IMPACT OF LONG-TERM ELECTROMAGNETIC FIELD EXPOSURE ON SACCHAROMYCES CEREVISIAE: CONTROLLING THE INOCULUM WEIGHT

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Abstract: Living organisms of Saccharomyces cerevisiae are subjected to extremely low-frequency electromagnetic field exposition during the workweek, followed by cultivation over the weekend. Initially, the weight of the inoculum with YPD agar is examined in the exposure setup without exposition between the first and the last day. Subsequently, the coil is supplied, generating a static and time-varying electromagnetic field. The theoretical assumption underlying this experiment is based on the modified ion parametric resonance theory, which specifically focuses on calcium ions at a frequency of 192.26 Hz. The time-varying magnetic field amplitude is set at 451.66 μT, accompanied by a static field of 250.92 μT. By employing an experimental protocol, the cells are exposed to these conditions for a long-term exposition within Petri dishes.

Keywords: extremely low-frequency electromagnetic field, exposition of cells, the mass ratio

1 Introduction

Electromagnetic field (EMF) exposure has been used in many different applications to modulate biological processes across industries. Pulse and alternating EMF have both been shown capable of stimulating and inhibiting different plant growth, with a focus on important agricultural crops such as corn and barley [1,2,3].

EMF treatment has been proposed as a cost and time-effective method to remove bacterial biofilm and scale build-up on ships and other infrastructure and has gathered increasing interest from both the military and commercial business [4]. Combined, these applications possess tremendous financial and economic impact in the orders of thousand-billions of United States dollars per year as seen in Figure 1. On more human-centric applications, pulse EMF has been shown to be able to induce vestibular response and offer a new method to study motion sickness and spatial disorientation [5]. In addition, EMF therapeutic applications can target issues such as pain and inflammation reduction, skeletal tissue healing, and even treatment of neurological diseases [6; 7; 8].

Taking a closer look at these applications, EMF of different parameters can have both positive and negative effects on biological systems, especially on cell activity and viability; and though well-observed, the EMF exact interacting mechanism with biochemical systems is still poorly understood. As such, it is a challenging task to set relevant safety limits for when considering its exposure effect on human health, a rising concern.

With the recent global commercialization of fifth generation (5G) telecommunication technology, there has been a renewed interest among the public in the health effect of EMF exposure. Looking broadly, in the modern environment, EMF can arise from a wide variety of sources and parameters, from commercial power distribution systems to your smartphones. According to the National Institute for Occupational Safety and Health, the three main categories of concern for EMF exposure are radio frequency, extremely low frequency, and static magnetic fields.

EMF exposure may be considered a cellular physical stressor and a possible carcinogen, though this issue remains controversial for non-thermal level of exposure. Thus, the exact cause-effect relation has yet to be established. Studies have shown that many processes and components have been identified to be able to be modulated by external magnetic field [9, 10, 11].

At the same time, many epidemiological studies have indicated a rise in human cancers occurrence since the advent of electricity, drawing correlation to the increased exposure to EMF in modern time [12]. A study from Shih et al. [13], shows that there is a significant correlation between smartphone usage and distance from their chest to increased incident of breast cancers at up to 5-fold.

Due to both the large amount of exposure parameters (different waveforms, frequencies, amplitude, time of exposure, and combination of the above and more) and the many feedback system present in cellular activities (not accounting for variation between cell types, species, and individuals), the observed result is non-linear and challenging to pinpoint the result to a single mechanism. Despite the difficulty mentioned above, investigating cellular growth rate is the first step to help fill in part of the puzzle, what is point of this contribution.

2 Materials and Methods

In the study conducted by Bajtos et al. [17], an experimental protocol was established to investigate the long-term exposure of cells cultivated on Petri dishes. The primary focus of the study was to address the following questions:

1. How does the mass of the YPD agar change over time?
2. How does the mass of the YPD agar with cell culture change over time?

