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Magnetic field gradient inhibits *Saccharomyces cerevisiae* growth

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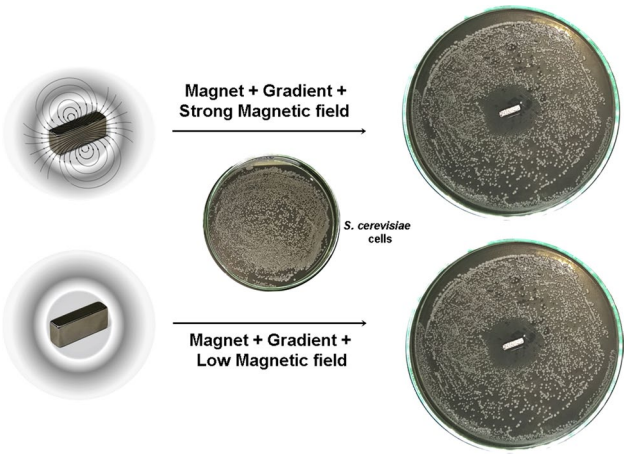
Abstract

The daily exposure of humans to artificial magnetic fields has inspired studies of their effects on biological systems. Different views are advocated by many research groups and few studies have clarified the role of magnetic field gradients in observed results. We investigated the effect of strong gradients and continuous magnetic fields in a cellular system. Colonies of *Saccharomyces cerevisiae* CCMB 355 were grown in solid and liquid media and exposed to the neodymium–iron–boron magnets. Notably, in solid medium, cells exposed to previously demagnetized NdFeB magnets or to the metals contained in magnets exhibited normal activities, but when exposed to the gradient, growth drastically fails near the magnet, even when the intensity of magnetism was near zero. Increasing the distance of the magnet to regions of weak magnetic field gradient caused decreased cellular malaise. When the magnet was removed, the cells were not capable of growing again, indicating that the gradient killed the exposed cells. In liquid medium, we observed a decrease in the absorbance values in the region of 560 nm when the substrate was directly permeated by a strong magnetic field gradient in comparison with a control without the magnet or with a magnet covered with a thin layer of silicone. This study helps to clarify the effect of the magnetic field gradient in biological systems.

Graphic abstract



Keywords NdFeB magnets · Magnetobiology · Yeast · Cell reduction · Biocide

1 Introduction

Humans are constantly surrounded by magnetic fields of various intensities and frequencies (Zablotskii et al. 2016a). Neodymium–iron–boron (NdFeB) magnets are crucial for electronic equipment such as hard disk drives, cell phones and allow the existence of most audio equipment such as

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speakers, phones and headphones (Du and Graedel 2011; Rollat et al. 2016; München and Veit 2017). Moreover, NdFeB magnets have low cost, produce a strong magnetic field and are increasingly used in household items such as clothes, toys, and jewels (Ryf et al. 2008).

The understanding of the "action mode" of magnetic fields is important for many purposes and is crucial to protect human health of possible damages (Dini and Abbro 2005). It was demonstrated that magnetic fields can also be used to manipulate the growth of cells and cellular components of industrial and biotechnological interest. Studies attempted to elucidate the effects of static magnetic fields (SMF), a magnetic field that does not change over time, on biological cells. However, contrasting results published in literature make the issue controversial, due to irreproducibility and lack of a theory that supports the results (Albuquerque et al. 2016; Zablotskii et al. 2016a). The SMF may be homogeneous or heterogeneous in space. Unsatisfactory experiments with strong homogeneous fields reveal that the biological effects are not produced by the magnetic field, but by the permanent magnetic field gradient (PMFG). The PMFG can be defined as the change of field intensity along the space (Kimball 1938).

Saccharomyces cerevisiae has been constantly used in investigations, because it is considered the eukaryotic biological research model. It provides a framework for research due to high conservation of many metabolic processes common between yeast and cells of multicellular organisms including humans (Cazzanelli et al. 2018; Nielsen 2019). Several experiments with this organism exposed to magnetic fields have been reported controversial results. While some studies showed an increase in ethanolic fermentation, production of glutathione and biomass (Motta et al. 2004; Muniz et al. 2007; Galonja-Coghill et al. 2009; Santos et al. 2010, 2012), other studies reported reduction of the proliferation in solid and liquid media (Kimball 1938; Iwasaka et al. 2004) and there are also results in which no changes in gene expression, viability, growth, morphology and fermentation were observed (Zablotskii et al. 2016a).

