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Effects of low-frequency magnetic fields on the viability of yeast *Saccharomyces cerevisiae*

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Abstract

A 50 Hz magnetic field effect on the growth of yeasts *Saccharomyces cerevisae* was studied. The cylindrical coil induced magnetic fields with inductions up to 10 mT. Duration of exposure varied up to 24 min. Exposure took place at laboratory temperature (24–26 °C) and the air ventilator maintained the temperature at the place of the sample. We measured the growth curves of yeasts in broth and we calculated the number of CFU (colony forming units) on solid soil. We found that magnetic field decreases the number of yeasts, and slowed down their growth. The result is similar to the experiments with bacteria *E. coli*, *S. aureus* and *L. adecarboxylata*. It seems that the magnetic fields kill a part of yeasts and the bigger part of them survives and continues in their growth.

Keywords: Low-frequency electromagnetic field; Yeast; Growth curve; CFU number; *Saccharomyces cerevisiae*

1. Introduction

Man-made low-frequency (up to 1 kHz) electromagnetic fields became an important part of our biosystem. They spread on the whole earth prepared to serve to human and his benefit. This contribution to our environment is relatively a new one. Organisms had to be adapted during their evolution to a natural magnetic field during many centuries and milleniums, but their exposure to magnetic fields has dramatically increased in the last century. They have to adapt to electromagnetic fields in a relatively short time [1,2]. Lots of papers were published in the previous years to find whether such fields can affect living organisms. They were first focused on the epidemiology and the connection between power-lines and human tumors and leukaemia. Later the research turned to the effects of electromagnetic fields in the molecular and cellular level.

The objects studied were cells [3], tissues [4], and whole living organisms [5,6]. The viability and proliferation [7], activity of enzymes [8], transport of ions [9,10] and gene transcription or expression [11–13] were investigated — with different results.

A lot of new results were presented in the 3rd international workshop on the biological effects of electromagnetic fields in Greece (October 2004) [14]. From 192 papers published in the proceeding of the conference 93 described experiments with electromagnetic fields (static, low-frequency or radio-frequency) and living systems. Approximately 68% of the papers using low-frequency fields reported significant effects (positive or negative) on organisms exposed. This “statistic” is only an approximation, it does not describe the strength of the effects but it shows that there are no negligible effects of EMF. 23% of the experimental works studied brain activity and nerve systems, object of 15% was epidemiology and 13% studied the effects on tumors and clinical applications of electromagnetic fields in medicine.

The importance of the research on the electromagnetic effects of EMF showed that the WHO (World Health Organisation)

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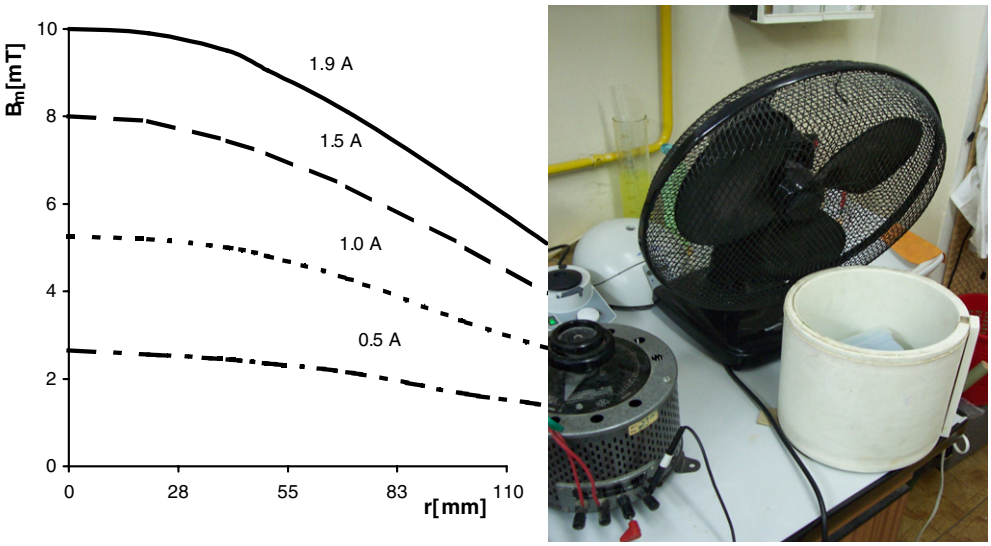

