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THE EFFECT OF ELECTROMAGNETIC FIELDS ON BAKER'S YEAST POPULATION DYNAMICS, BIOCATALYTIC ACTIVITY AND SELECTIVITY

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ABSTRACT. The influence of ultralow frequency in the range of 23.8 -35.7 mT electromagnetic field on growth dynamics of *Saccharomyces cerevisiae* cells and the activity and selectivity for the cellular bioreduction of the prochiral 1-(benzofuran-2-yl)ethanone has been evaluated. The interaction of cell populations with the electromagnetic field reduced their growth rate, their activity and selectivity. The effects directly depends on the intensity of the applied field.

Keywords: Saccharomyces cerevisiae, electromagnetic field, cell growth dynamics, activity, selectivity, bioreduction, biocatalysis

INTRODUCTION

Electromagnetic fields with low frequencies (50-60 Hz) and low intensity are associated with the production, transmission and use of electricity. They interfere with the frequency of many biological processes involving changes in the electrical charges movements of the biomolecules. Furthermore, nowadays, due to the antropic activities, a large spectrum of electromagnetic fields, differing in their frequency are present in the biosphere as presented in Table 1 [1]. Communication, remote controls are the most common such applications.

Being a part of our environment, these fields were considered among potential interference factors that can modify the behavior of organism. That explains the great number of studies on this topic, dedicated to cells [2], tissues,

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whole living organisms [3]. Also the cell dynamics, the ion transport or gene transcription and expression [4] were consistent study objects. The effects of magnetic fields are numerous and apparently contradictory, depending on their static or alternative nature, intensity and frequency, by influencing the production of certain metabolites [5] they can stimulate or inhibit the cell growth [6- 7].

Electromagnetic fields influence DNA structure even at extremely low frequency. Based on the reported effects, the International Agency for Research on Cancer (IARC) has classified the extremely low-frequency (ELF) electromagnetic fields (EMF) as "possible carcinogenic" [8].

Saccharomyces cerevisiae is a validated experimental model, being one of the most intensively studied eukaryotic organisms in molecular and cell biology [9]. Our interest was focused on the investigation of possible action of the electromagnetic field on the catalytic parameters (activity and selectivity) and growth dynamic of *Saccharomyces cerevisiae*.

Table 1. Clasification of electromagnetic fields according to the frequency and use [1]

Frequency zone	**Frequency**	**Wavelength in the air**	**Use**
Extremely low	0-300 Hz	>1000 km	Many biological processes, electricity transport
Low, middle, high	30 kHz-30 MHz	10 km -10 m	Amateur radio, remote controls
Very, ultrahigh	30-300 MHz	10-1 m	Radio/TV
Superhigh	300 Mhz- 30 GHz	1 m-10 cm	Satellite communication
Extremely high	30-300 GHz	10-1 cm	RADAR
Infrared	300 GHz- 300 THz	1 cm-100 mm	
Visible light	429-750 THz	700-400 nm	Light

RESULTS AND DISCUSSION

Literature data showed that in the range of extremely low frequencies associated to commercial electric power at low values of amplitude (0.2-12 mT), a slight stimulation of cell growth [10] and also dehydrogenase activity [11] may occur. We investigated the influence of a 50 Hz magnetic field, generated in a toroidal coil, on *Saccharomyces cerevisiae* cellular proliferation. The activity and selectivity of yeast dehydrogenases in the reduction reaction of a substrate was tested in both the presence and the absence of the electromagnetic field.