As the interaction between electric charges controls the biochemical processes of the cellular machinery, it is expected that, from a given intensity, the exposure to a magnetic field gradient affects the magnetic moments of ions and molecules and, consequently, changes cellular functionality by competing with electrical forces (Dürr et al. 2004; Pelloni et al. 2011; Zablotskii et al. 2018). However, few studies have quantified the effects of PMFG at the intracellular level (Zablotskii et al. 2016a). Notably, a moderate intensity SMF may affect the transmembrane ion flux and, in turn, changes in membrane potential may affect all cellular machinery (Santos et al. 2012; Zablotskii et al. 2016a). The interactions of molecules with a PMFG can be explained by the ability, at the appropriate intensity, to change the conformation and preferential orientation of organic molecules with diamagnetic anisotropy, such as microtubules, and lipid tubules (Santos et al. 2012; Albuquerque et al. 2016).

Cell growth is one of the most controversial results, half of the studies reported that cells were affected by magnetic fields and the other half reported no effects (Albuquerque et al. 2016). In this research, it was hypothesized that the absence of effects in studies on magnetic radiation in biological systems is a consequence of the absence or insufficient value of PMFG exposure. We propose to study the best conditions to evidence the effects of PMFG generated by commercial NdFeB magnets on the growth of *S. cerevisiae.*

2 Materials and methods

2.1 Neodymium magnets

In the following experiments N52 neodymium–iron–boron magnets (Nd2Fe14B), with dimensions 1.0 cm × 0.5 cm × 0.3 cm are used. This type of magnet was chosen because it is not expensive and presents an approximated value of remanence (Br) up to 1.4 Tesla. Moreover, the value of the coercivity is between 0.875 and 1.99 MA/m and the Curie temperature is 320 °C. This means that they are ideal to obtain the measurements proposed in this work. In accordance with the information of the seller, they are obtained for sinterization of the dust of an alloy, slightly magnetized, containing iron, neodymium and boron. The raw pieces thus obtained are machined to obtain the desired dimensions and coated with a nickel electrochemical process to avoid oxidation. Finally, they are magnetized with an intense external magnetic field applied perpendicularly to the major surface. Unfortunately, the magnetic field gradient associated with the magnets is not easily determinable, especially when they are introduced in the biological systems tested here. It must be considered that the environment is basically a culture broth with paramagnetic characteristics. For this reason, cells and enzymes are affected by a reduced gradient when compared to that produced by the same free magnet.

To obtain an estimated value of the free MFG in the space near the surface of the magnet and along the principal axes of symmetry, we use the SS495A electronic component: https://www.alliedelec.com/m/d/cd01bc793b6489eec67e2ac2cb0089ca.pdf. This is a linear Hall-effect sensor capable of measuring the value of the magnetic field in which it is immersed. The magnetic field measurement is carried out being proportional to the voltage on pin 3 of the sensor (pins 1 and 2 are reserved for supply energy) and it can be read with a simple voltmeter. According to the datasheet of the sensor, the measurement is linear in the range from −600 to 600 G, with a sensitivity of (3.125 ± 0.125) mV/G at 25 °C

(See graph of the data sheet: "TRANSFER CHARACTERISTICS AT Vs = 5.0 VDC").

This range is to be considered as the minimum interval guaranteed by the manufacturer, while the typical range is from − 670 to 670 G. These limits guaranteed the accuracy of the measurements of the magnetic field for a distance equal or greater than 1 mm from the surface of the magnet, where an intensity of 659.2 G was observed. For lower distances, the intensity of the field exceeds the limits of confidence of the sensor as seen in Table 1, where the decrement of the MFG as a function of the distance from the surface of the magnet is presented. The chosen direction is the one that corresponds to the principal axes of symmetry of the magnet.