Fig. 1. Cylindrical coil used for the generation of magnetic fields. Dependence of the magnetic induction on the distance from centre of the coil.

classified EMF to Class 1B — possible carcinogen. Research of EMF effects is in competence of International Commission for Non-Ionizing Radiation Protection (ICNIRP). The commission collects experimental data worldwide and establishes limits for public EMF exposure [15].

The results are still controversial. One possible reason for the controversy is that the experiments were not performed under well-defined conditions.

According to A. Markov et al.'s paper [16] it is important to study exposed organism not only on the cellular or tissue level but also the complex effects on the whole organism. From that reason bacteria [17–19], or yeast [20–23] — unicellular organisms — can be good subjects for the study of magnetic fields effects. In our previous works we studied the MF effects on bacteria. We described strain-dependent inhibition of bacteria in the presence of magnetic fields [24–26]. This work studies the viability of yeast *Sachcaromyces cerevisiae* after magnetic field exposure in order to compare its effects on eucaryotic and procaryotic cells.

2. Material and methods

2.1. Magnetic field

The magnetic fields were generated by a cylindrical coil (Fig. 1) powered by a transformer. The maximal effective current was 1.9 A (it corresponds to an amplitude of magnetic induction $B_m = 10$ mT) and the frequency was 50 Hz. The temperature during exposure was maintained in the range of 24–26 °C. The samples were placed on the nonconductive stand in the centre of the coil. Magnetic bias in the place of exposure caused by the ventilator or other electronic equipment was less than 0.2 μT (50 Hz) (five order of magnitude less than 10 mT) (Table 1).

Table 1
Parameters of cylindrical coil

Diameter	235 mm
Inner diameter	205 mm
Length	210 mm
Number of threads	880
Diameter of wires	2 mm

2.2. Yeast

The prototrophic tetraploid strain α/*A* of yeast *S. cerevisiae* CCY 21-4-59 (yeast collection, Institute of Chemistry, Slovak Academy of Science, Bratislava) was used. Yeast cultures were grown in malt extract broth (2% malt extract broth — Fluka, 2% glucose — Lachema Brno) in the thermostat with the temperature 30 °C. CFU — Colony forming unit were counted on the agar plates (2% malt extract broth — Oxoid, Basingstoke, Hampshire, England, 2% D-Glucose — Lachema Neratovice, Czech Republic, Agar No. 2 — Imuna, Šarišské Michal'any, Slovakia).

For all experiments fresh yeast cultures were used.

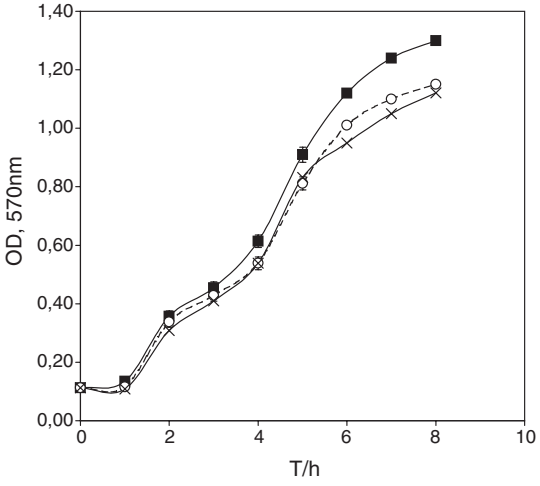

Fig. 2. Values of OD_{620} for the static yeast culture. Yeasts were exposed from 0 to 24 min (■ — control, o — 12 min, x — 24 min). Single experiment.

