, to date, no scientifc report has been found to corroborate the presence of some of these ferromagnetic molecules in these microorganisms. These magnetoreceptors have only been found in phytoplankton, which generally have a high concentration of magnetotactic bacteria (Yuan et al. [2020\)](#page-11-29).

On the other hand, in photoautotrophic cultures light plays a fundamental role by providing the photons that cells need to initiate photosynthesis. However, it also constitutes one of the essential factors to activate the formation mechanism of radical pairs together with the presence of chlorophyll molecules under the action of MF. Even though the radical pair mechanism contains elements that directly relate it to microalgae, such as the presence of chlorophylls, the results obtained so far related to this mechanism are not solid enough since it is not possible to establish the direct relationship between the photochemical efects found with the formation of the radical pair.

Finally, these cultures are carried out in liquid media disposed of by ions that can be infuenced, like intracellular ions, by the presence of an external MF, due to the Hall efect. However, no solid experimental evidence demonstrates the infuence of SMF on the ionic transfer from the culture medium to the cells or vice versa.

# **Efect of the magnetic feld on the microalgae and cyanobacteria cells**

The results obtained for microalgae and cyanobacteria suggest that the efect of MF on these microorganisms could involve the three mechanisms outlined above. However, there is not enough practical evidence to demonstrate this approach. Most of the efects found are associated with genetic, metabolic and physiological changes in microorganisms (Han et al. [2016;](#page-10-30) Shao et al. [2018](#page-11-10)), which can then be measured through the increase in the cell concentration of the cultures (Deamici et al. [2021](#page-10-16)), variations in growth parameters, changes in the biochemical composition of the biomass (da Costa et al. [2020](#page-10-20)) and improvement in the photosynthetic capacity of the microorganisms (Small et al. [2012](#page-11-30); Deamici et al. [2019a](#page-10-22)). Furthermore, these results can be grouped into three main topics which will be addressed further: (1) metabolic and/or genetic changes, (2) structural changes in cells, and (3) electron transition changes linked to reaction centres.

### **Metabolic and / or genetic changes**

Multiple studies have demonstrated that the application of SMF affects metabolic and/or genetic changes that positively stimulate cell concentrations in culture as well as their biochemical composition. For example, Han et al. [\(2016](#page-10-30)) explained that the changes in the counts of *Chlorella pyrenoidosa* could be attributed to the infuence of SMF on the biological functions of the organism by changes in hormones, the activity of some enzymes, the transport of membrane ions or the transcription of DNA. Shao et al. [\(2018\)](#page-11-10) related the increase in the growth rate of *S. platensis* (*A. platen*sis) during the exponential phase, with the efect of the SMF on possible improvement of metabolic pathways related to growth, which counteract the toxic efect of  $Cd^{2+}$  ions present in the culture medium. da Costa et al. [\(2020\)](#page-10-20) observed that with the application of an SMF of 30 mT in cultures of *C. minutissima* (*M. homosphaera*), the production of proteins and carbohydrates in cultures were stimulated. The authors relate the increase in protein and carbohydrate content to interactions between MF and cells, altering the intracellular macromolecule production. In addition, Deamici et al.  $(2016)$  evaluated the effect of different intensities of SMF (30 and 60 mT) and EMF (5 mT) on the growth and the composition of the biomass of *Spirulina* sp*.* In this research, the SMF of 30 and 60 mT stimulated cell growth and the content of proteins and carbohydrates, which was attributed to possible changes at the enzymatic level. However, MF intensities do not always relate in a linear way to cell concentrations and their composition. As such, Luo et al. ([2020](#page-11-31)) observed variability in their results when applying diferent intensities of SMF to cultures of *Chlorella vulgaris*. A wide variability in the content of extracellular polysaccharides and total proteins was seen at day 16 of the culture and an increase in cell concentration was not observed at all the intensities of the SMF applied with respect to the control cultures. The protein content and enzymatic activity of the treated cultures increased, which could be an indication of the modifcations at the metabolic level that MF can exert.