2.2 Theory

The well-established laws of electromagnetism can be used to predict the effects of PMFG on biological systems. In particular, the force F is calculated as the negative gradient of the potential energy E:

$$F = -\text{grad}(E). \tag{1}$$

For a particular direction, this equation is reduced to

$$F = -\partial E/\partial s, \tag{2}$$

where F is the force along the direction **s** and $s = |\mathbf{s}|$. The relation between the force applied on an electron and the magnetic field (B) in which it is immersed is:

Table 1 Estimated values for the magnetic field and its gradient as a function of the distance

Distance (mm)[a]	Voltage (Volt)[b]	Magnetic field (Gauss)[c]	Gradient (kGauss/m)[d]
0	4.89	> 764.8	
1	4.56	659.2	> 105.6
2	4.19	540.8	118.4
3	3.58	345.6	195.2
4	3.18	217.6	128.0
5	3.00	160.0	57.6
6	2.90	128.0	32.0
7	2.81	99.2	28.8
8	2.75	80.0	19.2
9	2.70	64.0	16.0
10	2.68	57.6	6.4

[a]Distance: distance from the surface of the magnet along the principal axis. [b]Voltage: voltage carried out from the SS495A sensor at the referred distance. [c]Magnetic field: corresponding value of the magnetic field obtained with the equation (Voltage − 2.5 Volts) × 1000/3.125 (See datasheet of the sensor SS495A for further details). [d]Gradient: MFG obtained dividing by 0.001 m the difference of the magnetic field in two consecutive points

$$F = -\partial E/\partial B \cdot \partial B/\partial s. \tag{3}$$

By a quantum point of view, the relation between the potential energy E of an electron and the applied magnetic field B is :

$$E = E_0 + g \cdot \mu_0 \cdot M_j \cdot B, \tag{4}$$

where E_0 is the energy of the electron in absence of magnetic field, g is the Landé Factor, Mj is the quantum number of the projection of the total angular moment and μ_0 is the Bohr magneton for the electron. Ultimately, by substitution of Eq. (4) in Eq. (3) we have:

$$F = g \cdot \mu_0 \cdot M_j \cdot \partial B/\partial s. \tag{5}$$

2.3 Yeast strain

The cells used in the experiments belong to the *S. cerevisiae* strain CCMB 355. *Saccharomyces cerevisiae* was chosen because it is a benchmark eukaryotic organism. Cryopreserved cells were supplied by the Collection of Cultured Microorganisms of Bahia, thawed, reactivated and cultured in Petri dishes with Sabouraud agar. Before using on petri dishes, cells were grown in liquid medium, nutrient broth, and quantified with a spectrophotometer at 560 nm. Only solutions with an absorbance around 0.1 were used. *Saccharomyces* is one of the yeast genera that exhibit better adaptations at different growth temperatures (Salvadó et al. 2011). Experiments were conducted at 37 °C because it is the temperature at which cells of the human body undergo the effects of magnetism, also allowing faster observable results.

2.4 Solid growth medium

Initially, the nutrient agar and the Sabouraud agar were tested three times each, to verify the growth of *S. cerevisiae* in these media. The latter was chosen to verify the effects of PMFG on this cell due to high efficiency. Rectangular parallelepiped magnets of type N52 (1.0 cm × 0.5 cm × 0.3 cm) were used. The magnetic flux at the surface was approximately 1T. The efficiency of this type of magnet in modifying the vitality of *S. cerevisiae* was tested in function of its position in Petri dishes. We studied the effect of the orientation exposing the cells to the three faces of the magnet. In addition, we tested efficiency when the magnet was not in contact with the medium (attached to the outside surface of the petri dish). Each experiment was repeated three times. The effect was also visible when the magnet was not in direct contact with the medium but was very weak when it was separated by the glass of the petri dish.

Therefore, in the following experiments, the vertical position (exposing the two larger surfaces to the medium) was

chosen to fix the magnet in the internal part of the petri dish. This position allowed a better observation of the effects of PMFG than other positions tested. To verify what was found by other researchers, the vitality of *S. cerevisiae* was tested with three series of experiments conducted at the same time and in the same conditions. In the first one, colonies of cells were cultured in presence of N52 magnets. In the second one, the same type of test was performed but the magnets used in the Petri dishes were preventively demagnetized with heat. For this experiment, an air jet heated to 300 °C was projected onto the magnets for a few seconds, taking care not to exceed the Curie temperatures. The resulting magnetic field along the perpendicular direction of the major surface of the magnet was reported in Table 2. In Fig. 1, the comparison of the magnetic fields reported in Tables 1 and 2 was presented. In the last, the magnets were covered with a thin layer of silicone to exclude the possibility of death by contamination or surface effects.

