Frequency Dependent Alterations of S. Cerevisiae Proliferation Due to LF EMF Exposure

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Abstract. *The presented paper deals with low frequency electromagnetic field application on Saccharomyces cerevisiae cells. Experiments performed through wide frequency range have shown selective frequency dependent biological response, which could be successfully predicted by ion parametric resonance theory proposed by V. V. Lednev. Although observed results give satisfying answer to questions whether or not electromagnetic fields could affect cell cultures even at non-thermal levels, the research presented herein opens a multitude of questions about the exact physical mechanisms underlying the observed microorganism behavior, as the theory discussed within the scope of this article is still not completely unambiguous.*

Keywords

Electromagnetism, low frequency electromagnetic fields, proliferation, cell membrane, ion parametric resonance.

1. Introduction

It is well known that the electromagnetic conditions in which we live nowadays are different from those in which people used to live in the past. The majority of living beings are confronted with artificial Electro-Magnetic Fields (EMFs), which complement the naturally occurring EMF of the Earth, and questions pertaining to influence thereof on biological systems are more frequent than ever.

The number of experimental studies investigating EMF effects on living cells, tissues or on human health in general, has grown rapidly over the last few decades. Much attention is currently being paid to research regarding potential connection between EMF exposure and cancer or other diseases, which has been discussed in various studies [1], [2], [3], [4], [5], [6] and [7]. Numerous works focusing on experiments with living cells have shown differences in the proliferation process between cells exposed to EMF and control (non-exposed) cells [8], [9], [10], [11], [12], [13] and [14]. Other works investigate animal behaviour when exposed to EMF [15] and [16] or possible genomic instabilities due to EMF exposure [17], [18], [19] and [20]. The results of these works are often inconsistent and, as stated in Buchachenko [21], the biological electromagnetic effects sometimes seem to be irreproducible and contradictory.

As pointed out in Markov [22], the problem is often discussed within the scope of thermal effects with minor interest on possible non-thermal mechanisms, even when considering currently accepted standards and recommendations (e.g. from International Committee of Nonionizing Radiation Protection - ICNIRP, 1998). Actually, the evaluation of EMF effects on biological objects is much more complicated due to principal differences in each approach, and no part of the problem should be neglected, especially when Low Frequency EMF (LF EMF) and weak signals, that are far below the ICNIRP recommendations, are considered. The complexity of this problem is documented by many authors [23], [24], [25] and [26], who tried to propose physical mechanism of biological LF EMF impact, but there is still lack of general acceptance of any proposed theory across the scientific community. This fact is at

the same time a strong motivational factor for further investigation in this field of research.

The Ion Parametric Resonance (IPR) theory, proposed by Lednev [25], [26] and [27], is one of the most discussed models of LF EMF interaction with biological objects. The assumptions of this theory are based on the Ion Cyclotron Resonance theory (ICR), first described by Liboff [24], speculating that the physiological activity of certain important ions can be altered when the frequency of applied time-varying magnetic field is equal to the frequency of ion motion in a static magnetic field. Ions in IPR model are represented by a harmonic oscillator, bound to a specific location at the surface of the cell membrane, and application of combined magnetic field should alter its oscillations. The mentioned physical model included several imperfections, particularly due to the problem of thermal noise, and was criticized by Adair [28] and [29]. The theory was defended by Engstrom [30] and mentioned imperfections were addressed by Lednev [26] and [31]. Despite criticism, there remains an impressive body of experimental evidence that can be taken as an empirical basis for ICR and IPR hypotheses [8], [9], [10], [11], [12] and [13]. In general, as Halgamuge et al. concluded [33]: "*... models based on electric interactions have difficulties to obtain a high enough signal-to-noise ratio at low field strengths. The fields that are needed to explain the opening of a membrane channel protein are unrealistically high. On the other hand, models based on magnetic interactions can easily obtain a large enough signal-to-noise ratio, because the level of magnetic thermal noise in tissue is low. The reason that these models work, is the same reason that makes the MRI technique possible.*"

Another attempt to shed more light into this research area is presented within this article. The authors opted to experimentally investigate non-thermal effects of LF EMF on Saccharomyces Cerevisiae cells, at various uncommon frequencies, often neglected in other research works. To gain better reproducibility of conducted experiments, authors used their innovative exposition system described in [33], proposed with the aim of achieving the highest possible homogeneity of magnetic flux density within the exposed volume, taking into account sample dimensions and incubator proportions. This system enables experiments with 3 petri dishes (diameter of 9 cm, height of 2 cm - each), each irradiated by a homogeneous magnetic field. Obtained results and observed biological reactions are then discussed within the scope of the IPR theory.