Although fndings in strictly controlled conditions show similar positive results, care should be taken when applying MF outdoors, where environmental characteristics cannot be controlled. In a study carried out by the Deamici research group (Deamici et al. [2021\)](#page-10-16) in in- and outdoor settings, it was observed that an application of 25 mT SMF during 1 h or 24 h to *C. fusca* (*D. abundans*), resulted in increases in the concentration of biomass and modifcations in the biochemical composition of the biomass, concerning the control cultures. These researchers attributed the observed efects to metabolic modifcations, such as variations in growth kinetics, which result from the applied SMF and do not occur linearly due to interference with environmental characteristics. Therefore, variations were observed between crops exposed outdoors and indoors. In addition, da Costa et al. ([2021\)](#page-10-31) explained that the efect of SMF on microalgae was also dependent on the moment in the light: dark cycle when it is applied. When the SMF is applied for 12 h in the light cycle cells alter their metabolic pathways and electron transport chain systems could be afected to keep metabolism functioning under the action of this physical agent. However, when the SMF is applied during 12 h in a dark cycle this does not happen.

Furthermore, the results changed when the SMF was applied for only 1 h since it stimulated cell growth during light and dark cycles. From these results, the SMF is a physical agent that stimulates the reactions associated with the photosynthetic apparatus of microalgae. Moreover, the degree of stimulus is directly related to the time the microorganisms are exposed to this treatment.

### **Structural changes in cells**

Other possible explanations that have been addressed about the effects of MF on microalgae cultures are related to modifcations of the membrane channels, which facilitate the transport of substances from the culture medium to the cells or from the cells to the culture medium. Deamici et al. ([2019b\)](#page-10-9) attributed the increased growth rate of cultures exposed to SMF to a possible enhancement of membrane permeability. Better absorption of nutrients by microorganisms could improve the efficiency of the metabolic reactions that microalgae develop during their growth. More recently, Chu et al. [\(2020\)](#page-10-15) evaluated the infuence of SMFs of diferent intensities (20, 30, and 40 mT) and nitrate concentration on the growth and lipid productivity of *Nannochloropsis oculata*. It was observed that the treatment carried out with the lowest feld induction (20 mT), was the one that stimulated the growth of *N. oculata*. This was associated with improving the permeability of the membrane of the algae, which could facilitate the transport of the substrate of the culture medium toward the cytoplasm of the cell. It could also be linked to the physical–chemical changes in the culture medium when an external MF is applied. This issue must be addressed so far and could constitute a key aspect in understanding the efects of MFs on microalgae cultures.

### **Electron transition changes linked to reaction centres of microorganisms**

Various authors have associated the results achieved in their research with electronic interactions and energetic changes generated in cells when the MF is applied (Small et al. [2012;](#page-11-30) Deamici et al. [2019a;](#page-10-22) Sukhov et al. [2021](#page-11-32)). First, it is necessary to explain that microalgae and cyanobacteria can absorb light energy through the chlorophyll molecules in their structure (Heimann and Huerlimann [2015](#page-10-32); Osanai et al. [2017](#page-11-0)). This absorbed energy begins a set of redox reactions, driven by the light energy in the reaction centers of photosystems I and II (Mamedov et al. [2015](#page-11-33)) as the frst stage of oxygenic photosynthesis. The efect of SMF on photosystem II has already been studied by various authors (Small et al. [2012;](#page-11-30) Deamici et al. [2019a;](#page-10-22) Sukhov et al. [2021\)](#page-11-32). The results have allowed us to conclude that the SMF positively afects PS II, increasing its quantum yield (Deamici et al. [2019a\)](#page-10-22). Although these results are closely linked to the mechanism that explains the formation of pairs of radicals, the experimental evidence that supports this relationship still needs to be improved. For example, Tu et al. [\(2015\)](#page-11-11) attributed the growth of algae and the production of oxygen to the stimulation of MFs in the energy fow and to the cyclic system of electron transfer in the photosynthesis process, which leads to the release of oxygen and the generation of electrons from high potential, so oxidation–reduction reaction process is facilitated. Likewise, Huo et al. ([2020\)](#page-10-8) relate the increases in cell concentration when the 30 mT SMF was applied to improving the bio-electrocatalytic reactions of a set of enzymes and other modifcations at electronic levels. The studies carried out by Deamici et al. [\(2016\)](#page-10-6) proposes a possible explanation related to the fact that SMF afects microalgae growth by changing the concentrations of free radicals or the reaction rates that involving these radicals.

