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REVIEW

Magnetic treatment of microalgae for enhanced product formation

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Abstract Static or modulated magnetic fields (MF) may interact with the biological system and affect the metabolism of microorganisms, such as their photosynthetic capacity or synthesis of carbohydrates. Their effects on microorganisms, which can be classified into inhibiting, stimulating and null, may be interpreted as the result of stress that cells undergo, thus, leading to responses through the same mechanisms. Biological effects of exposure to magnetic forces depend on magnetic intensity, frequency and exposure time. Modifications in these parameters may enhance product formation. Effects differ according to the form and application of MF characteristic parameters. Magnetic treatments have the advantages of being convenient and non-toxic, having low running cost, emitting no secondary pollution, enabling wide application and being easily shielded. MF application to the cultivation of microalgae, to improve the production of finished biomolecules, is a simple, inexpensive and powerful process. However, bioeffects of MF on microalgae need to be further investigated because there have currently been very few available reports in the literature. Thus, studies which aim at optimizing parameters involved in MF application must be developed in order to obtain the best conditions for the production of molecules with high economic potential.

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Introduction

Microalgae are a large and diverse group of aquatic organisms that lack the complex cell structures found in higher plants (Bowles 2007). Most species are photoautotrophic and convert solar energy into chemical forms through photosynthesis (Slade and Bauen 2013). These microorganisms are considered very promising for multi-purpose biomass production because they can grow rapidly, produce large quantities of lipids, carbohydrates and proteins, sequester and recycle CO_2 from industrial flue gases and remove pollutants from industrial, agricultural and wastewaters (Hunt et al. 2009). Although the commercialized production of microalgal biomass, as healthy food and valued-added additives is profitable, more efficient and inexpensive cultivation technique has been searched for (Zhang et al. 2015).

Magnetic fields (MF) have been applied during the growth of different species of microorganisms and different results have been found. Researches with MF application have shown differences in cellular behavior when cells are submitted to magnetic treatment. Its effect may be negative or positive, and may include the acceleration of growth and changes in metabolism (Hunt et al. 2009). According to Yang et al. (2011), these modifications depend on the intensity, frequency and the functional time.

The potential link between MF and the effect caused on living organisms is due to the fact that it causes oxidative stress, i.e., the MF can alter the energy levels and orientation of the spin of electrons, thus increasing activity, concentration and time life of free radicals (Repacholi and Greenebaum 1999; Sahebjamei et al. 2007).

MF exposure might enhance the growth of many species of microalgae and cyanobacteria, although a broader study of effects of MF is needed (Small et al. 2012).

Magnetic field

MF come from moving electrical charges. A charge creates an electric field, either at rest or in motion. However, a charge creates MF only if it is in motion (Halliday et al. 2012). MF can be generated by magnets or by electric current. Magnets are composed of different conductive materials, such as neodymium and ferrite. Each material has its characteristic magnetic intensity and generated MF are not uniform throughout the magnet. The generation of MF by electric current occurs by straight conductors, circular loop, flat circular coil, toroids, electromagnets and solenoids. A solenoid is a device constituted of a long conductor wire wound in the form of turns (Giancoli 1998). MF produced near the center of the solenoid, when being driven by an electric current, is practically uniform. In permanent magnets, electric charges are created by the movement of electrons in the iron atoms that constitute the magnet, while in the electromagnets, the electromagnetic charges area created by electrons movement in the conducting wire in coils. The unit in the International System is Tesla (T) and the Earth's MF near its surface is approximately 10^{-4} T (Halliday et al. 2012).

MF can be represented by field lines which are strong where the lines are closer to each other. They are weaker where the lines are separate, as shown in Fig. 1. The field lines emerge from the north pole (N), whereas the other end is the south pole (S).

Regarding to the intensity, MF may be classified into weak (<1 mT), moderate (1 mT to 1 T), strong (1–5 T) and ultrastrong (>5 T) (Dini and Abro 2005). MF can be uniform, i.e., they maintain the same field strength at a given location, continuous, i.e., they maintain certain intensity over time and alternating, i.e., the intensity varies. Effects of MF on cell growth can be classified into inhibitory, stimulant or null, and they can affect the direction of migration and alter the growth and reproduction of microorganisms, thus, generating a change in the rate of cell growth (Zapata et al. 2002, 2005).

MF interaction can be classified into three levels: (1) magnetic flux density, i.e., static MF exert forces capable of moving ions, such as electrolytes in solutions, leading to induction of electric fields and currents; (2) magneto-mechanical effect, in which uniform static MF produce twists in certain molecules and ferromagnetic materials, such as magnetite; and (3) electronic interactions in the body, since static MF can change power levels and direction of rotation of electrons (WHO 1987).