By systematically monitoring the mass variations of the YPD agar, in this contribution, it is aimed to understand the dynamics of the growth medium and its potential impact on cell culture.

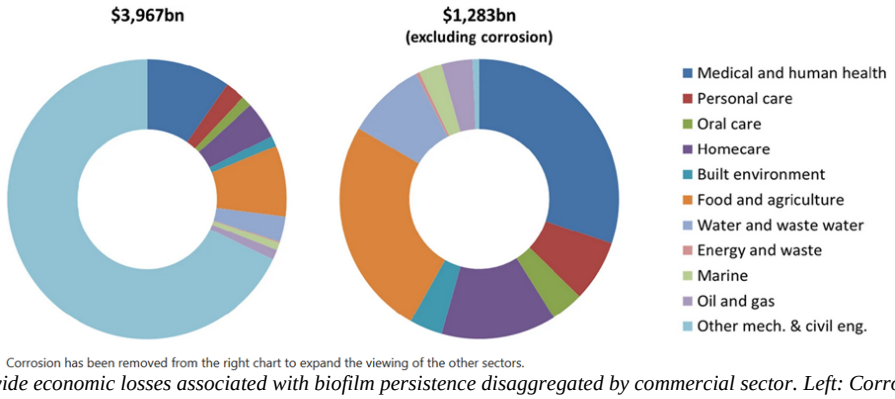

Figure 1. Worldwide economic losses associated with biofilm persistence disaggregated by commercial sector. Left: Corrosion (light blue) dominates industrial economic losses; Right: corrosion loss equivalents are excluded from chart to improve sector resolution. Adapted from [4].

Subsequently, two tests, named Test A and Test B, were carried out to examine the influence of extremely low-frequency electromagnetic field (ELF-EMF) on the growth of living organisms. The experiments were designed to explore the effects of ELF-EMF exposure on cell growth, considering the mass changes of the YPD agar.

The experimental protocol established in this study lays a solid foundation for the investigation of long-term effects of ELF-EMF on cell growth, presenting opportunities for scientific and practical advancements. To ensure reliable and accurate results, the experimental setup incorporates exposed and control coils for conducting paired experiments.

In terms of the biological component, the study employs *Saccharomyces cerevisiae* cells, commonly known as wine or beer yeasts. These cells serve as a suitable model organism due to their well-characterized biological properties and genetic tractability. By using Saccharomyces cerevisiae, it is possible to gain valuable insights into the effects of ELF-EMF on cellular processes and growth dynamics.

For more detailed information regarding the experimental setup, the biological procedure of YPD agar preparation, and cell handling, it is encouraged to refer to the work of Judakova [18]. Judakova's research provides additional information on the specifics of the experimental design, including the instrumentation, coil configurations, and other technical details essential for replicating and further exploring the study's findings.

Even though it is difficult to understand all processes in the biological aspect of how ELF-EMF can interact with the cells, it is probable, that more than 1 mechanism can occur. Some of the low energy electromagnetic effects on micro-biological systems are well documented in laboratory experiments but are difficult to translate to macro-biological system responses.

Reproducing the results may differ because of the difference in exposure setups, experimental conditions (alternative or static magnetic field), the frequency, intensity, duration time of magnetic field, the time of recovery, investigation targets, assay methods, etc. What more, when similar exposure time and intensity of magnetic field have been used in studies [19], [20] to determine the DNA damage, contradictory results attracted attention. The results should differ either because of the combination of different mechanisms responding to ELF-EMF.

