INVESTIGATION OF EXTREMELY LOW-FREQUENCY MAGNETIC FIELD BIOEFFECT AT CELLULAR LEVEL CONCERNING ICNIRP GUIDELINES AND EXPOSURE LIMITS

Marek Bajtos[1], Roman Radil[1], Klaudia Hargasova[2,3], Zuzana Judakova[1], Lucia Carnecka[1], Ladislav Janousek[1]

[1]Department of Electromagnetic and Biomedical Engineering, University of Zilina, Zilina, Slovakia

[2]Department of Systems Biology and Engineering, Silesian University of Technology, Gliwice, Poland

[3]Biotechnology Center, Silesian University of Technology, Gliwice, Poland

Abstract

The article deals with the possible influence of an extremely low-frequency magnetic field on cellular structures. The work is carried out to verify the change of growth dynamics of exposed samples by application of a time-varying magnetic field, with a specified action value of magnetic flux density, valid in the European Union. The frequency of applied field was preliminarily confirmed as biologically active, under conditions for Ion Parametric Resonance theory, in experimental work focused on targeting Ca^{2+} ions bound in specific positions on cellular membranes of eukaryotic cells. In thirty experiments, the authors use the dynamics of growth over a time horizon of eight hours to elucidate the effect of the field on the proliferation activity of the cells. Since living beings are exposed to similar electromagnetic fields daily, the presented article shed some light on whether, or not the irradiation has brought a statistically significant effect under conditions specified in ICNIRP guidelines and recommendations.

Keywords

extremely low-frequency electromagnetic field, ion parametric resonance, radical pair mechanism, Saccharomyces cerevisiae, action value

Introduction

All living organisms, including human beings, are exposed daily to various types of electromagnetic fields (EMF), which are characterized by parameters such as electric field intensity, magnetic flux density, or frequency. In today's fast-paced and technologically advanced society, with an increasing number of electrical devices, the importance of effectively investigating the effects of extremely low-frequency electromagnetic fields (ELF-EMF) on living organisms, beginning at the cellular level, should be carefully considered.

Many studies have shown a negative effect of these fields on organisms [1, 2], therefore, it is important to precisely define the exposure limit values for the individual field parameters. Exposure to ELF-EMF, which is defined within the frequency range of 0–300 Hz according to WHO [3], can be associated with various diseases, such as malignancies, or cardio-vascular and neurological disorders [4–7]. ELF-EMF is manifested mainly by non-thermal effects [6–10], a description of which is problematic. Nevertheless, the biological effect of ELF-EMF is observed even under these conditions of low-level electric intensity, magnetic flux density, and frequency, but its mechanism remains unclear and represents an unresolved biophysical and social issue. Despite the lack of wide scientific acceptance, there are still some research groups, which proposed plausible physical mechanisms of ELF-EMF action on biological samples at the cellular and sub-cellular levels.

One of these theories is based on ion parametric resonance (IPR). The model describes the mechanism of a parallelly combined time-varying (AC) and static (DC) magnetic field (MF) action on specific ions in biological structures (cells, tissues...). Activity in biological

Lekar a technika – Clinician and Technology 2021, vol. 51(1-4), pp. 29–34, DOI: 10.14311/CTJ.2021.1.04
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

structures is controlled by chemical reactions induced by enzymes (specific proteins) and certain ions act as cofactors within these reactions. The IPR model states, that during ion resonance, the activity of the transformed ions can change predictably in proportion to the frequency of time-varying MF and its magnetic flux density B_{AC}. If no biological ion resonates, the size of B_{AC} will not affect the biological activity of the structure [11]. For resonance activity of a specific ion, the following applies:

$$f_{AC} = \frac{1}{n}\frac{q}{m}\frac{B_{DC}}{2\pi}. \quad (1)$$

where f_{AC} is the frequency in [Hz] of the time-varying magnetic field, q is the electric charge in [C] of a specific ion, m is the mass of the ion [kg] and B_{DC} is the value of the static magnetic field, in the case of this article represented by magnetic flux density of geomagnetic field [T]. According to Belova and Panchelyuga [12], the most pronounced biological response could be observed at 1.8 times the ratio of these fields, and thus it holds that:

$$B_{AC} = 1.8 \cdot B_{DC}, \quad (2)$$

where B_{DC} is the value of the Earth's magnetic flux density, which in our case was measured in the incubator where the experiments were performed, as 39 μT.