Some studies have reported that MF intensity, production of by-products and growth of microorganisms have happened at high and low intensities. For instance, Singh et al. (1994) used 300 mT in *Anabaena doliolum* microalga, and found increase biomass, pigments, carbohydrate and protein concentrations. Tu et al. (2015) concluded that 100 mT increased the growth and oxygen production of *Scenedesmus obliquus*. Small et al. (2012) used 10 mT and found that the biomass nutritional value increased in relation to protein, pigment, Ca and Zn contents. Nimitan and Topola (1972) used only ~4.7 µT (0.0047 mT) in the culture of *Saccharomyces cerevisiae* and got increased alcohol production. These results showed that even at low intensities by-products of interest, may be produced, since MF act differently on microorganisms and high amounts of by-products can be obtained (10-100-fold higher).

Magnetic field interaction with biological systems

Microorganisms are formed by numerous systems. Their physiological and biochemical constitution may be susceptible to the action of magnetic forces. In general, all metabolic reactions are based on the difference of electrical charges and ions of the system (Teng 2005). All magnetic forces

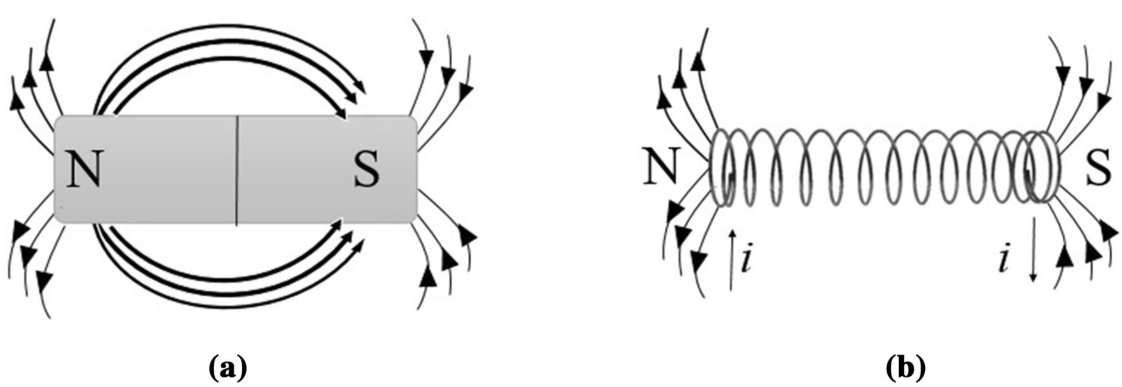

Fig. 1 Scheme of induction lines and location of magnet poles (**a**) and solenoid (**b**)

cause changes in metabolization in the biological cells and movements of electrons and ions, activities of free radicals, protein and enzymes, permeation of biofilms and cell growth (Yang et al. 2011).

Repacholi and Greenebaum (1999) reported that there are three established mechanisms by which MF interact with living organisms. The first one is the so-called magnetic induction, i.e., MF exert forces on moving ions in solution (e.g., electrolytes), which gives rise to induced electric fields and currents. The second is the magneto-mechanical effect, i.e., uniform MF produce torques on certain molecules and any ferromagnetic materials in the body. The third mechanism, which is often used for explaining the variation of biological reaction in cells, is electronic interaction, i.e., MF can alter energy levels and spin orientation of electrons. MF alter the spin states of free radicals, which in turn, change the relative probabilities of recombination and other interactions, possibly with biological consequences.

The effect of SMF on the concentration of free radicals is amplified if the radical pair is oppositely charged and physically confined (Engström 2006). According to the magneto-hydrodynamic effect theory proposed by Katz et al. (2005) and confirmed by Li et al. (2011), MF application in a solution perpendicular to the ion transfer direction, produces a magnetic force on the moving ions. The hydrodynamic flow results in decrease in the diffusion layer thickness, and leads to an accelerated transport of the electroactive species toward the electrode surface. The transition metal ions contained in some enzymes can be paramagnetic under MF, which results in increase in enzyme activity. In addition, Katz et al. (2005) and Li et al. (2011) confirmed that MF can affect the bioelectro catalytic transformations of several enzyme assemblies through enhancing electron transfer at the electrode solution interface. Therefore, it is expected that MF enhance cellular growth (Tu et al. 2015). However, Wang et al. (2006) reported that high MF intensities results in changes in enzyme conformation, which further impacts the intracellular biochemical reactions and inhibits algal growth.