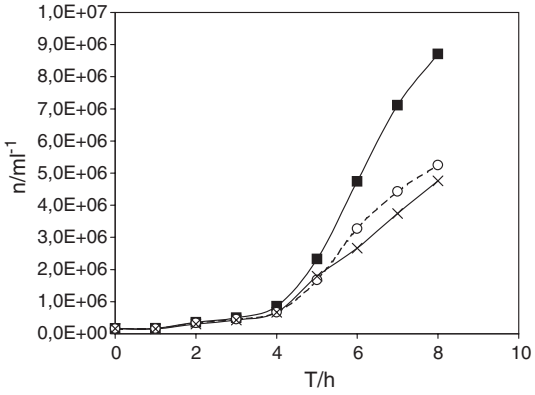

Fig. 3. The same experiment as on Fig. 3., *y*-axis shows the number of yeasts (■ — control, o — 12 min, x — 24 min)

Table 2
The ratio *n*(exp.)/*n*(contr.) of yeasts in yeast cultures after magnetic exposure B_m=10 mT (6, 12 and 24 min), 10 experiments

T/h	Number of exp./number of contr.		
	Duration of exp. 6 min	12 min	24 min
0	0.99±0.01	1.01±0.01	1±0.01
1	0.94±0.04	0.98±0.01	0,96±0.02
2	0.97±0.03	0.96±0.04	0,93±0.03
3	0.98±0.08	0.93±0.06	0,89±0.05
4	0.92±0.07	0.86±0.05	0,88±0.06
5	0.87±0.06	0.84±0.11	0,86±0.04
6	0.98±0.03	0.92±0.30	0,88±0.06
7	0.9±0.01	0.63±0.16	0,85±0.07
8	0.89±0.01	0.57±0.10	0,78±0.11
9		0.43±0.15	0,74±0.12

2.3. The determination of the number of yeasts in culture

2.3.1. Spectrophotometry

A Spectrophotometer Libra S 22 (Biochrom) was used to measure the optical density (OD) at wavelengths of 570 and 620 nm. As a reference sample clean broth was used. The calibration curve was repeatedly measured by taking the values of OD_{570} or OD_{620}. and by counting the corresponding number of CFUs (see below). The logarithmic calibration curves were calculated by the method of minimal squares from 36 measured data.

2.3.2. CFU counting

The bacterial culture was diluted to a final concentration of 2.10^2–10^4 cells/ml. 100 μl of the solution was spread on the agar plates (Petri dishes, radius=45 mm). After 1 day incubation of plates in the thermostat (30 °C) the number of colonies grown on the plate was counted.

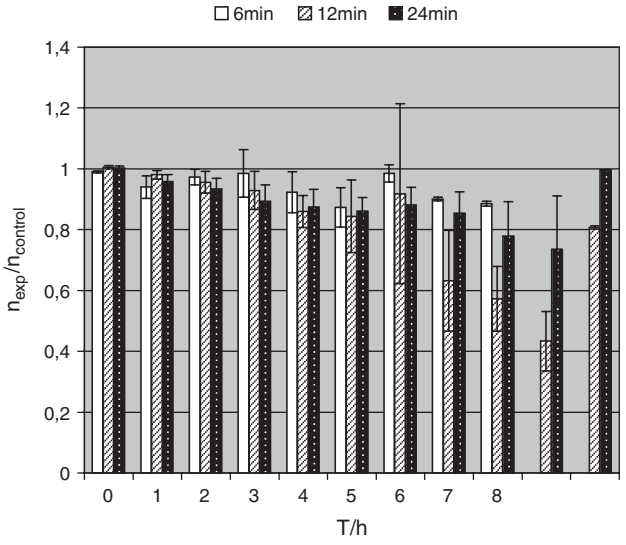

Fig. 4. Average of 10 experiments, the relative values of the number of yeasts (exp./contr) as a function of time since inoculation of yeast culture.