2.1 Mitochondria, Electron Transport Chain and Radical Oxygen Species

Mitochondria are organelles found in eukaryotic cells that play an important role in cell function. The most significant role of a mitochondrion is the process of obtaining energy. The energy is obtained by breaking down saccharides, lipids, or other energetically rich organic substances. Mitochondria are usually oval-shaped and have two membranes: an outer and an inner membrane. The outer mitochondrial membrane isolates the mitochondrion from the rest of the cell. The inner membrane is crimped, which makes mitochondrial cristae that make its surface larger. This membrane contains the enzyme system, which is responsible for intracellular respiration. This is the site where saccharides degrade, and the energy is released into adenosine triphosphate (ATP) molecules. Aside from that, each mitochondrion has its own DNA and ribosomes, allowing it to reproduce and synthesize proteins. [21]

The four protein complexes: Complex I, Complex II, coenzyme Q, Complex III, cytochrome C, and Complex IV, make up the electron transport chain (ETC). These complexes act as an electron transport system. The energy is released as ATP when the reduction potential is larger. The energy is used to drive a proton gradient by pumping hydrogen ions out of the mitochondrial matrix into the space between membranes. Different kinds of clusters participate in the reactions, which are responsible for the transport of electrons, along with the complexes themselves and their catalytic processes [22], [23].

Iron-sulphur (Fe–S) clusters are part of the metalloprotein class. There are different types of clusters, but the most common ones are [2Fe–2S], [3Fe–4S], and [4Fe–4S]. All these types of clusters play a crucial role in complexes of ETC. Their configurations specify the electron transport channels that power mitochondrial respiration. The structure of [2Fe–2S] is rhombic. It is part of ferredoxins and has a role in the synthesis of steroids. [4Fe–4S] clusters have a cubic structure, and they can be found in mitochondrial respiratory complexes. The other important role of clusters is in DNA metabolism, and they work as cofactors in the catalysis of enzymes. [23]

A set of metabolic processes in the mitochondria that produce ATP are referred to as “oxidative phosphorylation”. Nicotinamide adenine dinucleotide hydride (NADH) and flavin adenine dinucleotide (FADH2) are carriers of electrons. While electrons are passing through ETC, protons are pumping from the mitochondrial matrix between the membranes of the mitochondria, resulting in the formation of the transmembrane electrical potential. To help electrons go through ETC, Fe–S clusters are used. In Complexes I and II, Fe–S clusters transfer electrons from the carriers NADH and FADH2 to ubiquinone. In Complex III, the role of Fe–S clusters is to make the transfer of electrons to Complex IV easier. [23]

The production of ROS is the main problem with ETC. “Complex I is considered to be the main producer of reactive oxygen species within mitochondria.” [23] ROS can also be produced in Complexes II and III. This production is associated with the emergence of various diseases. Redox signalling in oxygen-sensitive tissues is significantly influenced by ROS generation from Fe–S clusters. Reversible redox alternation at the Fe–S clusters may play a critical role in sensing variations in oxygen tension. These changes in ROS generation may also affect the Fe–S clusters themselves.

The question is whether magnetic fields can affect Fe–S clusters in ETC. In the research of Toda et al. [24], the authors show the impact of the extremely low frequency weak magnetic fields (ELF-WMF) on mitochondria and complexes in ETC. In experiments, they used ELF-WMF in pulses of 10 μT and intensity at 1–8 Hz. ELF-WMF reduced the number of mitochondria. This field suppresses all four subunits that comprise Complex II. They also demonstrated that ELF-WMF induced mitophagy by hermetically stimulating mitochondrial ETC activity and inhibiting mitochondrial Complex II activity.

According to Barnes and Beeningham [25], transitions in weak combined steady and time-varying external fields can change the relative orientation of the total angular momentum of two radical pair members, which in turn can change whether their relative electronic states are S or T. This results in a change in the total molecular angular momentum **F**. For instance, if a process produces a pair of radicals that are in a T state with respect to one another, then the recombination rate of those radicals may be impacted if one encounters a time-varying field with a frequency corresponding to the hyperfine splitting brought on by the external static field, which shifts the combination to a relative S state. The modification will decrease the number of radicals that are free to drift off and increase the number that recombines into stable molecules for chemical reactions that initially generate radical pairs in T states. The same would apply to a pair that is initially created in a relative S state and is subsequently changed by an applied field into a relative T state.

