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LOW FREQUENCY ELECTROMAGNETIC FIELD EFFECTS ON GROWTH DYNAMICS OF YEAST CELLS

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Abstract: The paper deals with low frequency electromagnetic field effects on growth dynamics of yeast cells Saccharomyces cerevisiae. The current state of the research of electromagnetic field interactions with biological systems is briefly introduced. Several experiments are carried out in order to investigate influence of specifically adjusted artificial low frequency electromagnetic field on Saccharomyces cerevisiae growth dynamics. The experimental apparatus, materials, conditions and evaluation methods are described in the paper. The presented results indicate the differences in growth dynamics between cells exposed to magnetic field and the unexposed ones.
Keywords: electromagnetic field, yeast cells, exposed sample, control sample

INTRODUCTION

The influence of low-frequency electromagnetic field (LF EMF) on living organisms is widely discussed issue both in scientific area and in public. The interest in this topic has increased due to the rapidly extending daily use of electronic devices and generally increasing rate of human exposure to electromagnetic radiation from various artificial sources. There are numerous experimental studies investigating electromagnetic field (EMF) effects on living cells, tissues or on human health in general. Since number of studies [1-7] showed the potential connection between LF EMF exposure and cancer or other diseases, the interest in research of EMF interaction with biological systems has grown. Numerous works focusing on experiments with living cells showed the differences in the proliferation process between cells exposed to electromagnetic field and control (non-exposed) cells [8-14]. Other works investigate the animal behavior under the exposition of EMF [15] or possibility of genotoxic effect of EMF exposure [16]. The results of these works are often non-consistent and as Buchachenko [17] stated, the biological electromagnetic effects sometimes seem to be irreproducible and contradictory.

Besides experimental investigation, many authors tried to propose physical mechanism of biological LF EMF impact. One of the first suggested physical mechanisms was ion cyclotron resonance model (ICR) firstly proposed by Liboff [18]. ICR holds that the physiological activity of some important ions can be altered when the frequency of applied time-varying magnetic field is equal to the frequency of ion motion in static magnetic field (cyclotron frequency). The same assumptions are used in the ion parametric resonance (IPR) model [19, 20], but the interpretation of ion behavior is different. Ion in IPR model is represented by a harmonic oscillator and applied combined magnetic field should affect its oscillations. Both resonance models have been criticized due to several physical imperfections [21, 22], particularly due to the problem of thermal noise. Despite the lack of satisfactory physical explanation, there remains an impressive body of experimental evidence that can be taken as an empirical basis for ICR and IPR hypotheses [8-13]. The most accepted mechanism of biological EMF effects in recent years is the radical pair mechanism [23-26]. In this mechanism, it is proposed that magnetic field could affect the reactivity of biological radicals and alter their final concentration in biological milieu. Several authors [27, 28] expect that the final concentration of radicals could be changed in the range of 2 up to 40%, when the magnetic field is applied. Since radicals have an important role in biology it could lead to significant consequences for biochemical reactions in organisms.

Although an extensive theoretical and experimental research has been carried out, the unambiguous explanation of electromagnetic field influence on living structures is still lacking. Many open questions in this research field can be answered only by great amount of repetitively obtained experimental results. Results of experiments showing LF EMF effects on growth curve of cells *Saccharomyces cerevisiae* are presented in this paper. The growth curve of yeast cells is generally composed of five basic phases [29]. When the cells are inoculated into cultivation medium, they adapt to the medium (lag phase). The lag phase is followed by the exponential phase. The cells consume fermentable sugar, *e.g.* glucose, and the biomass concentration increases exponentially. When the glucose in medium is being limited, the diauxic phase occurs and the metabolism is shifted from anaerobic to aerobic pathway. In the post-diauxic phase the cells consume ethanol and other products of fermentation. Once these energy sources are exhausted, the cells enter the stationary phase and the maximal biomass concentration is reached. The representative growth curve of cells can be seen in the Figure 1.