2.4. Experiments

2.4.1. Growth curves — yeasts in broth

Growth curves were measured for static yeasts cultures. "Overnight grown" culture was mixed with clean broth in a proportion of 1:40. After mixing (time T=0 h) the new culture was divided into Erlenmeyer flasks, one control, the others were exposed in the coil for different times (3.1.1) or magnetic induction (3.1.2). All samples (including the sham-control) were kept at laboratory temperature during exposures, after the exposure of all the samples they were put into the thermostat without shaking. Every hour they were shaken and the optical density (OD) of all samples was measured and the corresponding number of cells was calculated from the calibration curve.

During dynamic experiments we exposed the yeast for 60 min to a magnetic field and the number of cells was measured every 10 min. The measurement continued for 30 min after turning off the field. The sham-control cells were kept in the same conditions (temperature, light, humidity) as those of the exposed samples. Control cells were exposed to a bias magnetic field (50 Hz) about B=0.2 μT.

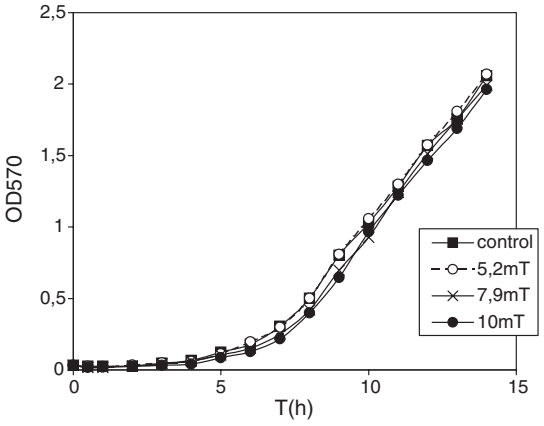

Fig. 5. Values of OD_{620} for the static yeast culture. Magnetic field induction varied from 2.7 to 10 mT (■ — control, o — 5.2 mT, x — 7.9 mT, ● — 10 mT). Single experiment.

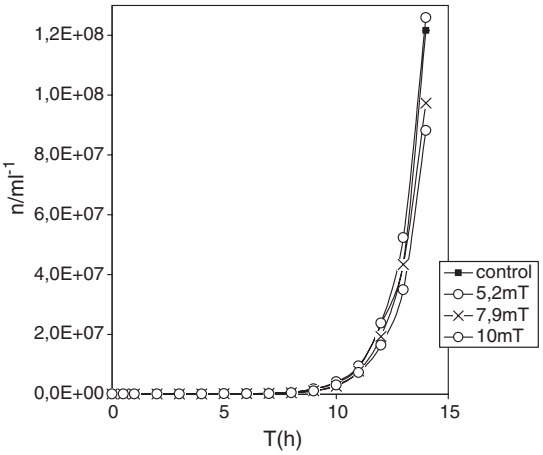

Fig. 6. The same experiment as on Fig. 5., *y*-axis shows the number of yeasts per ml (■ — control, O — 5.2 mT, x — 7.9 mT, ● — 10 mT).

2.4.2. CFU — yeasts on solid soil

"Overnight grown" culture was mixed with the broth in a proportion of 1:40. After mixing (time $T=0$ h) the new culture was grown in thermostat (30 °C). In the logarithmic phase of growth ($T=7$–8 h since its inoculation) the yeasts were put on the agar plates and were exposed to the magnetic fields.

For the statistical analysis of the results the *t*-test and Student's distribution were used ($P=0.95$).

3. Results

3.1. Growth curves — dependence on the duration of exposure

We exposed the yeast culture to the magnetic fields ($f=50$ Hz, $B_m=10$ mT). The duration of exposure varied in the range of 0–24 min. We observed a decrease of OD for all exposed samples. One of the experiments is shown in Figs. 2 and 3. Exposed samples have lower OD values at all times of duration of the exposure. We made 10 experiments, every point was measured at least 3 times. Single experiments with 3 measurements have relative standard deviations about 1%. Ratio exp./contr. for all experiments is shown in Fig. 4 (Table 2).