Another theory dealing with the action of ELF-EMF is the mechanism of radical pairs. Several authors [13–15] assume that changes in the concentration of radicals due to magnetic field can be in the range of 2–40%, depending on the parameters of the field and the length of exposure. An imbalance in the concentration of radicals can lead to the initiation of various diseases (disruption of lipid structures, proteins, DNA…). The mechanism of radical pairs suggests that MF can affect the concentration of radicals, which could be of clinical importance in various fields of medicine.

As aforementioned, research results within this scientific area provide some ambiguities, but these could be understood as strong motivational factors. Therefore, the presented work compares and discusses experimental results in terms of currently valid standards and recommendations regarding ELF-EMF exposure limits in the European Union. The main attention was paid to experimental verification of selected parameters combination of time-varying extremely low-frequency magnetic field (ELF-MF), which were defined as follows:

- frequency of the ELF-MF, which was proven by our preliminary experiments, as biologically active [16];
- the magnitude of magnetic flux density, related to its action value in the specified frequency band of ELF-MF following national and international standards and specifications.

The experimental part is performed by irradiation of eucaryotic cells, *Saccharomyces cerevisiae*.

Materials and Methods

The International Commission for Non-Ionizing Radiation Protection (ICNIRP) has specified basic limits and reference levels for short-term irradiation of CNS (Central Nervous System). These limits are presented in Table 1.

Table 1: Reference levels for general public exposure to time-varying electric and magnetic fields (unperturbed rms values) [17].

Frequency range	***E*** (V/m)	***B*** (μT)
0 Hz–1 Hz	-	4 · 104
1 Hz–8 Hz	10 000	3.2 · 104/f2
8 Hz–25 Hz	10 000	5000/f
0,025 kHz–0,8 kHz	**250/f**	**5/f**
0,8 kHz–3 kHz	250/f	6.25
3 kHz–150 kHz	87	6.25
150 kHz–1 MHz	87	0.92/f
1 MHz–10 MHz	87/f1/2	0.92/f
10 MHz–400 MHz	28	0.092
400 MHz–2 GHz	1.375 · f1/2	0.0046 · f1/2
2 GHz–300 GHz	61	0.2

To protect society from the possible negative effect of ELF-EMF, recommendations of ICNIRP for reference levels have been accepted by European Union for this purpose (1999/519/EC). For experimental verification of biological activity, one action value of magnetic flux density of 100 μT was chosen, concerning the directive 2013/35/EU, as well. The value corresponds to the power line frequency (50 Hz) and falls within the range highlighted in Table 1.

Irradiation system

Irradiation was performed using an exposure system, which incorporates a RIGOL DG4162 generator, the signal of which was amplified by HUBERT A 1100-16 amplifier and fed to an incubator containing 2 elongated solenoid coils (1 m each). The signal was connected to one exposure coil, while the other served as a control. For the continuous monitoring of the driving current, the KEYSIGHT Infinii Vision MSO-X 3012A oscilloscope was used. To achieve the specified magnitude of MF, the coil was driven by a harmonic sinusoidal current of 28.13 mA. The experimental setup can be seen in Fig. 1. There were 5 Erlenmeyer flasks in the cavity of each coil enabling to perform 5 pair experiments at a time, which is schematically shown in Fig. 2. The orange set in Fig. 2 was labeled as exposed, and the blue set as control.

Lekar a technika – Clinician and Technology 2021, vol. 51(1-4), pp. 29–34, DOI: 10.14311/CTJ.2021.1.04
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

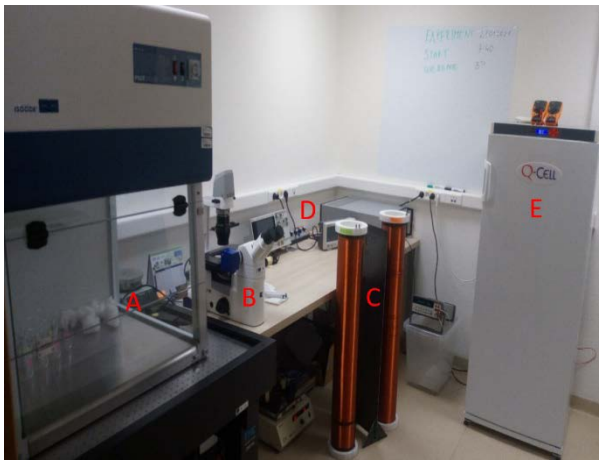

Fig. 1: Experimental set-up. A – PCR box, B – optical microscope, C – two identical MF applicators, D – EMF excitation system, which incorporates signal generator, amplifier HUBERT A 1100-16 (DC-1MHz), and digital multimeter for electric current monitoring, E – incubator Q-cell.