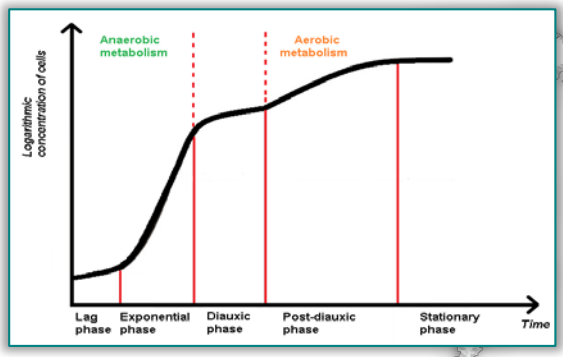

Figure 1. Representative growth curve of yeast cells (adopted from [29] and modified)

MATERIALS AND METHODS

The paper concentrates on experimental investigation of specific artificial LF EMF on growth dynamics of yeast cells. The cells are precultivated before the experiments. Two samples with the same initial concentration of cells are then used for experiments. One is exposed with artificial LF EMF of specific parameters, the other is shielded from the LF EMF. Details of experiments are described in details in this section.

A. Experimental Protocol

Yeast culture is stored in a refrigerator at 4°C. Yeasts are inoculated into glass Erlenmeyer flask (100 ml) with 10 ml of YPD medium (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) D-glucose in distilled water) and cultivated for 16 hours at 25°C on an orbital shaker at 180 rpm. Cell concentration in culture medium is counting after cultivation by using counting chamber (Neubauer) and microscope (Primovert, Carl Zeiss Microscopy, LLC). The same volume of culture medium with cells is transferred into two Erlenmeyer flasks (100 ml) with 30 ml of fresh YPD in order to ensure the same initial concentration of cells in two experimental samples.

Parallel measurements of cell concentration are performed. One sample is exposed to magnetic field generated by coil (exposed sample) and one sample is shielded from the magnetic field (control sample). Concentration of cells from both samples is measured every two hours during 8 hours. The samples are cultivated at 25°C and are bubbled by air in order to ensure oxygen intake and to avoid sedimentation of cells.

B. Measurement Equipment

Measurements take place in double-chamber incubator in which Erlenmeyer flasks with cell cultures are placed. The previously designed and fabricated exposure coil is used as a source of low frequency magnetic field. The homogeneity in exposure area achieves ~90%. Schematic drawing of the measurement system can be seen in the Figure 2, and photography in the Figure 3.

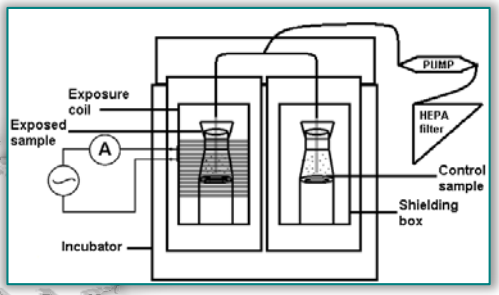

Figure 2. Measurement system schematic

Figure 3. Measurement system photo

C. Experimental Conditions

Experimental conditions are set up based on the previous results of authors [8, 30]. These conditions arose from assumptions of the ion parametric resonance model [20] and observed different growth areas of cell cultures between exposed and control samples by specific ratios of frequencies and magnetic flux density of applied magnetic field. The harmonic driving signal with frequency 1600 Hz is generated by a signal generator (Agilent E4436B, Agilent Technologies, Inc.) and amplified by a linear amplifier (Hubert A1110-05, Dr. Hubert GmbH). Magnetic flux density within the exposed volume varies spatially between 2.2 and 2.3 mT.

RESULTS AND DISCUSSION

To investigate the LF EMF influence on growth dynamics of yeast cells, the concentrations of cells are measured and growth curves are then constructed. The ratios of cell concentrations of exposed to control samples during the experiments are calculated and discussed.