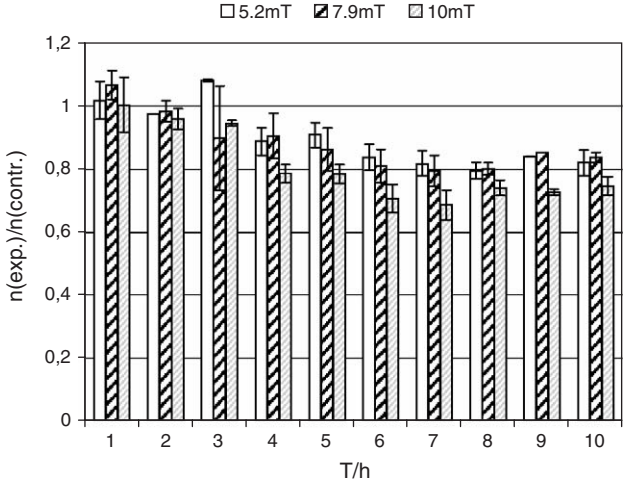

Fig. 7. Average of 4 experiments, the relative values of the number of yeasts (exp./contr) as a function of time since inoculation of yeast culture. (5.2, 7.9 and 10 mT)

Table 3
The ratio n(exp.)/n(contr.) of yeasts in yeast cultures after magnetic exposure $t=12$ min ($B_m=5.2$,7.9 and 10 mT)

T/h	Number of exp./number of contr.		
	$B_m=5.2$ mT	7.9 mT	10 mT
0	1.02±0.05	1.07±0.04	1.00±0.04
1	0.98±0.06	0.98±0.04	0.96±0.09
2	1.08±0.01	0.90±0.03	0.94±0.03
3	0.89±0.02	0.90±0.15	0.78±0.04
4	0.91±0.05	0.86±0.08	0.78±0.04
5	0.84±0.04	0.81±0.08	0.71±0.04
6	0.82±0.05	0.80±0.05	0.69±0.06
7	0.80±0.05	0.80±0.06	0.74±0.07
8	0.84±0.03	0.85±0.02	0.73±0.03
9	0.82±0.02	0.84±0.03	0.75±0.03

3.2. Growth curves — dependence on the magnetic field induction

We exposed the yeast cells culture to the magnetic fields ($f=50$ Hz, $t=12$ min)(Figs. 5 and 6 show one single experiment). Magnetic field induction varied in the range of 0–10 mT. We observed the decrease of ODs in all samples. The effect was stronger with higher magnetic inductions. We made 4 experiments, every point was measured at least 3 times. Ratio exp./contr. for all experiments is shown in Fig. 7 (Table 3).

3.3. The study of growth dynamics

The ODs were measured during the exposure of the yeast culture and were compared with the control ones. Magnetic field ($B_m=10$ mT, $f=50$ Hz) was turned off 60 min after the beginning of the exposure. After exposure we continued with the measurement of the OD for 30 min. We observed a significant decrease of the number of yeasts about 20 min after the beginning of the exposure. The ratio between the control and

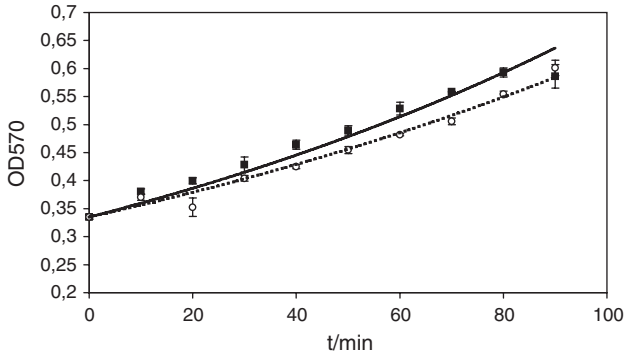

Fig. 8. The study of the dynamics of the growth. Magnetic field was switch off 60 min since the begging of exposure ($B_m=10$ mT). ■ — control (approx. $Y=0.335.10^{0.0071}x$, $R^2=0.9555$), o — exposed ($y=0.335.10^{0.0062}x$, $R^2=0.9762$). Magnetic field was switched off in $t=60$ min.