Also in the research carried out by Huo et al. ([2020](#page-10-8)) an increase in the concentration of biomass was observed, when the flamentous microalga *Tribonema* sp. was grown at low temperatures (25 $\degree$ C) with the application of the SMF. These researchers attribute this behavior to the fact that MF can afect the bio-electrocatalytic transformations of various sets of enzymes in algae cells by enhancing electron transfer at the interface of the electrode solution. In the study carried out by Deamici et al. ([2019a\)](#page-10-22) with the cyanobacterium *A. platensis*, it was observed that during the action of a SMF of 30 mT the photochemical processes in the electron transport chain improved, compared to the control groups. However, the absorption fux per reaction centre was not afected.

Another critical point is that application of MF might trigger the formation of pairs of radicals in the media the cells are cultured in, which might afect cell growth. Chu et al. [\(2020](#page-10-15)) considered that a decrease in the specifc growth rate could be related to an increase in free radicals in the media due to applying an SMF with intensities of 30 mT and 40 mT. As mentioned before, it is clear that the application of MF does not have a linear behavior in terms of positive observed results (Zhang et al. [2017;](#page-11-9) Deamici et al. [2021](#page-10-16)). To protect cells from the stress they are exposed to, such as radicals in the media, MF might mediate metabolic reactions in the cells, ultimately limiting their growth and altering their biochemical composition. So, investment in defense mechanisms is at the expense of cellular growth.

With the results of the phototaxis and hypoosmotic shock, experiments reported for the dinofagellate *Gymnodinium catenatum*, Vale ([2017](#page-11-34)) presented a hypothesis for two separate damaging mechanisms that condition the natural blooms of *G. catenatum*. One mechanism depends on solar radiation, and the other relates to geomagnetic activity. In the particular case of geomagnetic activity, the researchers explained that EMFs could alter the activity of ion channels, specifcally voltage-gated calcium channels. Variations in EMFs caused by tectonic activity may infuence the physiology of *G. catenatum* (Vale [2017\)](#page-11-34).

To better understand the processes triggered during the light phase of photosynthesis when SMF is applied, it is necessary to explain some basic processes related to light capture by photosynthetic organisms. From the moment, we will focus only on the photochemical process that occurs in the RCs of the PS II, which is where the efect of the SMF has been evaluated.

During the light reactions of photosynthesis the light captured by the light-harvesting complex (LHC) is transferred to the reaction centers (RC) of photosystem II (PS II) (Junge [2019](#page-10-33)). Special chlorophyll P680 is found in the RCs of PS II (Vinyard et al. [2013\)](#page-11-35). These special chlorophylls will be excited by the absorption of a photon of light energy, generating the oxidized form of chlorophyll (P680**<sup>+</sup>**) (Mamedov et al. [2015\)](#page-11-33). From this moment on, the excited chlorophylls of the RCs will reduce to a pheophytin molecule (Phe), forming the radical pairs  $P680^{\bullet+}$  and Phe<sup>•−</sup> in the PS II (Barber [2003;](#page-10-34) Mamedov et al. [2015\)](#page-11-33). The radical Phe**.−** reduces a plastoquinone molecule bound to the protein quinone A  $(Q_A)$ , beginning an electron transfer process between photosystem II and I (PS II and PS I) where quinone  $B(Q_B)$  also intervenes through the electron transport chain (Mamedov et al. [2015;](#page-11-33) Shen [2015](#page-11-36)). These oxidation–reduction reactions in a chain release the energy necessary for synthesizing adenosine triphosphate (ATP) (Leister [2022\)](#page-11-37). Then the ATP generated together with nicotinamide adenine dinucleotide phosphate (NADPH) will be used by the cells as a source of chemical energy during the dark phase of photosynthesis.