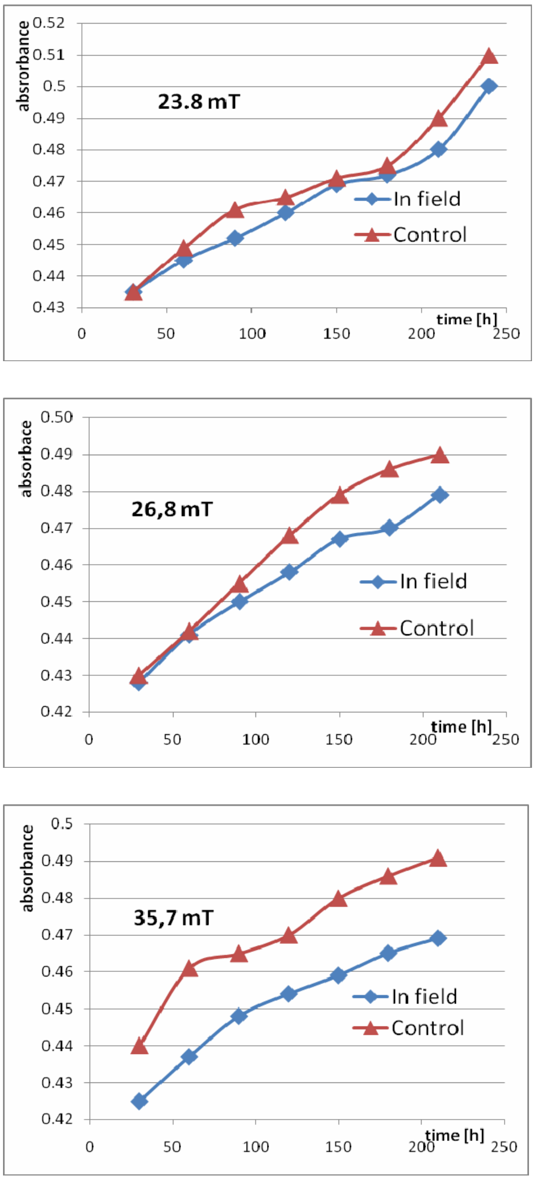

Figure 1. Comparative cell population dynamics in a magnetic field

a) The effect of the electromagnetic field on Saccharomyces cerevisiae growth dynamics

The cellular growth rate was slowed at all tested intensities of the electromagnetic field with the frequency of 50 Hz. As shown in Figure 1, the growth curves of the exposed yeast populations had lower values than the curves of the control populations.

In all cases the growth was positive, but the electromagnetic field showed a slight inhibitory effect.

The slowing of the cell growth was proportional with the intensity of the field.

b) The effect of the electromagnetic field on enzyme activity and selectivity

Biocatalyst-assisted conversion of the substrate is an estimate of the biocatalytic activity of the cells.

Parallel determinations in presence of the electromagnetic field at 26.8 and 35.7 mT and in unexposed cultures showed that in the range of these intensities the field reduced the catalytic activity (Figure 2) and selectivity of yeast dehydrogenases (Table 2).

The selectivity of the biocatalytic reaction was estimated by measuring the enantiomeric excess of the bioreduction product using as substrate 2-acetyl-benzofuran (Figure 3).

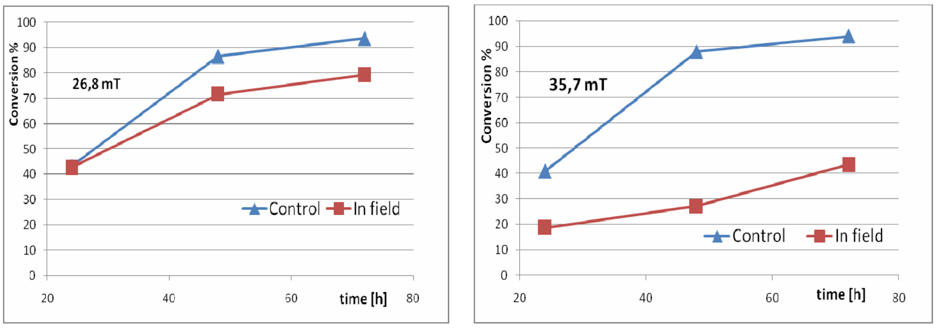

Figure 2. Influence of electromagnetic field on the conversion of the bioreduction of 2-acetylbenzofuran

As for enzymatic activity, determinations showed that samples exposed to the electromagnetic field, in the range of 26.8 and 35.7 mT intensity, lead to enantiomeric excesses of the product under the values found for the unexposed samples.

1-(benzofuran-2-yl)ethanone (*S*)-1-(benzofuran-2-yl)ethanol

Figure 3. Cellular reduction of 2-acetyl-benzofuran

Table 2. Enantiomeric excesses of the reaction product obtained with exposed and unexposed cells

Enantiomeric excesses (ee%)	**1.8 A**			**2.4 A**		
	24h	48h	72h	24h	48h	72h
Control sample	72%	73%	75.4%	66%	71%	77%
Exposed sample	67%	72%	74%	38%	44%	45%

CONCLUSIONS

The electromagnetic field generated by an alternative current of 50 Hz, applied at intensities in the range of 23.8-35.7 mT on *Saccharomyces cerevisiae* suspensions produced significant alterations in the growth dynamics of the cell populations. The catalytic activity and the selectivity in the reduction of the investigated prochiral 1-(benzofuran-2-yl)ethanone were impaired.

The results obtained in parallel experiments, with samples unexposed and exposed to the electromagnetic field, are in aggreement with the literature data and demonstrate the lowering of the growth dynamics.