Beruto et al. (2014) concluded that the effect of the low intensity electromagnetic field on the growth of *Chlorella vulgaris* did not act on the mitotic division, but MF played a significant role in promoting cell clusterization in the liquid phase.

Bioeffects on microalgae

The effect on the metabolism of algae and microalgae due to the action of MF has been studied since the last decade, with an optimization of biotechnological processes, such as wastewater treatment (Tu et al. 2015), protein production (Yang et al. 2011), biomass production (Luna et al. 2011; Small et al. 2012; Deamici et al. 2016a) and pigments concentration (Deamici et al. 2016b).

Different exposure time, microalga species and MF intensity have given distinct responses. Thus, different strategies of MF application, such as, continuous exposition or application for a certain period of time during cultivation have been carried out. Luna et al. (2011) observed in a culture of *C. vulgaris* that permanent exposition to MF led to a prolonged disturbance that is imposed on the cultivation. Therefore, microalgae immediately adapt to this perturbation and no measurable metabolic response has been observed. The authors concluded that MF application for 1 h could be a good application protocol for batch cultivation. However, for continuous and semi-continuous cultures, permanent application would be useful to promote protein accumulation.

Small et al. (2012) suggested that increased oxidative stress and free-radical effect appears to be the most likely mechanism for the effect of MF on microalgae. Thus, MF treatment affects the microalgae by changing free-radical concentrations.

Chlorella kessleri, Scedesmus armatus and *Chlamydomonas reinhardtii* have been grown phototrophically from 0.02 to 0.2 mT. Data indicate that the rate of cell division is larger at 0.02 than at 0.2 mT. No reliable effect is found on pigment contents (Pazur and Scheer 1992).

Hirano et al. (1998), in the cultivation of *Spirulina platensis* IAM M-135, observed decrease in the carbohydrate content and growth inhibition when this microalga was subjected to MF at intensities above 10 mT. According to theses authors, the inhibition of the reaction system of the Calvin–Benson cycle, in which the carbohydrate is synthesized from CO_2, probably was one of the causes for the growth inhibition of *Spirulina platensis*. The consequent response is the growth inhibition at intensities above10 mT.

Application of 0.25 T enhanced growth of *Spirulina platensis* and higher algal cell density was achieved in a shorter culture time. In addition, the nutritional composition of this microalga was improved in essential amino acids (histidine) and trace elements (Ni, Sr, Cu, Mg, Fe, Mn, Ca, Co and V) (Li et al. 2007).

According to Zhi-Yong et al. (2007), enhanced growth rates may be associated with increased uptake of nutrients due to permeability of the membrane. This fact has been reported in the case of *S. platensis* cells exposed to electromagnetic fields. Other authors explain that in assays performed with this cyanobacterium, rapid growth is due to the high rate of nitrogen and phosphorus consumption (Li et al. 2007).

Wang et al. (2008) investigated the effect of MF on the antioxidant defense system of *C. vulgaris*. The activity of superoxide dismutase and peroxidase increased significantly at 10–35 mT. However, a remarkable increase in catalase activity occurred at 45–50 mT. Thus, MF application

Table 1 Studies of magnetic field application to microalga culture

Microorganism (reference)	MF intensity (mT)	Exposure time (h)	Positive results	Negative results
Spirulina platensis (Li et al. 2007)	0–550	3	250 mT increased cell dry weight in 22% and improved essential amino acids and trace elements (Ni, Sr, Cu, Mg, Fe, Mn, Ca, Co and V)	550 mT induced cell death after the 4th day
Chlorella vulgaris (Wang et al. 2008)	0–50	12	Specific growth rate and growth was significantly increased; its antioxidant defense system was regulated at10-35 mT	Specific growth rate was lower at 5, 45 and 50 mT and DPPH activity decreased at 45 and 50 mT
Haematococcus pluvialis (Luna et al. 2009)	30	0.15, 0.30,1 and 24	MF for 0.15 h increased growth, cell division and pigment synthesis	MF for 0.15, 0.30 and 1 h decreased maximum cell density but did not enhance chlorophyll content
Chlorella kessleri (Small et al. 2012)	10	Every 18 min (recycle)	Fourfold biomass and lipid production rate and increased protein, pigment, Ca and Zn contents	Increase in oxidative stress was measured indirectly as decrease in antioxidant capacity: decrease in cell size and changes in chloroplast organization
Scenedesmus obliquus (Tu et al. 2015)	50–500	0.5	100 mT stimulated both algal growth and oxygen production. There was 11.5% increase in chlorophyll-*a* content after 6 days	400 and 500 mT inhibited growth and chlorophyll-*a* content
Chlorella fusca (Deamici et al. 2016a)	30, 60	1 and 24	60 mT (24 h) increased biomass concentration, besides carbohydrate and protein contents	30 mT (1 h) decreased the carbohydrate content
*Spirulina*sp. LEB 18 (Deamici et al. 2016b)	5, 30 and 60	1 and 24	MF for 24 h stimulated growth and increased productivity, besides protein and carbohydrate contents	30 mT for 24 h inhibited growth
Chlorella kessleri (Bauer et al. 2017)	30, 60	1 and 24	60 mT (1 h) stimulated biomass concentration in 83.2%, besides stimulating synthesis of lipids, pigments and antioxidants	MF for 24 h enhanced neither growth nor carbohydrate content