One magnet was fixed at the center of each petri dish (1 cm high, diameter 9 cm) with the characteristics described in Table 1. Anchorage was guaranteed by the solidification of the Sabouraud agar media in the petri dish. After solidification, the solution containing *S. cerevisiae* was spread over the surface of the medium with a Drigalski spatula in a sterilized laminar flow cabinet. All petri dishes received 50 µm of the liquid medium with *S. cerevisiae* and the same number of cells, previously quantified spectrophotometrically by SP—2000UV (Spectrum, Shangai, China) using the 560 nm region and with absorbance around 0.1. The

Table 2 Estimated values for the magnetic field and its gradient as a function of the distance for partially demagnetized magnets

Distance (mm)[a]	Voltage (Volt)[b]	Magnetic field (Gauss)[c]	Gradient (kGauss/m)[d]
0.0	0.88	132.3	
1.0	0.91	130.0	2.3
2.0	1.06	118.5	11.5
3.0	1.46	87.7	30.8
4.0	1.88	55.4	32.3
5.0	2.05	42.3	13.1
6.0	2.22	29.2	13.1
7.0	2.30	23.1	6.1
8.0	2.35	19.2	3.8
9.0	2.40	15.4	3.8
10.	2.45	11.5	3.8

[a]Distance: distance from the surface of the magnet along the principal axis. [b]Voltage: voltage carried out from the SS495A sensor at the referred distance. [c]Magnetic field: corresponding value of the magnetic field obtained with the equation (Voltage − 2.5 Volts) × 1000/3.125 (See datasheet of the sensor SS495A for further details). [d]Gradient: MFG obtained dividing by 0.001 m the difference of the magnetic field in two consecutive points

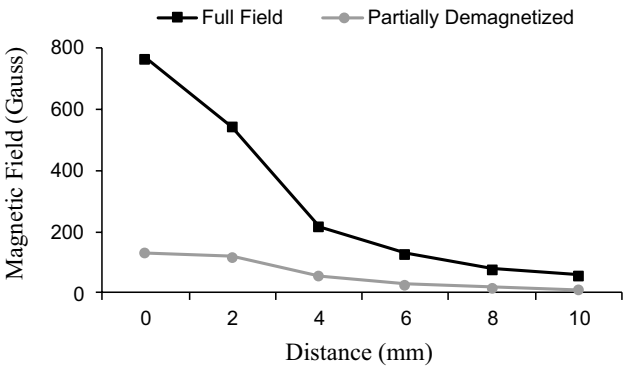

Fig. 1 Comparison between magnetic fields of the magnet as it comes from the factory and after thermal demagnetization. See text for details

petri dishes were maintained at 37 °C for 24 h in a constant temperature incubator.

To verify if the thin silicone layer covering the magnets would affect the cells, petri dishes were prepared with a blob of silicone of the same dimension and shape of the used magnets. After 24 h of growth, no effect was visible. Moreover, petri dishes with no magnet, with and without cells, were also prepared and maintained in the incubator as a control and to discard the possibility of contamination. Finally, cells were cultivated (as described above) with demagnetized and crushed magnets to confirm that the metals do not affect the growth.

In all the experiments, the magnets were disinfected with 70% alcohol before being placed in the petri dishes and successively exposed to UV radiation for 15 min with Sabouraud agar. To verify if the gradient generated by the magnet causes the death of *S. cerevisiae* or only stagnates the growth, after 24 h growing in solid medium, the magnet was removed with metal tongs and the petri dish was left for a further 24 h in a constant temperature incubator.

2.5 Liquid growth medium

Falcon tubes were used to compare the following treatments:

1. cell-free nutrient broth
2. nutritious broth with magnet
3. nutritious broth with silicone-coated magnet
4. nutritious broth with cells
5. nutritious broth with cells and magnet
6. nutritious broth with cells and silicone-coated magnet

Previously, tubes were prepared with 3 mL of nutrient broth and cells of *S. cerevisiae*. The tubes were incubated under stirring in hybridization incubator COMBI-SV12 (FinePCR, Seoul, Korea) at 40 rpm for 24 h at 37 °C. The solutions of tubes were quantified spectrophotometrically at

560 nm and one of the tubes with absorbance around 0.1 was randomly chosen. Subsequently, the tubes of the treatments were prepared in the same way with 50 µL of solution containing the cells of *S. cerevisiae* and incubated as described above. The magnets used in these experiments were of the same type as those used in the solid medium experiments. The position of the magnet inside the tube was held by magnetic interaction with other magnet positioned on the outer side. After 48 h, the solutions were quantified spectrophotometrically at 560 nm. For statistical reasons, ten replicates were performed for each treatment above.