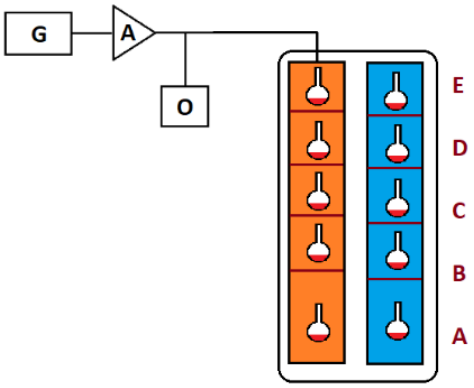

Fig. 2: Schematic representation of the signal input to the coil in incubator, where G – generator, A – amplifier, O – oscilloscope.

Cultivation protocol and temperature monitoring

The model organism of experiments were yeast cells of *Saccharomyces cerevisiae*, since their genome has been sequenced, its genetics is easy to manipulate, and these cells have a similar structure to higher eukaryotic cells.

For yeast cultivation and propagation YPD (Yeast-Extract-Peptone-Dextrose) solution was used. Substances required to prepare the YPD medium include 2% peptone, 1% yeast extract, 2% glucose, and 95% distilled water.

Prior to each experiment, a 24-hour pre-cultivation process at chamber temperature was performed, followed by dilution to achieve an equal initial concentration of experimental samples. Afterward, the 8 hours of continuous irradiation was performed with temperature in the incubator set at 30 °C, maintaining the same ambient conditions for both control and exposed samples.

The temperature for experiments was chosen based on [18], where authors found that the optimal temperature for the fastest *Saccharomyces Cerevisiae* initial growth is within the interval of 30 °C to 35 °C. Since yeasts are sensitive to temperature changes, it is even one of the most important factors influencing the growth and proliferation of their cultures, it is necessary to keep it at a constant level. This was achieved by temperature monitoring using a control device developed by the authors. The setup incorporates an Arduino platform and two DS18B20 sensors, which were used, for exposed and control samples temperature monitoring during an 8-hour experiment. Data were real-time displayed on the OLED display SSD1306 and stored in a file on an SD card.

Evaluation method

The evaluation methodology was established on a manual count of cells on Bürker chambers, followed by a calculation of the growth coefficient, which indicates how many times more, resp. fewer yeast cells have been observed within the solution after the experiment duration. An example of one exposed sample growth coefficient calculation is represented by equation (3):

$$E_{\mathrm{X}} = \frac{\sum_{i=1}^{6} E_{\mathrm{after}\,(i)}}{\sum_{i=1}^{6} E_{\mathrm{before}\,(i)}}, \qquad (3)$$

where E_{after} stands for the number of cells in the 6 Bürker chambers after the experiment, while E_{before} represents the number of cells from 6 counting squares before the experiment for the exposed sample. E_{X} is the partial growth coefficient for the exposed sample. The same calculation applies to each exposed and control sample. A more detailed description of this methodology could be found in the previous work of the authors [19].

Results

The comparison of growth coefficients of the 25 individual experiments is shown in Fig. 3. The blue bar shows the ratio between the concentration of cells in the control, non-irradiated samples, and the orange bar shows the ratio between the concentration of cells in the exposed, irradiated samples. Error bars show standard error, calculated from the standard deviation. The standard deviation for the set of data Control after/before was 2.32 and for data Exposed after/before 2.31. In the set of 5 preliminary experiments, the value of B_{AC} was changed to strictly follow the IPR theory. The value of the parallel component of the magnetic flux density of time-varying MF was set to 70.2 μT and its frequency to 29.89 Hz, which corresponds to the IPR

Lekar a technika – Clinician and Technology 2021, vol. 51(1-4), pp. 29–34, DOI: 10.14311/CTJ.2021.1.04
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

model resonance-based predictions calculation for the calcium ions Ca^{2+} resonant frequency. In this set of experiments, the standard deviation for the Control after/before data equals 0.34 and 0.43 for the Exposed after/before data. Results after 8 hours of irradiation are presented in Fig. 4.