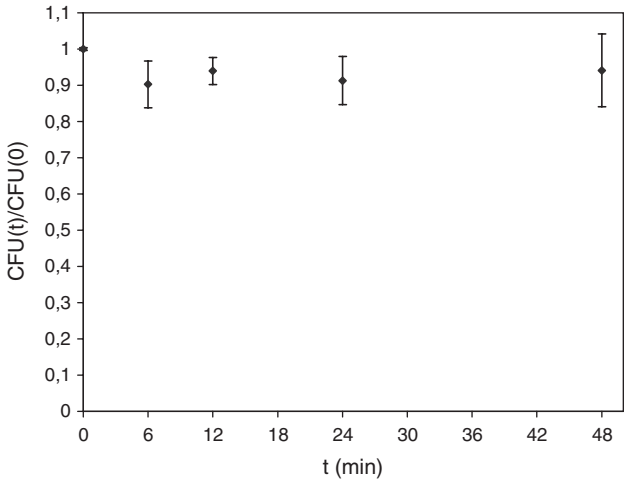

Fig. 9. Relative number exp./contr. of CFU in dependence on the duration of exposure, All data are statistically significant value with respect to a control sample ($\alpha=0.05$).

the exposed sample was later almost constant (Fig. 8). We made 6 experiments.

3.4. Exposure on the agar plate, dependence on the duration of exposure

Agar plates with yeast cells were exposed to the magnetic fields. Duration of the exposure varied up to 48 min. Magnetic induction was 10 mT. Each point was determined from at least 4 plates. We made 10 experiments. To summarize the results we calculated the ratio exp./contr. The *t*-test ($\alpha=0.05$) was applied on the data set. The number of CFUs of exposed yeast differ from the control ones significantly for all the durations of exposure. There is no significant difference between exposed samples (Fig. 9) (Table 4).

4. Discussion

All experiments showed inhibiting effects on the growth of the yeasts *Saccharomyces cerevisiae* after their exposure to magnetic fields ($B_m \leq 10$ mT, $t \leq 60$ min, $f=50$ Hz, temperature during the experiments was maintained at 24–26 °C during the exposures). From all results it seems that the inhibition can be seen immediately after putting the yeast culture into the magnetic fields. The graphs (Fig. 8) show that the difference between the control and the exposed samples is present in the very first measured point after exposure. Looking at Fig. 8 it can

Table 4
Dependence of relative numbers of CFU *n*(exp.)/*n*(contr.) on the duration of exposure

Duration of exposure/min	*n*(exp.)/*n*(contr.)
0	1.00±0.07
6	0.84±0.06
12	0.82±0.14
24	0.83±0.09
48	0.86±0.08

Table 5
Comparison of parameters *A* describing magnetic field effects on different type of organisms

	E. coli	L. adecarboxylata	S. aureus	S. cerevisiae
Parameter A (min^{-1})	0.0302	0.0121	0.0096	0.0021

Equation $(n(t)/n(0)=1\cdot e^{-A\cdot t})$.

be seen that the difference between the control and the exposed samples is evident after 20 min of exposure and the ratio exp./contr. remains almost the same for the rest of the experiment. It seems that the magnetic field (or the current induced by the magnetic field) kills a part of yeasts (maybe more sensitive to external fields) and the rest of them continue developing without any disturbances. Because the growth of the yeasts is based on geometric progression, the small change of the number of cells on the beginning of the growth can dramatically change the whole growth curve. Results of the experiment on the solid soil show that the CFU numbers decrease significantly after the shortest exposure, but with longer exposure these remain unchanged.