Our original experiments on the biocatalytic activity and selectivity showed that the interference of the electromagnetic field with the biochemical process resulted the decrease of the substrate transformation rate and also reduced the discrimination capability of the enzymatic equipment for the two enantiotope faces of the substrate.

EXPERIMENTAL SECTION

The electromagnetic field was generated in a toroidal coil powered by an autotransformer at three values of the AC power: 1.6 A, 1.8 A and 2.4 A, which produced in the coil axis magnetic inductions of 23.8, 26.8 and 35.7 mT, respectively.

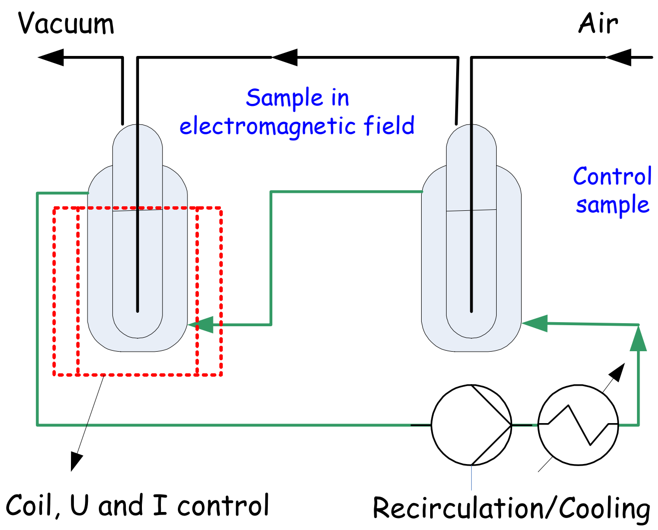

Figure 4. Experimental design for studying the effect of the electromagnetic field on certain functional parameters in *Saccharomyces cerevisiae*

A glass vial (bioreactor) provided with a cooling jacket and an air bubbler was introduced in the axis of the coil. An identical inexposed vial was used as control reactor. Both vials were termostated at 25 ^{o}C. The yeast suspensions were agitated with the same rate by air bubbling using an arrayed assembly as shown in Figure 4. Two experiments were performed, one at three different intensity values of the field and the second at two values of field intensity. In the first experiment it was investigated the influence of the intensity of the field upon the cell population growth and in the second one the effect of the field upon the stereoselectivity and the reaction rate for the cellular bioreduction of 2-acetyl-benzofuran was monitored. In all experiments commercially available baker's yeast was used.

For the growth experiment first a calibration curve was draw on, using the correlation between absorbance at 600 nm, and cellular concentration (gl^{-1}). The absorbance was measured using an UV-VIS Perkin-Elmer, type Lambda 1 spectrophotometer.

A suspension with the initial concentration of 0.500 gl^{-1} yeast and 2.5 gl^{-1} sugar was introduced in the two bioreactors. Samples were taken every 30 min. from both bioreactors, for a period of 240 min.

In the selectivity experiment, the investigated reaction was the enantiotope selective reduction of 2-acetyl-benzofuran (1-(benzofuran-2-yl)ethanone). The synthesis and the reduction of acetyl-benzofuran was synthesized according to literature data in the literature and was performed as earlier described by us [12].

The non-fermentative system was used in these experiments which provide a superior selectivity, but a prolonged reaction time in comparison with the fermenting system.

The solution of 2-acetyl-benzofuran (50 mg) in absolute ethanol (5 ml) was added into each bioreactor containing a suspension of 20 g yeast in 100 ml distilled water. Samples (1 ml) taken perodically (24h) from both exposed and unexposed bioreactor to appreciate the difference in biocatalyst selectivity due to the action of the electromagnetic field were extracted with ethyl acetate (3ml). The organic layer was dried over anhydrous sodium sulphate and the solvent was eliminated by rotary evaporation. The crude solide was redissolved in hexane (0.5 ml) and the solution was subjected for chromatographic analysis using an Agilent 1200 HPLC. Using a Chiracel OJ-H analytical column and a mixture of hexane:isopropanol 90:10 (v/v) as eluent previously tested succesfully for the enantiomeric separation of the racemic 1-(benzofuran-2-yl) ethanol, the enantiomeric excess of (*S*)-1-(benzofuran-2-yl) ethanol produced in both bioreactors were measured.

The conversion of the substrate was calculated from using the area of the alcohol and prochiral ketone chromatograpic signals corrected with the *ratio* of the specific molar absorbance of the compounds.

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