Results have verifed that variations in the primary photochemistry of PS II occur when SMF is applied to cells. These variations are related to the increase in the respiration rate and the photosynthetic evolution of oxygen (Small et al. [2012](#page-11-30)). This photosynthetic oxygen increase process constitutes evidence demonstrating that the efects of SMF have a direct relationship with light absorption by microorganisms; they are also associated with the photochemical processes that occur in PS II, which is where the photolysis of water takes place. The photolysis of water is produced by breaking the chemical bonds that constitute the water molecule due to the incidence of light energy. This photochemical reaction gives rise to hydrogen and electrons as reaction products, which will form part of the subsequent reactions, in addition to the gaseous dioxygen that is expelled into the medium (McEvoy and Brudvig 2006).

On the other hand, the primary photochemistry of PS II has also been studied by Deamici et al. [\(2019a\)](#page-10-22). They observed that when the SMF is applied for 1 h and 24 h per day, the maximum quantum yield for primary photochemistry  $(\varphi_{\text{no}})$  increases concerning control cultures. This improvement in PS II photosynthetic efficiency is closely related to the increase in the probability that a trapped exciton moves an electron into the electron chain beyond  $Q_A$  ( $\psi_o$ ) and quantum yield for electron transport ( $\phi_{eo}$ ). However, there is another parameter that these authors evaluated that is closely related to the formation of radical pairs, and that was not analyzed in depth. This parameter is the absorption fux per reaction center (ABS/RC) and is considered an estimate of the antenna size of the photosynthetic apparatus. The ABS/RC value is inversely proportional to the number of active RCs of the PS II. The results showed that with the application of the SMF, the ABS/ RC decreased for all the cultures exposed to the magnetic treatment. This fnding indicates that in PS II there is an increase in active RCs. The concentration of the oxidized form of chlorophyll (P 680**<sup>+</sup>**) increases, which will then reduce the pheophytin molecule, increasing the concentration of the radical pairs P680•+ and Phe•− in the PS II. With this interpretation of these results, it could be said that the mechanism of action that best explains the photochemical processes of SMF in microalgae is the formation of radical pairs of the RC of PS II.

# **What happens to the microalgae and cyanobacteria culture medium under the action of MF?**

Water is one of the essential substances for the development of any living organism. Therefore, any external or internal disturbance exerted on this substance could afect the development of living organisms. The efects of MFs on the physical–chemical characteristics of water have already been confrmed by several authors (Wang et al. [2018;](#page-11-38) Yuan et al. [2020\)](#page-11-29). However, it has been verifed that the efects of magnetically treated water on biological systems are generally associated with the presence of dissolved salts in the liquid medium.

For example, Abdulraheem and Jameel [\(2021](#page-9-3)) evaluated the effect of water treated with different MF intensities (1000) and 3000 G) and diferent salinities with electrical conductivities of (0.7, 4, and 8 dS m<sup>-1</sup>) on the growth of the sunflower plant. They observed that the use of saline water with magnetic treatment could increase germination, root depth, stem height, and humidity of both the aboveground parts and the roots of the sunfower plant at diferent levels depending on the part of the plant, the value of the electrical conductivity of the irrigation water and the intensity of the MF. Similar results were obtained by Samarah et al. ([2021](#page-11-39)) in the evaluation studies of the application of magnetically treated saline solutions on tomato seeds. Magnetic treatment of water or seeds improved seed germination percentage, germination rate, and seedling growth in two laboratory experiments, especially under salinity stress of 5 and 10 dS m−1. Based on these results, we could say that MFs applied to saline solutions induce the formation of an electric feld due to the "Hall efect", discussed in the mechanisms section. Nevertheless, more studies are still needed to corroborate this statement and its relationship with the triggered biological processes.

The previous results are vital for microalgae and cyanobacteria cultivation since any variation can positively or negatively infuence these microorganisms in the culture medium. That is why this knowledge constitutes the basis for the development of technologies for wastewater treatment. The research carried out by Poshtarenko [\(2021](#page-11-40)) has verifed that MF technology in wastewater treatment reduced the turbidity of the water and eliminated its impurities, mainly in the presence of iron ions. This author addressed the polarizing effect of the magnetic field on ions and water molecules as a possible hypothesis for the efect of MF on wastewater.