Finally, it is proposed that observable magnetic field effects may typically occur in conditions where an organism is stressed by other factors that change the radical concentrations, pushing the radical concentrations outside the range of values where they can be mitigated by radical scavengers.

If this is the case, it may help to explain why it can be difficult to obtain repeatable results because the initial conditions in biological systems are difficult to duplicate. In experiments

human related, this is particularly true. In many animal experiments, the conditions are designed to eliminate other stresses so that the compound effects of magnetic field stress and other stresses are not seen. It is to be noted that radicals are generated as a part of the metabolic processes and their values may vary by more than a factor of 10.

However, while these radicals are produced, a signal is sent to produce radical scavengers, and the concentrations are reduced to a baseline level. Long-term elevations of radical concentrations can damage and reset the baseline [26]. This in turn may lead to the reason why long-term exposure to cell phones may be different from short-term exposure.

Many interesting molecules' and radicals' coupling constants are not measured. Although the interdependence between nuclear and electronic states in solution is obvious and measurable, for example, in line shifts and broadening of NMR and EPR spectra, some of the measurements or theories used are for isolated molecules and may not be directly applicable for the case of molecules and radicals in physiological settings. Understanding of how weak magnetic fields can affect biological processes could be greatly improved by conducting experimental tests of the proposed hypotheses and variations in the theories that include excitations at both RF transition frequencies for the Zeeman states of the electrons and modulation at frequencies in the 5-500 Hz region.

2.2 Experimental Setup

In the experiment, a Q-Cell series incubator (Hanwha Solutions, China) is used. It is an equipment made to store samples and incubate microorganisms at a precisely controlled temperature while upholding the strictest standards for stability and homogeneity. Q-Cell incubators have a combined cooling and heating system with forced air circulation. It allows for operation at temperatures that are like or below ambient. The typical temperature range is from 3 to 40 °C, with an optional increase to 50 or 60 °C. The temperature can be regulated through an LCD display with 0.1 °C precision.

In the Q-Cell incubator in the laboratory, two coils are located: one powered and one controlled. Inside the coils is the support system for 10 samples of storage (each coil contains 5 samples). Between the coils is a shielding plate. Each coil is made of wound copper wire with an average diameter of 1 mm and a length of 912 m for each coil. The number of windings is 2000. The incubator with the coils is shown in Figure 2.

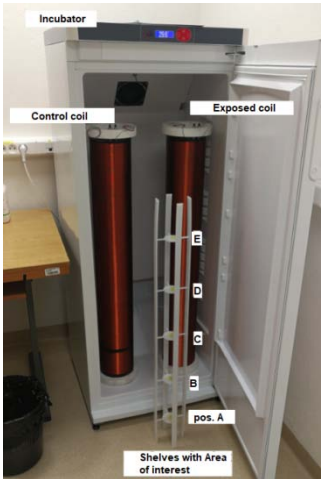

Figure 2. Experimental setup used for the experiments.

For the coils, it is necessary to consider their current carrying capacity. The current carrying capacity of the conductor is based on the cross-section of the conductor and the material from which it is made. As mentioned above, the material is copper, and the average diameter is 1 mm. For calculating the conductor cross-section, the elementary formula for calculating the content of a circle is used. According to the table in the book by Meravy and Kroupa [27], for a cross-section of 0.75 mm^2, the current carrying capacity for copper wire is 6 A.

In the experiment, an extreme-broadband power amplifier model HUBERT A1110-16-E (Dr. HUBERT GmbH, Germany) is used. With this model, both a voltage amplifier and a current amplifier can be used. For inductive loads, the current amplifier provides a constant, frequency-invariant output current.