In our previous work we stated, that the magnetic field effect depends on the bacteria strain. Comparing results for three bacterial strains and yeast *S. cerevisiae* we can state, that the bacteria were more sensitive to magnetic field exposures than the yeasts. Dependences $(n(t)/n(0)=f(t))_{B_m}$=const. for all the bacterial strains exposed and for the yeasts were approximated by the exponential function $y=1\cdot e^{-A\cdot t}$. In Table 5 parameters *A* are compared. It can be seen that the biggest effect was observed for bacteria *E. coli*, less for *L. adecarboxylata*, *S. aureus* and the minimal effect was observed for yeast *S. cerevisiae.* The difference between the bacterial strains is probably caused by their different shape. Differences between the bacteria and the yeasts can be caused by the type of cell (eucaryotic or procaryotic) (Fig. 10).

To confirm the previous statement it is necessary to make experiments with different shapes of yeast cells. The influence of shape on bacterial cells and magnetic fields exposure was already studied in our laboratory [27].

In the work of Bellia et al. [20] *S. cerevisiae* was exposed to 50 Hz magnetic field with an induction 0.5 mT and a static field

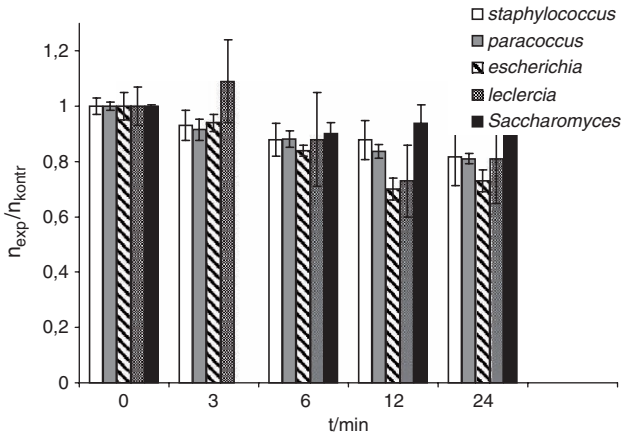

Fig. 10. Comparision of magnetic field effect on bacteria (*E. coli*, *L. adecarboxylata*, and *S. aureus)* and yeasts *S. cerevisiae.*

in the range of 0.1–100 mT. The measured parameter was duplication time. They concluded that there was no significant differences between the exposed and not exposed samples. But experimental error for these measurement was 28% and the experiment was not able to detect smaller changes in the growth of the yeasts — for example we detected changes about 20% with a relative error of 5%. It seems that CFU counting is a better method to look for changes of the growth of *S. cerevisiae.*

Iwasaka et al. [21] exposed *S. cerevisiae* to a static magnetic field of 14 T. The rate of yeast proliferation under the magnetic fields (9–14 T) decreased after 16 h of incubation compared to that of the control group. From the analysis they expect that the changes in the growth were due to the changes of the magnetically changed gas-transport processes, hydrostatic pressures acting on the yeast, and changes in the distribution of the yeast sedimentation, as well as the possible effects of magnetic fields on the yeast respiratory systems in the observed disturbance of the proliferation.

Ruiy-Gomés [22] reported the growth effects induced by static and sinusoidal 50 Hz magnetic fields (MF) on the haploid yeast strain *S. cerevisiae* WS8105-1C. The experiments were performed at 0.35 and 2.45 mT, and the yeasts were exposed to MF for 24 and 72 h in the homogeneous field area. Growth was monitored by measuring the optical density at 600 nm. The data indicated that static and sinusoidal 50 Hz MF (0.35 and 2.45 mT) did not induce alterations in the growth of *S. cerevisiae*.

On the other hand, Bininger and Ungvichian [23] reported that gene expression in *S. cerevisiae* is after exposure to 20 μT 60 Hz magnetic fields altered over a period of 15 cell generation. It can be seen, that similar experiments do not lead to the same results. Differences in strain and in used magnetic fields play an important role in the results.

It might be early to answer the question if such fields can have some effect on humans. First, the field with an induction of 10 mT is very rare in our environment. We can detect it very seldomly in the vicinity of welding machines or induction furnaces. The ICNIRP limit for personal exposure is 100 μT, it is one hundred times lower.

On the other hand it is important to do similar experiments with strong, maybe stronger fields, to determine the mechanisms of possible reactions of the external fields on organisms.

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