2.6 Statistical analyses

For the analysis of solid medium results, the presence, absence and size of inhibition halo in comparison with controls were considered. Using Magview® green film, it was possible to see the gradient generated and compare it with the halo format (see online supplementary material). The data of the liquid medium experiments were analyzed using Kruskal–Wallis, a non-parametric test to detect significant differences in absorbance measurements among treatments and control. All statistical analyses were conducted in the R statistical computing environment version 3.5.3 (R Core Team 2018).

3 Results

3.1 Solid growth medium

An approximately circular cell growth inhibitory halo was observed around the magnet. The cells were affected in an inhomogeneous manner according to the reduction of the force of the field gradient over space. Where the field gradient loses force, the colonies were smaller than those of the remainder of the petri dish. Halos of similar size, approximately 2.5 cm in diameter, were also observed in the media in which the magnets had the magnetic field previously reduced with heat while the field gradient remained approximately unchanged (Fig. 2), proving that the magnetic field was not responsible for cell death.

Another important result was that the cell death drastically stops at a distance of about 1 cm from the surface of the magnet, where the calculated value of PMFG was 6.4 kGauss/m (Table 1). Interestingly, the corresponding value of the magnetic field was 57.6 Gauss but, comparing Fig. 2b with Table 2, it is observable that the mortality induced by the magnet with magnetic field that has been thermally reduced, continues with a value of 23.1 Gauss where the PMFG was 6.1 kGauss/m. Unfortunately, due to the inherent difficulty of biological experiments, it was very difficult to obtain a more accurate value keeping the system

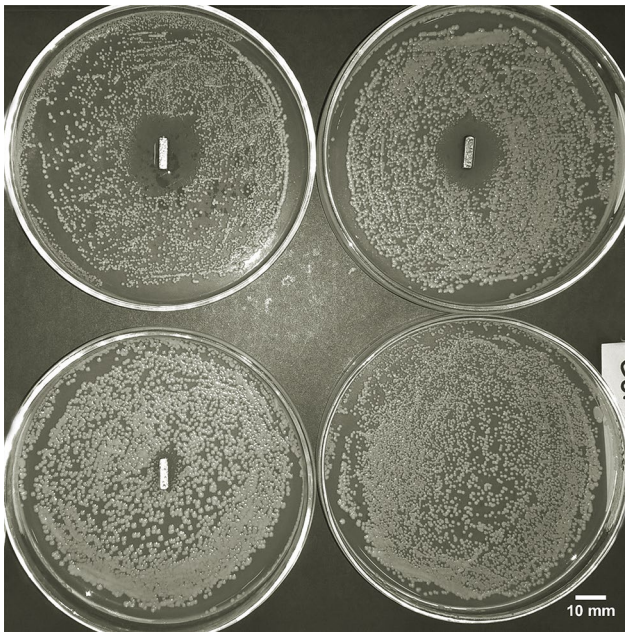

Fig. 2 Experiment made in solid medium. The magnets were used in vertical position because previous experiments showed better efficacy in highlighting cell death. **a** Top left photo: experiment with the bare magnet. **b** Top right photo: Experiment with magnet previously heated. After heating, the magnetic field was strongly reduced, however, the inhibition halo was similar to that formed by the bare magnet in the top right photo. The size of the halos was similar because the field gradient was little influenced, confirming that the cellular inhibition was not caused by the magnetic field. **c** Bottom left photo: the magnet was coated with a silicone layer. The small halo of inhibition near the magnet shows that both the magnetic field and its gradient are reduced. **d** Bottom right photo: petri dish without magnet. The normal cellular development was visible in the whole surface of the solid medium

intact. A reduced halo was also found around the silicone-coated magnets (Fig. 2), indicating that chemical contamination was not responsible for cell death. Furthermore, the cells presented a regular development around the metals of the magnets previously crushed and completely demagnetized. Additionally, to confirm whether the effect would be biocidal or biostatic, the magnets were removed from the media and the petri dishes were put back in the incubator for 24 h. This experiment demonstrated that the PMFG had a biocidal effect on *S. cerevisiae* because the halo cells failed to grow again (see supplementary material).