2. Materials and Methods

Saccharomyces cerevisiae cells (brand VIVO – commercially available from Lesaffre Slovakia, Inc.) were selected as the target biological object for irradiation by LF EMF. The reason for this choice is the growth analogy with cancer cells [34], as well as previously published works regarding LF EMF effects on yeast cells [35] and [36].

2.1. Experimental Protocol

Chemical laboratory testing protocol for the mentioned VIVO strand of S. Cerevisiae is publicly available from company Mikrolab, LLC. This means that the microbiological, physical and chemical properties and parameters of investigated microorganisms were known prior to the experiments.

Cells were cultivated on GKCH agar, in accordance with STN EN ISO 7218 (560104) and STN ISO 7954 (560087) standards and specifications. Cultivation was performed under identical ambient conditions for 72 hours, with temperature of both exposed and control samples set slightly above room temperature, specifically to 27 °C. Continuous temperature monitoring was performed using three Negative Temperature Coefficient (NTC) thermistors and processed and logged in real-time. Two of the NTC thermistors were placed within the incubator chambers to monitor the temperature of both exposed and control samples, and the third thermistor was placed outside the incubator as a reference sensor. To avoid any effects from airflow, humidity, or changes of light conditions, the exposed samples were held in a solid plastic holder, matching the diameter of the coil cavity.

2.2. Experimental Setup and Evaluation Methods

The experimental setup, used for all of the presented experiments and detailed in [37], is schematically shown in Fig. 1. The setup was specifically designed for paired sample experiments, conducted on two sets of three Petri dishes inoculated by S. Cerevisiae.

One set was placed within one chamber of incubator, housing the mu-metal magnetic shielding box. This set was the control, or reference sample - being shielded from the generated LF EMF. The second set of samples, representing the exposed cells, was placed at specific position within the already mentioned developed coil system, depicted within the real scheme of experimental setup on Fig. 2. The coil system was driven by harmonic current (generated by the Agilent 33220A signal generator, manufacturer Agilent Tech-

nologies, Inc.), and amplified using a linear amplifier (Hubert A1110-05, manufacturer Dr. Hubert GmbH) - the resulting amplitude I_m being in the 0.96–1.09 A range. The exact value of excitation current depended on the impedance matching and frequency used, so as to achieve the required magnitude of magnetic flux density. The experiment was repeated for 5 times at said frequency.

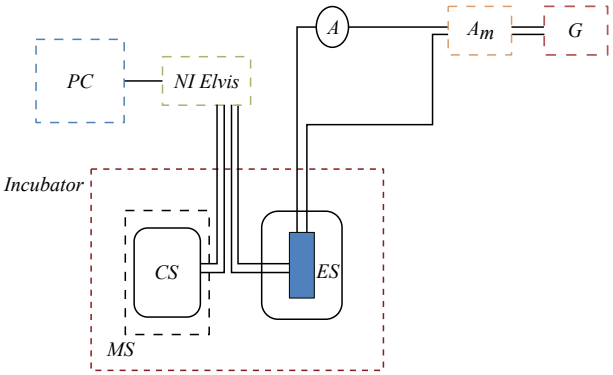

Fig. 1: Experimental setup: G - signal generator Agilent 33220A; Am - amplifier; A - ampere meter; Incubator with two chambers: ES - exposed samples placed within the excitation coil, CS - control samples placed within the MS - magnetic shielding box; NI Elvis II - development board for temperature data acquisition; PC - temperature monitoring and visualization.

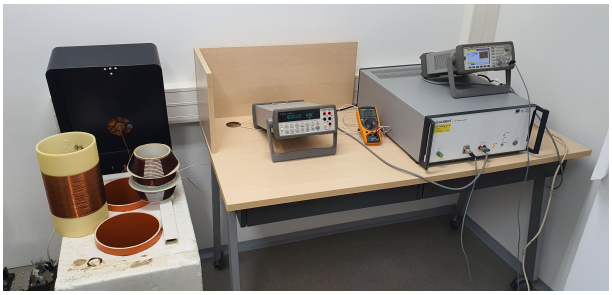

Fig. 2: Propopsed and constructed experimental setup showing the applicator used in preliminary experiments and the newly designed applicator.

From the afore mentioned it is evident that samples were irradiated by LF EMF with magnetic flux density $\vec{B}$, which varies spatially within the exposed volume. The exact value of $\vec{B}$-field produced by the coil system was validated by measurements, using sensitive fluxgate sensors. Since this method is described in more detail in [33] and [38], for the purpose of this article, it is sufficient to mention the conclusion that real-life measurements re-confirmed values obtained from numerical simulations, documenting minimum magnetic flux variations (max. 5 %) within the irradiated volume, when using the proposed excitation coil system. The value of magnetic flux density varied between $\vec{B} = 2.33$–2.45 mT, and was frequency independent.