increases the microalgae growth and regulate its antioxidant defense system to protect cells efficiently.

The effect of 0.03 T on *C. vulgaris* cultivation using soy waste as cultivation medium was evaluated and high cellular densities in a shorter culture time were obtained. The quality of the biomass of *C. vulgaris* is evaluated from the biochemical point of view and the highest lipid concentration was observed when MF were applied to the stationary phase (Luna et al. 2011).

The maximum protein content was reached when 0.24–0.3 T was applied on *C. vulgaris*. Analysis indicates that weaker magnetic induction density can speed up *Chlorella* growth and protein content. If 0.24 T was applied, protein and oxygen are produced properly. However, if MF from 0 to 0.18 T were applied, protein content in *Chlorella* increase and from 0.24 to 0.30 T, maximum content of protein was reached (Yang et al. 2011).

Small et al. (2012) exposed *Chlorella kessleri* to a uniform MF in laboratory-scale raceway. Ten mT more than doubled the growth rate by comparison with the control and increased carbohydrate, protein, chlorophyll *a* and chlorophyll *b* contents. According to the authors, MF exposure increased chloroplast and thylakoid area, but thylakoids became disorganized, with more abundant chloroplast starch granules. The use of permanent magnets could reduce the cost of algal oil production from about $1.80/L to about $0.45/L, below the cost of palm oil, neglecting the cost of downstream processing. Closed bioreactors or open raceways ponds can be used to increase biomass production with MF application. Fifty percent increase in respiratory rate than growth rate could be due to an increased need for nicotinamide adenine dinucleotide phosphate (NADPH) to reduce nitrate for the increased protein content.

Scenedesmus obliquus was grown in municipal wastewater with MF application. The calculation of growth and oxygen production rate was based on the change in dissolved oxygen concentration. Results indicated that magnetic treatment stimulates both algal growth and oxygen production. Application of 100 mT in the logarithmic growth phase for 0.5 h increased the chlorophyll-*a* content in 11.5% over the control after 6-day growth. In addition, magnetization enhanced the oxygen production rate in 24.6% over the control (Tu et al. 2015).

MF and exposure time have a significant effect on the growth of *Chlorella fusca* LEB111. An application of 60 mT throughout all cultivation increased biomass concentration in 20.5% by comparison with control cultivation, which resulted in the largest increase of carbohydrates (24.8% more than those obtained in control) (Deamici et al. 2016a).

Growth of *Spirulina* sp. LEB18 was influenced by the action of MF. Thirty and 60 mT for 1 h d^{-1} stimulated growth, thus leading to higher biomass concentration by comparison with the control culture. Increases in productivity (105.1%, 60 mT, 1 h d^{-1}), protein (16.6%, 60 mT for 24 h d^{-1}) and carbohydrate contents (133.2%, 30 mT for 24 h d^{-1}) were observed. Results showed that MF may influence the growth of *Spirulina* sp., since it triggers a stimulating effect and can leads to twofold biomass concentration in equal cultivation time periods (Deamici et al. 2016b).

Some of responses obtained with MF application to microalgae culture are summarized in Table 1. As with other organisms exposed to the action of MF, in microalgae, effects caused by the action of MF (Table 1) depend on the application strategy and exposure time.

Conclusion

Studies have shown that the MF application to microalga culture has significantly influenced the growth of these microorganisms as well as the production of compounds of interest. However, effects of this application may stimulate or inhibit the growth and production of biocompounds. In order to obtain satisfactory results, for each species of microalga, MF intensity, exposure time, period of application during cultivation and devices used to apply MF must be evaluated. Since MF can be achieved through magnets or solenoids, for each type of photobioreactor, these devices around the photobioreactor or with the external circulation of the culture medium must be adapted. MF application in microalgae is a promising process, as it has already demonstrated that it can increase growth of microalgae and consequently the yield of products with low costs of process modification.

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