It is a four-quadrant voltage and current amplifier with a maximal output voltage of 75 V_p and a maximal output current of 28 A_p. In the current amplifier mode for inductive loads, there are six configurable compensation networks. When operating in current control mode, the A1110-16-E operates similarly to a voltage-controlled current source and provides an inductive load with a practically frequency-independent constant load current.

The 34401A Digital Multimeter (Keysight Technologies, USA) is a multimeter with a 6.5-digit resolution. It offers 10 measurement functions, including DC/AC current, DC/AC voltage, 2-wire and 4-wire resistance, frequency, period, diode, and continuity. The maximal voltage input is 1000 V, and the maximal current input is 3 A. Its accuracy is 0.0035% for DC and 0.06 for AC.

The RIGOL DG4162 generator (RIGOL, Technologies, China) from the DG4000 series was used as a generator. DG4000 series is a multifunctional generator that can be used as a harmonic, pulse, function, or arbitrary waveform generator, analog/digital modulator, and counter. The generator has two channels, and the maximal frequency is 160 MHz.

2.3 Test A – Growth Rate Without EMF Exposure

To ensure that cells remain in the stationary phase rather than entering the phase of death during the 7-day cultivation period, Test A was conducted to determine the optimal growth conditions. Based on the findings of Lin et al. [28], it was determined that maintaining the temperature within the range of 10 to 20 °C in the incubator is crucial. To extend the stationary phase of yeast cells and slow down their growth, a temperature of 15 °C was selected for cultivating the samples. As this experiment does not involve exposition, the hypothesis suggests that both control and exposed samples placed on different coils will exhibit similar decreases in mass.

In Test A, two YPD mediums were prepared, differing in the duration of pre-cultivation on a shaker. The first medium was agitated for 26 hours, while the second medium underwent agitation for 20 hours. Subsequently, a dilution of 1:10 was performed for both mediums to ensure similar initial conditions for the samples. The concentration of the first medium was measured to be 317,778 cells/ml, whereas the concentration of the second medium was determined to be 247,333 cells/ml.

The incubator was set to a temperature of 15 °C, and 10 Petri dishes (PDs) were inoculated with the first medium, marked as number 1, while another set of 10 PDs were inoculated with the second medium, marked as number 2. Each inoculum consisted of 100 µl. To account for different conditions, five control samples and five exposed samples were prepared from each concentration. These samples were labelled as Control coil (C) or Exposed coil (E) depending on the coil they were placed in. The final designation of each sample included the coil (C/E), the medium used (1/2), and the position in the coils (A/B/C/D/E).

During Test A, the samples were placed in the incubator, and their weights were measured at the beginning and end of the 7-day experiment. Although the incubator temperature rose to 21.9 °C briefly when the samples were initially placed, it stabilized at 15°C after 22 minutes.

2.4 Test B – Growth Rate With EMF Exposure

In Test B, the preparation of YPD medium and agar plates takes place one day before the inoculation. The YPD medium is

prepared by placing one loop of yeast on a shaker for 24 hours. On the day of inoculation, a dilution of 1:10 is performed, and the concentration of the resulting solution is determined using Formula 2.1, which yields a concentration of 266,667 cells/ml.

$$V_2 = V_1 \cdot \frac{c_1}{c_2}, \quad (2.1)$$

where V_1 [µl] is the original volume used in the previous experiment, V_2 [µl] is the required volume needed to ensure the same beginning condition as in the previous experiment, c_1 [cells/ml] is the concentration calculated in the previous experiment, and c_2 [cells/ml] is the new calculated concentration. The volume for the first group of samples is 119 µl and 93 µl for the second group.

The null hypothesis for this experiment in Test B is like that of Test A. The prediction states that the mass of the exposed samples will undergo changes like the mass of the control samples. However, in this case, the exposition is turned on, meaning that the cells are subjected to the EMF.

In Test B, the samples are labelled following the same convention as in Test A: using the coil designation (C/E), the medium used (1/2), and the position in the coils (A/B/C/D/E). Once labelled, the samples are measured and then placed in the incubator, where the temperature is maintained at 15 °C, as in previous test.