3.2 Liquid growth medium

At the significance level, $\alpha = 0.05$, the Kruskal–Wallis test revealed a significant difference among the absorbance measurements ($\chi^2 = 36.7$, $df = 5$, $p < 0.05$). The absorbance of tubes with cells exposed to the magnet was significantly higher than those with nutrient broth and cells without magnet. However, the absorbance of the tubes with nutrient

broth and cells, where the magnets were coated with silicone, did not differ from those with magnet uncoated or without magnets. There was no difference in treatment with cells exposed to the magnet compared to the treatments with magnet coated and uncoated in tubes without cells. Measurements of the magnetic fields revealed that the layer of silicone drastically reduced to zero when the magnetic field was already at a distance of 1 mm from the major surface of the magnet (Table 3).

However, the absorbance value of the control with only nutrient broth differed significantly from other treatments (Fig. 3).

Table 3 Estimated values for the magnetic field and its gradient as a function of the distance for magnets coated with silicon

Distance (mm)[a]	Voltage (Volt)[b]	Magnetic field (Gauss)[c]	Gradient (kGauss/m)[d]
0			
1	2.51	4.6	
2	2.50	0.0	4.6
3	2.50	0.0	0.0
4	2.50	0.0	0.0
5	2.50	0.0	0.0
6	2.50	0.0	0.0
7	2.50	0.0	0.0
8	2.50	0.0	0.0
9	2.50	0.0	0.0
10	2.50	0.0	0.0

[a]Distance: distance from the surface of the magnet along the principal axis. [b]Voltage: voltage carried out from the SS495A sensor at the referred distance. [c]Magnetic field: corresponding value of the magnetic field obtained with the equation (Voltage − 2.5 Volts) × 1000/3.125 (See datasheet of the sensor SS495A for further details). [d]Gradient: MFG obtained dividing by 0.001 m the difference of the magnetic field in two consecutive points. In the distance sero, it was impossible to measure values due to the thin layer of silicone

4 Discussion

In the present study, we investigated the effects of the PMFGs generated by commercial NdFeB magnets on the growth of *S. cerevisiae* in solid and liquid culture media. In both, it was possible to verify that cells were affected. In the solid medium, the cells near the magnet did not grow. The effect was most evident when the two surfaces of the magnet were in contact with the medium (vertical position) and the MagView® green film allowed to confirm that the cellular inhibition halo around the magnet corresponds to the PMFG generated by the magnet (see online supplementary material). Subsequently, the experiment where the magnet was removed from the solid medium during cell growth and the exposed cells around the magnet were unable to grow again, allowed to confirm that PMFG killed the cells, showing biocidal effect (see online supplementary material).