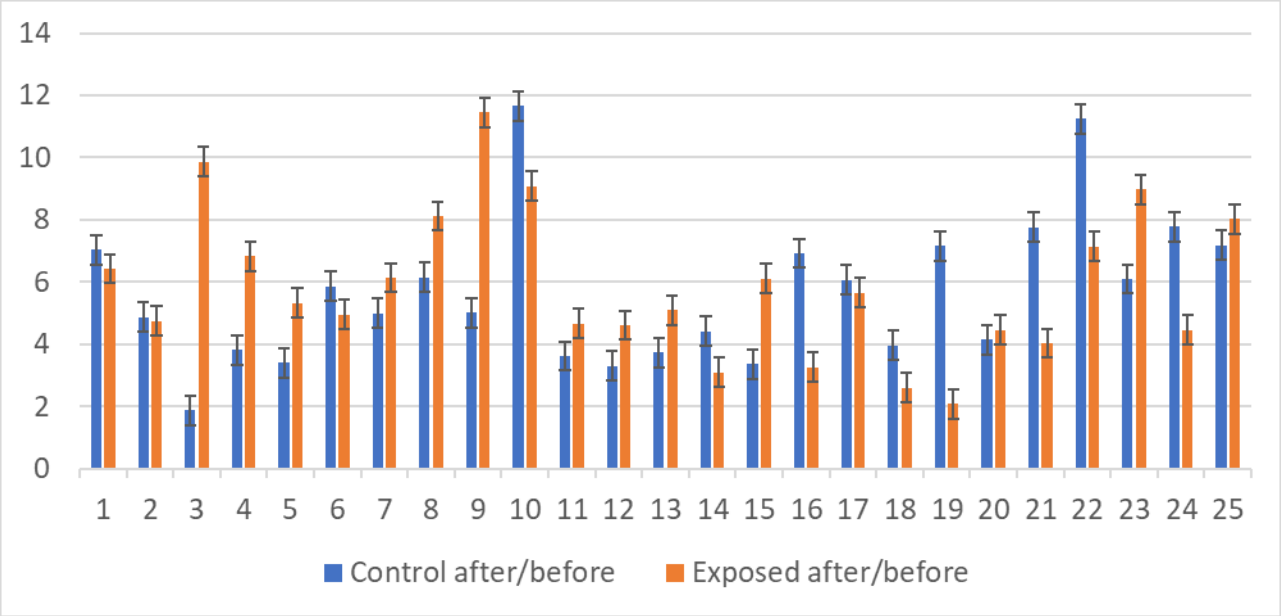

Fig. 3: Comparison of growth dynamics for 25 control and exposed experiments (f_{AC} = 29.89 Hz, B_{AC} = 100 μT).

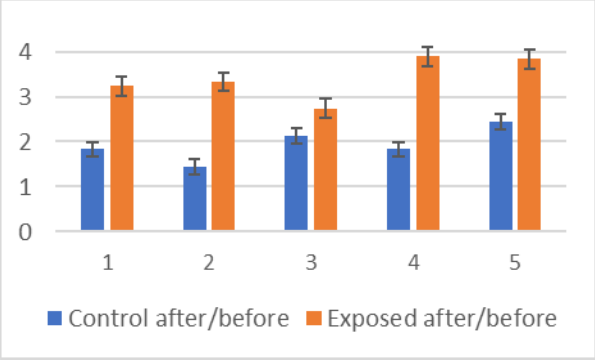

Fig. 4: Comparison of growth dynamics for each of 5 preliminary experiments: control (blue bar) and exposed (orange bar) samples (f_{AC} = 29.89 Hz, B_{AC} = 70.2 μT).

Discussion and Conclusion

From the presented results could be concluded, that in thirteen experiments, the stimulation effect of the MF on the proliferative activity of yeast cultures was observed, in twelve experiments this effect could be defined as proliferative inhibition. The results were indicated by the statistical evaluation, where the Willcoxon (Mann-Whitney) test was used, which at the 5% level of significance accepts the null hypothesis, and thus it could be concluded, there is no statistically significant difference between the growth coefficients of exposed and control samples. In general, the presented results suggest that ELF-MF with a frequency value of 29.89 Hz and signal amplitude of 100 μT after 8 hours of irradiation did not show a statistically significant effect on the proliferation response of *S. Cerevisiae* cultures. However, since our research suggests the existence of a fundamentally selective mechanism of influence on biological samples, we must admit, that the first set of 25 experiments was performed with signal amplitude, which was selected as an action value for ELF-EMF for the power line frequency (50 Hz). The combination of frequency and magnetic flux density in the main set of experiments did not meet the exact conditions for ion parametric resonance theory, which predicts, that magnetic flux density for targeting calcium cations at power line frequency, should be set at 70.2 μT, what is currently the target of further investigations of authors. The logic, or motivation behind the magnetic flux density change at the frequency of 29.89 Hz could be found in the results of previously published works by authors [19, 20]. The research of mentioned works was focused on low-frequency MF magnetic flux density change maintaining one so-called bioactive frequency of driving signal, which resulted in interesting observations of cell cultures growth inhibition.