Although still with a limited number of studies, some authors have already begun to report changes in the microalgae culture medium when this physical agent is applied. Han et al. ([2016](#page-10-30)) investigated the cultivation of *Chlorella pyrenoidosa* in wastewater and the application of an SMF of 0.18–0.70 T. They reported that magnetic treatment of wastewater resulted in a 9% signifcant reduction in water turbidity. This efect is not only scientifcally crucial from a chemical point of view. Also, from a physiological point of view, a decrease in the turbidity of the wastewater is of great importance since there will be a larger availability of light for the microorganisms to carry out photosynthesis. Moreover, it was observed that the pre-treatment of

municipal wastewater before microalgae cultivation had a more beneficial effect on the accumulation of biomass and lipids (Feng et al. [2020](#page-10-35)). However, there were no signifcant diferences when the magnetic treatment was applied in an installation with wastewater and microalgae. These experimental results prove that MF application on culture media from the chemical point of view afects its physical and chemical characteristics.

# **General conclusion and future perspectives**

This review discusses the main biological effects induced by magnetic felds (MFs) in microalgae and cyanobacteria. Currently, only three mechanisms are reported to attempt to explain the efects of MFs on biological systems, including (1) magnetic induction, (2) magneto-mechanical efect, and (3) the formation of radical pairs. Despite the existence of these mechanisms, the technology currently available has not allowed the scientifc community to directly evaluate them. Most effects observed in microalgae and cyanobacteria are based on biochemical responses and cell growth, which have been manifested through metabolic and/or genetic changes, electron transition changes linked to reaction centers of the microorganisms, and structural changes in cells. Evaluating the efects of static magnetic felds (SMF) at an electronic level is a complex process that requires adequate equipment for experimental studies and correct and profound interpretation to establish relationships between the experimental results obtained with the proposed mechanism of action. The most robust mechanism discovered so far is related to the formation of radical pairs associated with electron transition changes linked to the reaction centers of the photosynthetic apparatus of microorganisms.

On the other hand, many questions about the effects of magnetic felds (MF) on microalgae and cyanobacteria and all the factors associated with their cultivation remain unanswered, such as the efects of MF on culture media. These questions can be resolved by standardizing the results obtained by diferent authors and reporting essential parameters such as magnetic intensity, type of magnetic feld, type of spatial distribution of the magnetic feld, and chemical composition of devices used for magnetic treatment. Furthermore, future studies should report the specifc strain used in their research, as even within the same species, variations may depend on the physiological state of the microorganisms and their isolation source, among others. Other crucial factors to consider are the growth phase at which the MF is applied to the microorganism and the duration of the exposure to the MF.

Many emerging concepts focus on new approaches for the application of MF on microalgae, ranging from the production of magnetically modifed cells (Savvidou et al. [2019\)](#page-11-41), to the development of techniques for focculation of these microorganisms from magnetic nanoparticles (Álvarez-Manzaneda and de Vicente [2017](#page-9-4); Yin et al. [2020\)](#page-11-42) and also as pre-treatment for the wet extraction of the biomass, observing excellent results during the extraction (Guo et al. [2019](#page-10-36)). Magnetic treatment technology has the added value of solving signifcant environmental problems, such as wastewater treatment integrated with microalgae cultivation. In addition, this technology has the advantages of low operating cost, no proven toxic efects, does not produce secondary contamination, provides a wide range of applications, and has a long service life (Zhang et al. [2017\)](#page-11-9). Therefore, commercially exploiting the full potential of products derived from microalgae and applying MFs can improve the economic feasibility of microalgae and cyanobacterial biorefneries.

**Authors contribution Y.S. Font:** Conceptualization, Investigation, validation, visualization, writing-original Draft **Y.O. Díaz:** Conceptualization, writing – Review and editing **A. Cuypers**: Conceptualization, Resources, Writing – Review and Editing, funding acquisition, **E.I Alemán:** writing – review and editing, validation, funding acquisition, supervision; **D. Vandamme**: conceptualization, resources, writing – Review and editing – supervision – funding acquisition.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Declarations**

**Conflict of interest** None.

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