After the samples are properly positioned, the parameters of exposition determined in the next chapter are configured in the generator, and the amplifier is switched on. This ensures that the desired EMF exposition is established.

The exposition is initiated every working day for a duration of 6 hours, beginning at the same designated time. Initially, the exposition time is set for 5 consecutive days.

Following the 5-day exposure phase, the subsequent two days serve as rest days, constituting the weekend. After the weekend period, the samples are measured again to assess any changes that may have occurred because of the electromagnetic field exposition.

2.5 Exposition Setting

For Test B, the establishment of parameters for exposure is crucial, and these parameters are determined based on the Ion Parametric Resonance (IPR) theory. In this study, the focus is on the role of Ca^{2+} ions, as they play a significant role in proper human body function, as proposed in the IPR model [29], [30].

To calculate the AC frequency, Formula 2.2 from the IPR model is employed. This is essential for determining the parameters of exposure, specifically for characterizing the frequency variation over time, denoted as f_{AC} [Hz]:

$$f_{AC} = \frac{1}{n} \cdot \frac{q\boldsymbol{B}_{DC}}{2\pi m} \quad (2.2)$$

where $\boldsymbol{B}_{DC}$ [T] is the DC magnetic flux density, q [C] is the charge of the charged particle, m [kg] is the mass of the charged particle, and n [-] is the frequency index. This formula describes how the frequency of an applied AC field depends on the static magnetic field density. According to the study of Bajtos et al. [30], the designated AC frequency adjusts proportionally with the change of the magnetic flux density.

It is important to note that the frequency variation over time, as described by Formula 2.2, impacts the static magnetic flux density. The frequency is one of the parameters that need to be set in the system to achieve the desired exposure. As noted by Bajtos et al. [30], increasing the static component of the magnetic flux density can help mitigate the effects of the Earth's magnetic field, which changes over time. These considerations and the utilization of Formula 2.2 allow for the determination of appropriate parameters for the exposure in Test B.

Considering the limitations in increasing the static magnetic flux density, this research specifically focuses on ELF-EMF and its impact on living cells. According to a study conducted by Touitou and Selmaoui [31], extremely low frequencies ranges between 1 and 300 Hz. Therefore, the calculated frequency must fall within this required limit.

In laboratory, the magnetic field strength of the Earth is measured to be approximately 50.92 µT. For this experiment and to satisfy one of the postulates of IPR, which is the collinearity of $\mathbf{B}_{DC}$ (static magnetic field) and $\mathbf{B}_{AC}$ (applied AC magnetic field), a magnetic flux density of 200 µT is chosen. This value ensures the neglect of the Earth's magnetic field. Subsequently, in Formula 2.2, the sum of the mentioned values, 250.92 µT, is substituted [30], [32].

To further calculate the AC frequency in Formula 2.2, additional values need to be included. As for the charge of the charged particle, *q* [C], the smallest positive electric charge is considered. Since calcium has an oxidation number of 2+, the charge is multiplied by 2. As for the mass of the charged particle, *m* [kg], the atomic mass of calcium, which can be obtained from the periodic table, is utilized. The periodic table generally provides the relative atomic mass, which represents the ratio of an element's average atomic mass to the unified atomic mass unit (Dalton unit). To calculate the mass of the calcium ion, the relative atomic mass is multiplied by the unified atomic mass unit, which is approximately $1.66 \cdot 10^{-27}$ kg. Lastly, the frequency index, *n* [-], is selected as 1. The calculation of the AC frequency can be performed using Formula 2.3 [29], [30]:

$$f_{AC} = 1 \cdot \frac{2 \cdot 1.602 \cdot 10^{-19}[C] \cdot 250.92 \cdot 10^{-6}[T]}{2 \cdot \pi \cdot 40.08 \cdot 1.66 \cdot 10^{-27}[kg]} = 192.26\ [Hz]. \quad (2.3)$$

The calculated frequency falls within the range of ELF, indicating that the selection of an increased static magnetic flux density was appropriate. This ensures that the experiment aligns with the desired frequency range. It is expected that the biological reaction will be more pronounced when this condition is fulfilled.