The results of 25 experiments described within this work are still in good correlation with previous works of authors [19, 20], and other research groups [21, 22], which point to the selective response of biological systems to the application of external ELF MF. Simply, if the combination of ELF-MF parameters does not fulfill certain specific conditions, the biological reaction would be weak, or would not occur at all.

Lekar a technika – Clinician and Technology 2021, vol. 51(1-4), pp. 29–34, DOI: 10.14311/CTJ.2021.1.04
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

The biological reaction selectivity is also supported by data set of preliminary experiments which meet the demands of the IPR predictive model of magnetic field interactions with biological systems targeting Ca^{2+} ions. The data show a statistically significant effect of growth stimulation in the whole experimental series, but still, further research must be conducted to statistically prove obtained results.

Finally, in future experiments, it would be interesting to appropriately change the frequency of the signal by magnetic flux density corresponding to IPR theory targeting different ions. For 70.20 μT this would lead to frequencies of 16.8917 Hz targeting chloride anions, 20.7607 Hz for iron cations, 18.258 Hz for zinc cations, and 593.6943 Hz for hydrogen cation H^+ or 85.7006 Hz for lithium cations, where all mentioned ions are present in the human body [11].

Apart from the IPR theory, fields with magnetic flux density with magnitudes of thousands of μT are used in magnetic therapy. It could be appropriate to increase the value of the investigated MF magnitude in the experimental protocol, but also the frequency accordingly, and monitor the effect of the field with new parameters.

The future direction of work could be found, especially in long-term exposure of samples to extremely low-frequency magnetic fields and to focus on irradiation of one sample for weeks or for months, to see if there is any possibility of cumulative effect. Although the original 8 hours of irradiation represents the time when the yeast cultures are the most metabolically active, at extremely low frequencies a more pronounced effect could be observed in long-term exposure. On the contrary, at higher frequencies and amplitudes of the field (915.66 Hz, 1.195 mT, and 2.33 mT), a statistically significant effect has been observed after the specified eight hours in our previous works [19, 20].

Acknowledgement

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-19-0214.

A preliminary version of the results published in this article was presented at the Trends in Biomedical Engineering 2021 conference.

References

[1] Pall ML. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverseeffects. J Cell Mol Med. 2013 Aug;17(8):958–65. DOI: 10.1111/jcmm.12088

[2] Zheng Y, Ma XX, Dong L, Ma W, Cheng JH. Effects of uninterrupted sinusoidal LF-EMF stimulation on LTP induced by different combinations of TBS/HFS at the Schaffer collateral-CA1 of synapses. Brain Res. 2019 Dec 15;1725: 146487. DOI: 10.1016/j.brainres.2019.146487

[3] Electromagnetic fields and public health intermediate frequencies (IF) [Internet]. World Health Organization; 2005 [cited 2022 Jul 12]. Available from: https://www.jeic-emf.jp/assets/files/Fact%20Sheets/facrt_sheet_IF.pdf

[4] Mustafa Y, Riad S, Abdel-Rahman S, El-Demellawy M, El-Messeiry S. Effect of Low Frequency Electromagnetic Fields (LF-EMFs) on c-myc Oncogene Expression Level in Peripheral Blood Mononuclear Cells (PBMCs). Biotechnol J Int. 2014 Oct 30;4(12):1263–71. DOI: 10.9734/BBJ/2014/14106

[5] Jeong JH, Kim JS, Lee BC, Min YS, Kim DS, Ryu JS, et al. Influence of exposure to electromagnetic field on the cardiovascular system. Auton Autacoid Pharmacol. 2005 Jan;25(1):17–23. DOI: 10.1111/j.1474-8673.2004.00328.x

[6] Misek J, Belyaev I, Jakusova V, Tonhajzerova I, Barabas J, Jakus J. Heart rate variability affected by radiofrequency electromagnetic field in adolescent students. Bioelectro-magnetics. 2018 May;39(4):277–88. DOI: 10.1002/bem.22115

[7] Misek J, Veterník M, Tonhajzerova I, Jakusova V, Janousek L, Jakus J. Radiofrequency electromagnetic field affects heart rate variability in rabbits. Physiol Res. 2020 Aug 31;69(4):633–43. DOI: 10.33549/physiolres.934425