After continuous irradiation, the growth dynamics parameter of both control and exposed samples was quantified. Specifically, growth dynamics were computed via semi-automated software called PetriCounter, which was developed as a replacement for commercially available counters. Algorithm thereof is detailed in [39]. Principally, it allows detection and counting of yeast growth areas, using methods of image processing, analysis, and feature extraction, to enable experimental results quantification and comparison of observed effects. Statistical significance of observed results was finally evaluated using Student's T-test for paired samples, with significance level $\alpha = 0.05$.

3. Results and Discussion

As was mentioned in previous section of this article, experiments were focused on the behaviour of Saccharomyces cerevisiae cells influenced by exogenous LF EMF of different frequencies produced by the designed coil system. Our work follows previously conducted experiments published in [40], [41] and [42], but the frequency range differs, and is wider in comparison with preliminary experiments. At first, we re-evaluated the base frequency of 1.6 kHz, which was experimentally proven as statistically relevant for the inhibitory effect on S. Cerevisiae proliferation. The first set of experiments was then conducted with frequencies of 1.6, 0.8, and 0.4 kHz to verify hypothesis regarding effects of subharmonic frequencies and correlation thereof with IPR theory, presented in [30]. Results of these experiments are shown in Tab. 1.

Tab. 1: Comparison between experimental results observed at irradiation signal frequencies and IPR predicted frequencies at specified magnetic flux density targeting Ca^{2+} ion.

Magnetic flux density (mT)	2.39	2.39	2.39	2.39
Frequency generated (kHz)	1.600	0.800	0.400	0.200
Frequency predicted (kHz)	1.596	0.798	0.398	0.204
Growth area ratio (exposed/control)	0.59	0.54	0.64	0.80

From the experimental results it is clear that all subharmonic frequencies have shown inhibitory effects and statistically significant biological responses are present at frequencies 0.8 and 0.4 kHz, as well as the base frequency. These frequencies correspond to the ion parametric resonance frequency of Ca^{2+} ions, and harmonic components thereof in accordance with Lednev's IPR theory from [25], [26] and [31] and the Eq. (1):

$$f = \frac{f_c}{n} = \frac{1}{n} \cdot \frac{q}{2\pi m} \cdot B_{gen}, \tag{1}$$

where f_c represents the cyclotron resonance frequency of the ion, q is the elementary charge of the ion, m is

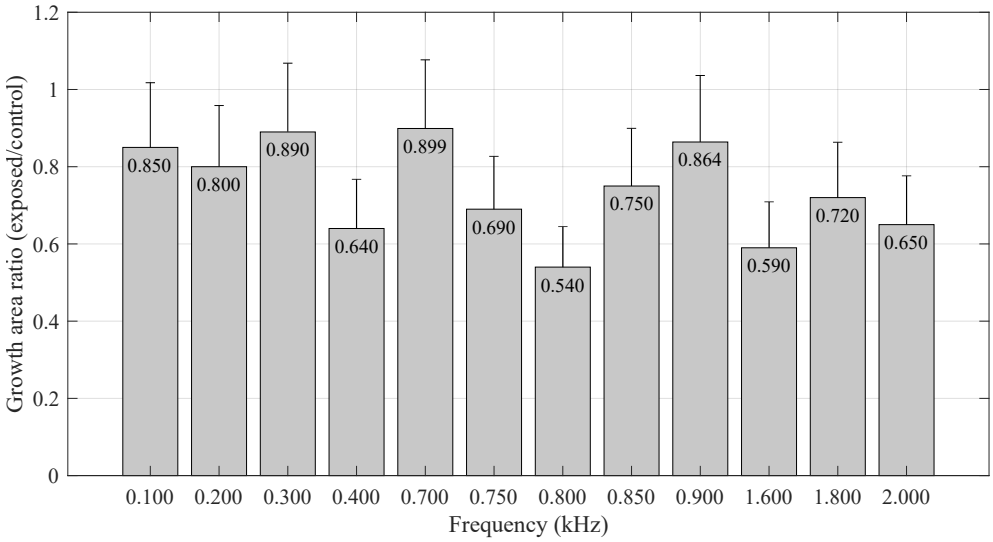

Fig. 3: Graphical interpretation of proliferation response of irradiated samples within the investigated frequency range of applied time-varying LF EMF.

mass of the ion and n denotes the corresponding harmonic component. B_{gen} is the mean value of generated magnetic flux density, which in our case was 2.39 mT.