To calculate the currents, both static and varying magnetic flux densities are required. In this case, the varying magnetic flux density is determined to be 1.8 times the static value. This choice is made with the expectation that the biological reaction will be stronger under these conditions [33]. The specific value for the AC magnetic flux density can be obtained using Formula 2.4 [32].

$$B_{AC} = 1.8 \cdot 250.92\ [\mu T] = 451.66\ [\mu T] \quad (2.4)$$

Utilizing Formula 2.5, one can determine the AC and DC currents.

$$I = \frac{B - 20.443}{2183.6} \quad (2.5)$$

Using Formula 2.4, the DC, I_{DC} [A], where the value of B_{DC} [µT] is substituted for the position of *B*, is 105.55 mA. The AC, I_{AC} [A], where the value of B_{AC} [µT] is substituted instead of *B*, is 197.48 mA [33].

3 Results

The primary objective of this study is to examine the cellular proliferation response when exposed to EMF. The proliferative response may vary among cells. In a study conducted by Carnecka et al. [32], it was observed that exposure to ELF-EMF had an inhibitory effect on two samples, while three other samples exhibited a stimulating effect. Their study focused on the impact of Mg^{2+} in a liquid medium and was conducted under conditions similar to those employed in this present work.

This work has the objective of determining the exposure parameters and evaluating the results based on the IPR theory.

The weight of the samples is chosen as the primary parameter for comparing the results before and after exposure. The yeast strain *S. cerevisiae* is cultivated on PDs for a duration of 7 days. Prior to each experiment, a null hypothesis is established, and its validity is assessed by comparing the p-value to a significance level of 0.05.

The data obtained from each experiment do not exhibit a normal distribution, as indicated by the Kolmogorov-Smirnov test. To compare multiple samples, the Kruskal-Wallis's test is employed, while the Mann-Whitney test is used for comparing two samples. All the necessary calculations are performed using MATLAB (Mathworks, USA) [35].

For Test A, the results after 7 days of cultivation at 15°C are shown in Figure 3.

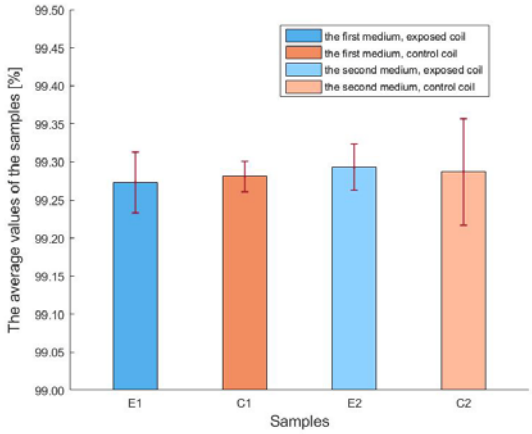

Figure 3. The mass ratio average values of the samples of two different concentrations after 7 days of cultivation at 15 °C in incubator with no exposition.

To evaluate the validity of the second hypothesis, we compare the samples of the first and second concentrations between the E and C coils using the Kruskal-Wallis's test. The results of the test indicate a p-value of 0.64. Since this value exceeds the significance threshold of 0.05, we conclude that there are no significant differences observed. Therefore, it is likely that the second hypothesis, which suggests that the change in mass for all samples is the same, holds true.

During Test B, the concentration of the diluted inoculum is determined to ensure consistency with Test A. By calculating the necessary volumes, the desired concentrations are achieved and subsequently pipetted onto agar plates. Additionally, parameters are configured to enable the exposition.