[8] Lewczuk B, Redlarski G, Zak A, Ziółkowska N, Przybylska-Gornowicz B, Krawczuk M. Influence of electric, magnetic, and electromagnetic fields on the circadian system: current stage of knowledge. Biomed Res Int. 2014;2014:169459. DOI: 10.1155/2014/169459

[9] Balassa T, Szemerszky R, Bárdos G. Effect of short-term 50 Hz electromagnetic field exposure on the behavior of rats. Acta Physiol Hung. 2009 Dec;96(4):437-48. DOI: 10.1556/aphysiol.96.2009.4.4

[10] Shin EJ, Jeong JH, Kim HJ, Jang CG, Yamada K, Nabeshima T, et al. Exposure to extremely low frequency magnetic fields enhances locomotor activity via activation of dopamine D1-like receptors in mice. J Pharmacol Sci. 2007 Dec;105(4):367–71. DOI: 10.1254/jphs.sc0070348

[11] Blanchard JP, Blackman CF. Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems. Bioelectromagnetics. 1994;15(3):217–38. DOI: 10.1002/bem.2250150306

[12] Belova NA, Panchelyuga VA. Lednev's model: Theory and experiment. Biophysics. 2010;55(4):661–74. DOI: 10.1134/S0006350910040263

[13] Timmel CR, Till U, Brocklehurst B, Mclauchlan KA, Hore PJ. Effects of weak magnetic fields on free radical recombination reactions. Molecular Physics. 1998;95(1):71–89. DOI: 10.1080/00268979809483134

[14] Usselman RJ, Hill I, Singel DJ, Martino CF. Spin biochemistry modulates reactive oxygen species (ROS) production by radio frequency magnetic fields. PLoS One. 2014 Mar 28;9(3): e93065. DOI: 10.1371/journal.pone.0093065

[15] Simkó M. Cell type specific redox status is responsible for diverse electromagnetic field effects. Curr Med Chem. 2007;14(10):1141–52. DOI: 10.2174/092986707780362835

[16] Hargasova K. Vyšetrovanie biologickej reakcie buniek na časové rozdiely v kontinuite ožarovania nízkofrekvenčným elektromagnetickým poľom [diploma thesis]. [Zilina]: University of Zilina; 2021. Available from: https://opac.crzp.sk/?fn=detailBiblioForm&sid=ECC3D3F0B3159C4F3712EB027BE4&seo=CRZP-detail-kniha

[17] International commission on non-ionizing radiation protection. ICNIRP guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz). s.l.: HEALTH PHYSICS, 1998.

[18] Walsh RM, Martin PA. Growth of saccharomyces cerevisiae and saccharomyces uvarum in a temperature gradient incubator. Journal of the Institute of Brewing. 1997;83:169–72. DOI: 10.1002/j.2050-0416.1977.tb06813.x

[19] Radil R, Barabas J, Kamencay P, Bajtos M, Hargasova K. Targeting Ca2+ and K+ ions using LF EMF to induce proliferation response of S. Cerevisiae. In 12th International Conference on Measurement 2019 May 27 (pp. 119–22). IEEE. DOI: 10.23919/measurement47340.2019.8780087

[20] Radil R, Barabas J, Janousek L, Kamencay P. Investigation of Magnetic Flux Density Variation Influence on the Biological Response of Cell Cultures. In 13th International Conference on Measurement 2021 (pp. 186–9). IEEE. DOI: 10.23919/Measurement52780.2021.9446789

[21] Bereta M, Teplan M, Chafai DE, Radil R, Cifra M. Biological autoluminescence as a noninvasive monitoring tool for chemical and physical modulation of oxidation in yeast cell culture. Sci Rep. 2021 Jan 11;11(1):328. DOI: 10.1038/s41598-020-79668-2

[22] Zastko L, Makinistian L, Moravčíková A, Jakuš J, Belyaev I. Effect of Intermittent ELF MF on Umbilical Cord Blood Lymphocytes. Bioelectromagnetics. 2020 Dec;41(8):649–55. DOI: 10.1002/bem.22302

Ing. Roman Radil, PhD.
Department of Electromagnetic and Biomedical Engineering,
Faculty of Electrical Engineering and Information Technology,
University of Zilina,
Univerzitna 1, 01026, Zilina, SR

E-mail: roman.radil@feit.uniza.sk
Phone: +421 415 132 113