Predicted frequencies for the biological response based on this model are also shown in Tab. 1. It is evident that experimental frequencies at which statistically significant biological effects were observed are closely related to their theoretically predicted counterparts.

Also apparent from the presented set of experiments is that the biological effect tends to disappear at lower frequencies, as was the case in experiments using 0.4 kHz. To verify this observation, we opted to investigate a wider frequency range, with focus on low frequencies and also on frequencies around the experimentally proven biologically active frequency of 0.8 kHz.

This set of experiments is presented graphically in Fig. 3, for better illustration and interpretation thereof. Observed results once again show inhibitory effects. More interestingly, there are signs of periodicity within the experimental data, with strong peaks at previously described resonance frequencies of Ca^{2+} that could be predicted by IPR model. Furthermore, the previous assumption, regarding the biological effect extinction at lower frequencies, seemed to occur at frequencies below 0.4 kHz.

Another interesting observation concerns the neighbourhood of the frequency 0.8 kHz, where the proliferative response is weak in comparison with the resonance (base) frequency, albeit still present. This observation points to the window theory, mentioned by Markov in [22], but could be also explained as a cumulative effect of other ions, which play an important part in cell proliferation processes.

Since frequency dependent biological response is evident from the presented results, we opted to also investigate the influence of magnetic flux density $\vec{B}$ of the generated LF EMF. For this purpose, we chose the frequency of 0.8 kHz, where the biological response seemed to be most significant, and altered the value of $\vec{B}$ to 50 %, 25 % and 10 % of its original value. These alterations were technically performed by amplitude changes of effective value of driving current, which directly influences the amplitude of $\vec{B}$ in the coil cavity and thus the exposed volume.

Results presented in Fig. 4 show that significantly strong proliferative response is present only with original magnetic flux density of 2.39 mT. The biological response is weak or no longer observed at given frequency due to modified magnetic flux density of the applied LF EMF.

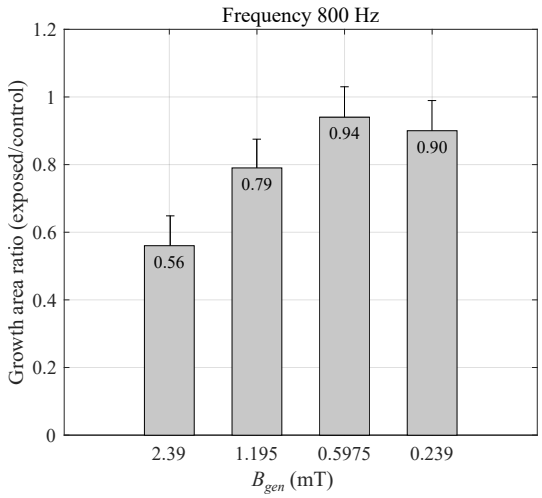

Fig. 4: Graph of proliferation dependence of S. Cerevisiae on B_{gen}, at the frequency of 800 Hz.

4. Conclusion

The presented article discusses chosen theories related to LF EMF mechanisms of interaction with biological objects, represented herein by Saccharomyces cerevisiae cells. Repetition of previously conducted experiments published in [8], [38], [40], [41] and [42] confirmed inhibitory effect on the proliferation response at the frequency of 1.6 kHz. Furthermore, the experimental results clearly demonstrated non-thermal effects of externally applied time-varying LF EMF on the proliferation process of cultivated cells. Our findings regarding the Ca^{2+} are also in correlation with research presented in [43]. The observed biological responses could be referred to as frequency selective because statistically significant effects occur only at specific frequencies, which points to the resonance character of the observed bio-effects.

The mechanism of action influencing the behaviour of the irradiated biological samples could be theoretically explained by the IPR theory. This model successfully predicts observed biologically active frequencies, considered resonance frequencies of Ca^{2+} ions and harmonic components thereof. From this point of view, we can draw the conclusion that Ca^{2+} ions play an important role in cellular proliferation processes, and could be exploited as targets for irradiation by external LF EMF tuned to parametric resonance frequency thereof, which corresponds with findings presented by Belova [27]. The relevance of V. V. Lednev's theory can be also confirmed by results of our investigation of magnetic flux density changes with respect to proliferation response at unchanged frequency. This means that when expecting biological response in accordance with the IPR theory and Eq. (1), any change of $\vec{B}$ necessarily leads to change of the ion parametric resonance frequency, but not every change of frequency must lead to change of magnetic flux density.

Despite the findings presented within this article, an unambiguous explanation of EMF influence on living structures is still lacking. Thus, many questions are still open in this field of research and can only be answered by great amount of successful and repetitively obtained experimental results from which conclusions and theories can be drawn.

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