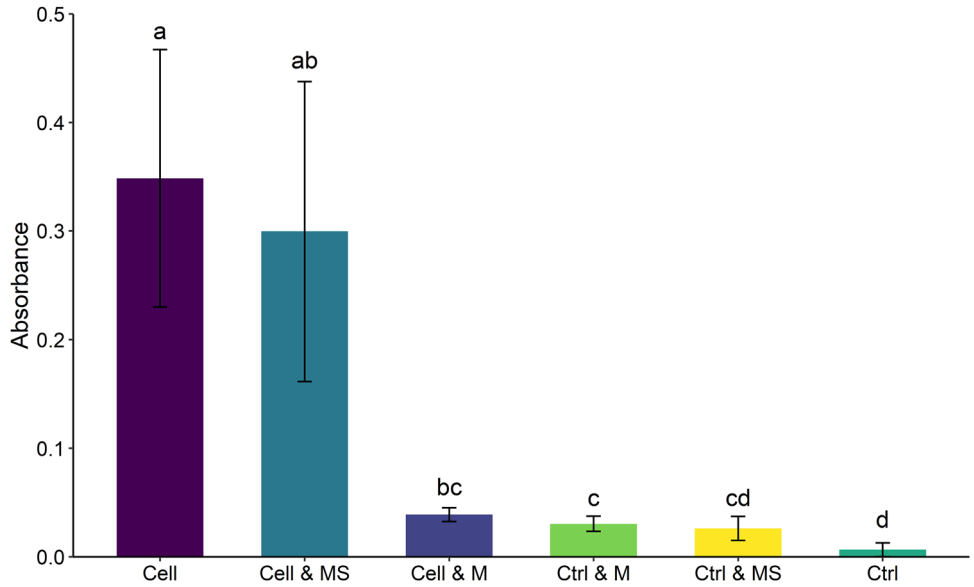

Fig. 3 Comparative analysis of the absorbance (in the region of 560 nm) in different liquid medium treatments to evaluate the effect of PMFG on *S. cerevisiae* growth. Cell: the tube with nutrient broth and cells. Cell & MS: the tube with nutrient broth, cells and a magnet coated with a thin layer of silicone. Cell & M: the tube with nutrient broth, cells, and a bare magnet. Ctrl & M: the tube with nutrient broth and a magnet. Ctrl & MS: the tube with nutrient broth and a magnet coated with a thin layer of silicone. Ctrl: only nutrient broth. Thin black lines represent the standard errors. Different letters above the bars indicate significant differences among treatments (Kruskal–Wallis test; $p < 0.05$)

Furthermore, in the next solid medium experiment, the inhibition halo was generated by both the heat-reduced magnetic field and the normal magnet, which is further evidence that it was not the magnetic field but the field gradient that affected the cells.

In the liquid medium, a similar effect was shown by the number of cells exposed to the magnet, which was statistically reduced compared to the control. In literature, there are reports of both absence and the presence of effects of magnetic fields on biological systems (Albuquerque et al. 2016). Ambiguities arise by observing that many studies evaluated the effects of magnetic fields, but not their gradients. Many studies reported no effects of static magnetic fields on cell growth and survival, or minimal effects as a function of the intensity (Kohno et al. 2000; Nakahara et al. 2002; Ruiz-Gómez et al. 2004; Miyakoshi 2005, 2006; Zablotskii et al. 2018).

The absence of biological effects can be attributed to exposure to homogeneous or quasi-homogeneous fields (Zablotskii et al. 2016a). However, in agreement with our results, when the gradient field was applied, inhibition effects on cell growth were observed (Neurath 1968; Wang et al. 2009). Moreover, decrease of *S. cerevisiae* activity (Kimball 1938; Rosen 2003) is probably observed because the gradient can affect the magnetic moment of biomolecular structures (Neurath 1968) and disrupt cellular machinery that fails to function properly. The effects are dependent on the value of the gradient and a strong magnetic field gradient can alter the functionality of cells and tissues (Zablotskii et al. 2018).

Our results include the comparison between weak and strong magnetic fields. The first one was obtained demagnetizing the magnets with heat. It is remarkable that they confirm the biological effects depending on the gradient value rather than the magnetic field strength. The effect of the gradient on the cells was maintained even with the reduction of the magnetic field, proving that the effect was in consequence of the former. Although reduced with heat, the gradient pattern was similar to that of the original one. In this way, it should be expected that the halos induced by strong and weak magnetic fields are comparable. These results are in accordance with previous studies where it was only possible to verify biological effects when a magnetic field gradient was provided (Hirose et al. 2003; Zablotskii et al. 2014; Wang et al. 2009).

In this study, we demonstrate that in solid medium, the cell activity is directly affected by the inhomogeneous magnetic field, which was stronger near the surface of the magnet and loses intensity by increasing distance. Far from the magnets, where the PMFG is strongly reduced, the cells showed normal development and formation of colonies and we also observed that the halo of cells inactivity followed the field lines (Egami et al. 2010; Zablotskii et al. 2013). The cell migration to areas where the gradient magnetic field is strongest can be attributed to interaction with cytoskeleton and intracellular F-actin reorganization (Zablotskii et al. 2016a, 2018). Further confirmation is that the gradient that induces cell inactivity comes from the experiment with a fully demagnetized and crushed magnet. The development of cells on the metals of the magnet indicates that it was not the metal components that caused the inhibition of *S. cerevisiae*. NdFeB magnets have been used to clinical applications (Yuksel et al. 2018), especially in orthodontic approaches (Mancini et al. 1999; Phelan et al. 2012), and our results agrees with other studies that reported negligible toxicity of their components to cells (Bondemark et al. 1994; Rogero et al. 2003; Prijic et al. 2010).