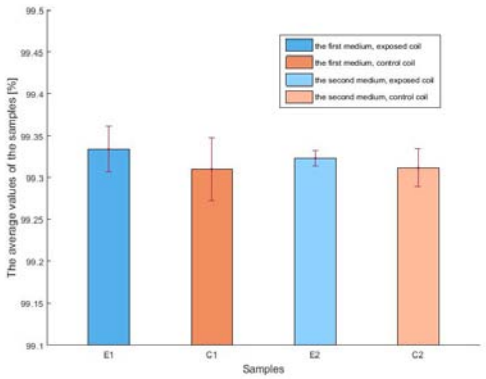

Figure 4. The mass ratio average values of the samples of two different concentrations after 7 days of cultivation at 15 °C in incubator with the exposition.

The obtained results from Test B are then compared and visually presented in Figure 4 allowing for a comprehensive analysis and interpretation.

The comparison of the control and exposed samples for the first concentration yields a p-value of 0.31, while for the second concentration, it is 0.57. Furthermore, when considering all samples from both the control and exposed coils, the Kruskal-Wallis's test calculates a p-value of 0.42. These results indicate the absence of a significant effect. Thus, it supports the prediction that the hypothesis stating an equal decrease in all samples holds true.

To provide a more detailed depiction of the findings, Figure 4 presents the average values and standard deviations (SDs) of the samples. Notably, the exposed samples exhibit higher average values compared to the control samples. Additionally, the SDs of the exposed samples are smaller than those of the control samples. This observation contrasts with the results from Test A, as depicted in Figure 3. Although these changes could potentially be random, they might also indicate a proliferative change in cells under the influence of electromagnetic fields.

4 Discussion and Conclusion

This contribution focuses on investigating the potential impact of ELF-EMF on living organisms. To conduct the study, an experimental protocol is developed based on the IPR theory. The model organism selected for this investigation is *S. cerevisiae*, cultivated on PDs.

The proliferative activity of the cells is monitored by measuring the mass of the samples. The confirmation or refutation of hypotheses related to Test A and Test B is determined based on the p-value, considering a significance level of 0.05. Additionally, standard deviations (SD) are calculated for each test.

Test A examines the behaviour of cells in the incubator without any exposure to ELF-EMF. Test B is designed to simulate Test A but with the inclusion of exposure. In the control coil, the control samples are subjected to normal growth conditions. The experiment begins with five consecutive days of exposure, where each day follows the same schedule of six hours of exposure. The following weekend is designated as a non-exposure period. At the end of the experiment, the mass of the samples is compared to the initial mass. Based on the calculated p-value, no significant changes in the mass ratio between the control and exposed samples were observed during the exposition.

It is worth noting that the impact of ELF-EMF on the proliferative activity of cells may be more evident when the cultivation temperature is higher or when different parameters of exposure are employed. During this research, other potential parameters for comparing the results were discovered. Notable changes were observed in the appearance of the samples. While the growth area of cells reaches full coverage during cultivation at room temperature, at 15°C, distinct regions of varying growth levels can be observed. Other interesting parameters to monitor include the concentrations of yeast metabolic byproducts, as well as the size, shape, and number of budding cells observed under a microscope.

Nowadays, a good question stays: how complex could biological cells be in context with their interaction with ELF-EMF? Despite IPR theory, the resonance of molecules could occur due to a magnetic moment associated with the nuclear spin of a molecule/compound in the biological system. This resonance could be aided in changing the rate of transitions in a molecule and hence changing the rate of recombination in a chemical reaction. The next interaction could be explained by The Feedback Effect. For example, if you push a swing at the top, you increase the amplitude and if you push at the bottom in the same direction, you decrease it. [25] Therefore, the timing of an electromagnetic pulse concerning oscillating biological processes can lead to either positive or negative effects. Two or

more different mechanisms can likely be causing the response when exposed to magnetic fields.

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Primary Paper Section: B

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