Five experiments with the same exposure conditions are performed. The concentrations of both control and exposed samples of Saccharomyces cerevisiae are measured and analyzed. The growth curves presented in the Figure 4 are constructed from average values of cell concentrations, measured at selected time points (0 h, 2 h, 4 h, 6 h, 8 h) during five experiments. The error bars represent standard deviation of the measured cell concentrations. The ratios of cell concentration in exposed sample (c_{exp}) to cell concentration in control sample ($c_{control}$) can be seen in the Table 1. The bar graph in the Figure 5 shows the ratios of exposed to control cells at the end of each experiment.

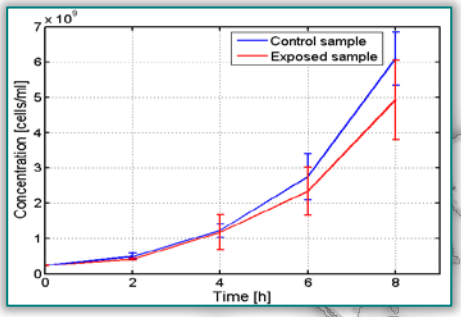

Figure 4. Growth curves of control and exposed sample

Table 1. Ratios of cell concentrations during measurements

	Ratios $c_{exp}/c_{control}$ in selected time points				
Measurement No.	0 h	2 h	4 h	6 h	8 h
1	1.0	0.83	1.66	0.93	0.75
2	1.0	0.80	0.67	0.83	0.92
3	1.0	0.73	0.85	0.62	1.01
4	1.0	0.96	0.88	0.74	0.52
5	1.0	0.94	0.77	1.18	0.87

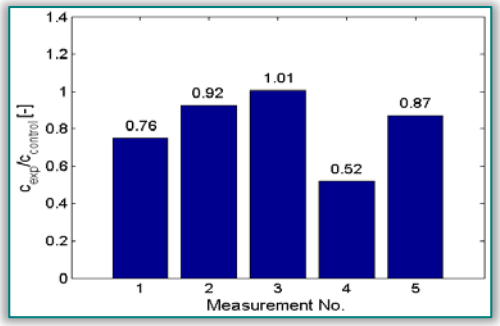

Figure 5. Ratios of cell concetrations in exposed sample to control sample at the and of each experiment

The dynamics of cell growth between exposed and control sample is slightly different. The increase of cell concentration in control sample is faster compared to the exposed sample. The final concentration of cells in exposed and control sample would very likely reach the same values, due to the identical initial conditions for cells. Since the difference of concentrations in exposed and control sample increases with time, it is probable that the control sample enters the stationary phase earlier than the exposed sample. This fact would imply slower growth dynamics of cells in exposed sample. One could then assume that the EMF in these experimental conditions has an inhibitory effect on cell proliferation processes. The ratios of concentrations of exposed to control sample presented in the Tab. 1 and Figure 5 are in most of cases lower than 1.0. It is in accordance with the previous assumption about an inhibitory effect of EMF. On the other hand it is necessary to notice very high standard deviations of measured cell concentrations (Figure 4). To eliminate this imperfection and to be able to state any satisfactory conclusion about the biological LF EMF impact, additional experiments with the same exposure conditions are needed to be performed. Furthermore it would be beneficial to evaluate several extra parameters characterizing growth dynamics of cell culture, such as absorbance, dissolved oxygen concentration, etc. Nevertheless, these preliminary results like the recent experimental studies realized at our department [8, 30] indicate the detectable interaction of electromagnetic field in low frequency range with living structures.

CONCLUSION

The presented paper dealt with low frequency electromagnetic field influence on growth dynamics of *Saccharomyces cerevisiae* cells. The preliminary results indicated, that LF EMF affect the cell proliferation processes. The obtained results implied the differences in growth dynamics of cells exposed to time-varying magnetic field (1600 Hz, ~2.3 mT) compared to control cells. The growth curves and ratios of exposed to control samples implied the inhibitory effect of EMF on cell growth. It is necessary to realize additional experiments with the same conditions and to perform proper statistical evaluation in order to state some unambiguous conclusion of the LF EMF influence on growth dynamics of cells.

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