The results of the liquid and solid media experiments are in accordance. There was a significant reduction in the number of cells in the treatments under the PMFG generated by a bare magnet compared to the treatment in which the cells grew without the magnet. In addition, in both liquid and solid media, the layer of silicone reduced the effect of the field gradient. Although the effect has been pronounced in solid medium, the silicone-coated magnet did not differ from cells without magnet and neither from cells exposed to the bare magnet in the liquid medium. According to Zhang (1997), magnetic properties of magnets coated with silicone do not change much compared to bare magnets. However, it should be considered that the results obtained were dependent on the viscosity of the solid and liquid media which probably allowed different magnetic permeability. Additionally, liquid medium was stirred and the cells may have been differentially exposed to the gradient which might have influenced the results. In solid medium, the reduced halo suggests that the silicone decreased the magnetic field and also the gradient (Table 3 and Fig. 2c).

Growth inhibition and cell death reflect a set of gradient effects at the intracellular level. The intracellular components of cells behaved differently when exposed to PMFG because they show distinct magnetic susceptibilities and electrical charges. The ferromagnetic and paramagnetic components tend to be translocated to the region where the field is strongest while the diamagnetic provide resistance to field lines (Abdel Fattah et al. 2016). However, the cells operate as neutral electromagnetic units (Zablotskii et al. 2018) and the effect of the gradient magnetic field can be dependent on the type of cell exposed because each type has its own dielectric properties (Buemi et al. 2001; Daoud et al. 2012).

Unlike a homogeneous magnetic field, the field gradient can submit parts of cells to distinct stresses, causing: cell re-orientation and migration toward areas where the field gradient is stronger (Kimball 1938; Zhang et al. 2003; Zablotskii et al. 2018), displacement of elements, interaction with metallic cofactors, increase of free radicals, induction

of mutations (Okano 2008; Zablotskii et al. 2014; Wang and Zhang 2017), change selectivity of ion channels, unbalancing the ion flux (Fanelli et al. 1999; Zablotskii et al. 2016b), changes in the cell membrane potential (Polyakova et al. 2017), cytoskeleton remodeling, loss of membrane integrity (Wang et al. 2014; Wosik et al. 2018; Zablotskii et al. 2018), among other consequences that explain the malfunction of the cellular machinery. These effects probably act synergistically and if the intensity and time of field gradient exposure are large enough it can result in cellular death (Zablotskii et al. 2018).

Albuquerque et al. (2016) claim that the effects of magnetic fields would be stochastic, but we disagree. We argue that effects depend on the exposure to a gradient rather than a spatially homogeneous field. The discrepancy of the results in the literature shows that other non-standard variables interfere in biological systems exposed to SMF. Despite the possible harmful effects of daily exposure to PMFGs on human cells, they may be targeted to affect the viability, proliferation, and apoptosis of leukemic cells, for example (Zablotskii et al. 2014). This indicates possibilities of gradient field application in the manipulation of the cellular machinery. Besides, the use of magnetic fields has been prospected in medicine (Zablotskii et al. 2014, 2018) and further studies are necessary to clarify the effects at the cellular and intracellular levels. With standardization of cell type, temperature, exposure time, magnetic field gradient intensity and direction (Tian et al. 2018), it is possible to establish patterns in the effects of exposure to SMFs in living organisms.

As discussed in a previous section, cell death stops drastically at about 1 cm away from the magnet surface. This particular condition suggests that further studies are needed to establish the exact behavior of cells around this PMFG limit value. To define the experiment necessary for this purpose, many factors must be considered such as temperature, sterilization, sensor storage temperature among others. From a technological point of view, this kind of research could be promising.

5 Conclusions

Our results support that the magnetic field gradient, generated by type N52 rectangular NdFeB magnets, kills *S. cerevisiae* cells in regions where it is stronger, making their growth unfeasible. In regions farther from the magnet, the cells are not under the effect of the gradient and grow normally. This could be verified in the solid medium, where the effect of the gradient is spatially observable. The reduction of the magnetic field does not necessarily reduce its gradient (calculated by the difference between the intensity of the field at two proximal points divided by the distance), which is responsible for the effects on the biological systems. Studies about the role of field gradients should be considered to reduce and explain controversial results present in the literature. There is much to clarify about the effects of magnetism on cells and their components, but certainly, several biotechnological and medical applications may arise from the use of magnetic field gradients in various elements of biological systems.

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Compliance with ethical standards

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