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KNJIGA RADOVA

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Novi Sad 8. i 9. juni 2018.
Novi Sad, Serbia, June 8-9, 2018

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ЕНЕРГИЈА ЗНАЊА

MINAQUA

TIMIZOLIRKA

Influence of the low frequency 10-1000 Hz magnetic field on *Saccharomyces cerevisiae* respiration activity

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Introduction

Thanks to the fast technological development, a man-made low frequency electromagnetic fields have become a very important part of environmental polution.[1] Therefore, it is not surprising that effects of electric, magnetic or electromagnetic fields on different microbes have become a very popular topic nowadays, mostly because the mentioned physical fields could potentially act as a stress factors and hence affect the survival of the microbial cells as well as their behavior and metabolism.[2] Influence of static and low frequency magnetic field has been investigated on many microorganisms such as *Saccharomyces cerevisiae*[1,3,4,5], *Enterococcus faecalis*[2], *Escherichia coli*[6] and *Spirulina sp.*[7]

Ruiz-Gomez *et al.*[3] studied the influence of long-term exposure to static (0 Hz) and 50 Hz sinusoidal MF induced by a pair of Helmholtz coils (0.35 mT and 2.45 mT) on the growth of *Saccharomyces cerevisiae* by measuring the optical density of the suspension at 600 nm. Authors concluded that neither static nor 50 Hz sinusoidal MF induce alterations in the growth of *S. cerevisiae*.[3] Similarly to the work of Ruiz-Gomez and his coworkers, Novak *et al.*[1] investigated the influence of the 50 Hz MF on the growth of *S. cerevisae,* but the MF was induced in a cylindrical coil (10 mT). Contrary to the first study, they concluded, based on the serial dilution method and measurements of the optical density at wavelengths of 570 and 620 nm, that magnetic field decreases the number of yeast cells, and slows down their growth.[1] Mentioned papers represent only some examples with conflicting results of the bioeffects of the applied magnetic fields. Potential reasons for that could be the use of different cell types, magnetic field exposure protocols, intensities, frequencies *etc.*

Besides magnetic field examinations on the growth of *S. cerevisiae* by the optical density measurements, Motta *et al.*[4] also studied effects of the static MF (220 mT, exposure time 24 h) on ethanolic fermentation by *Saccharomyces cerevisiae* measured by gas chromatography, and concluded that ethanol concentration was 3,4 times higher in magnetized cultures.[4] Nakasono and coworkers[5] performed detailed study of the effect of 50 Hz magnetic-field exposure on genome-wide gene expression, of the yeast *Saccharomyces cerevisiae*. Their results indicate that a 50 Hz magnetic field below 300 mT did not act as a stress factor like heat shock or DNA damage.[5]

In this study the influence of magnetic field on yeast cells is examined by measuring respiration activity. This is the first experimental report of the changes in respiration influenced by magnetic field which was measured by Micro-Oxymax© respirometer, to the best of our knowledge. Also, contrary to the previously performed studies, in this study the

low frequency magnetic field with constant frequency scan regime from 10 to 1000 Hz was used.

Experimental part

Prior to the experiment, *S. cerevisiae* was grown on the malt extract agar. In order to prolong log phase of cell division which will be monitored in experiments, the diluted (1:1) Sabouraud dextrose broth (SBD) was inoculated with overnight culture suspension. All experiments were performed in pair: control (CC) and magnetic field exposed cells (MFEC) and lasted 24 h (Figure 1). As it can be noticed from Figure 1, CC and MFEC bottles were installed in a glass water recirculation jacket and were mutually connected in line with a thermostat in order to minimize possible temperature differences between samples.

Low frequency magnetic field was generated by wrapping one bottle together with recirculation jacket in Cu-coil. Inside of the mentioned Cu-coil 10-1000 Hz magnetic field was induced and generator was used to set up a frequency range and a scan interval to 100 s. An amplifier was used to set up a maximal effective current trough the coil (2 A which corresponds to magnetic induction of 33 mT), and an oscilloscope was used to control changes in effective current during frequency scanning.

Respiration activity of CC and MFEC was continuously measured by a twelve-channel Micro-Oxymax® respirometer (Columbus Instruments, USA). All experiments were performed in two light-proof 5 mL glass bottles with 3 mL of the inoculated SBD medium. Constant temperature at 28 °C was maintained by thermostat (Julabo, F12 Refrigerated/Heating Circulator, Germany). Cell respiration was measured every 20 min during 24 h and experiments were performed in five replicates. Cumulative O_2 consumption and cumulative CO_2 production (in μL) were determined.

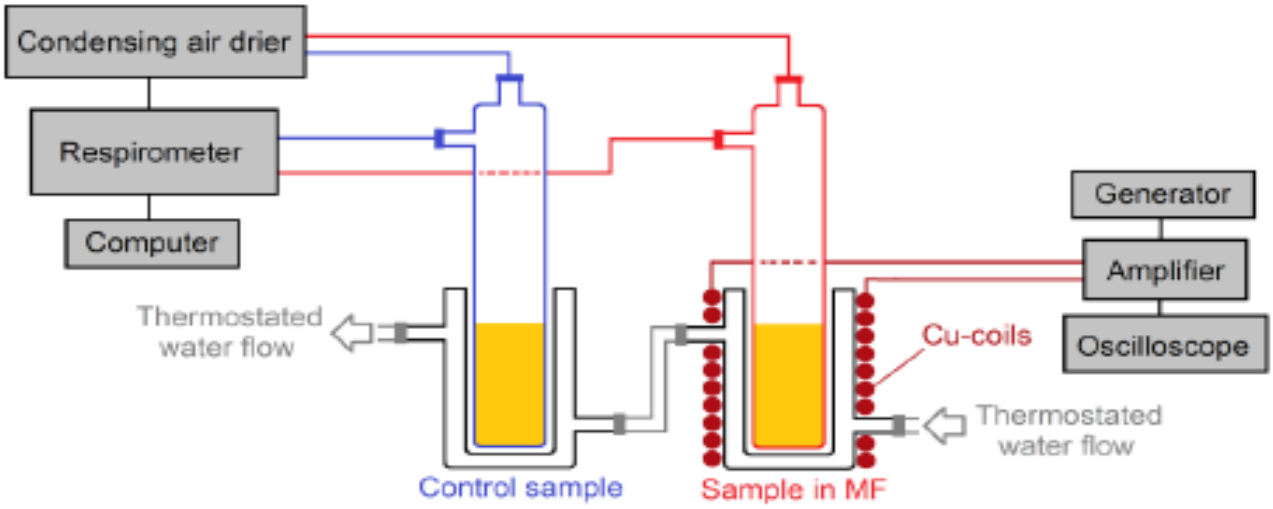

Figure 1. Schematic view of the experimental setup.

Results and discussion

In this paper the influence of the magnetic field with constant low frequency scan regime from 10 to 1000 Hz on yeast cells respiration activity was examined. Like it was mentioned in Experimental part, cumulative O_2 consumption and cumulative CO_2 production were monitored in CC and MFEC during 24 h. It should be stressed out that for all frequency ranges experiments were repeated five times.

The Figure 2. shows cumulative O_2 consumption and cumulative CO_2 production in CC and MFEC over 24 h, obtained in all five repeated experiments.

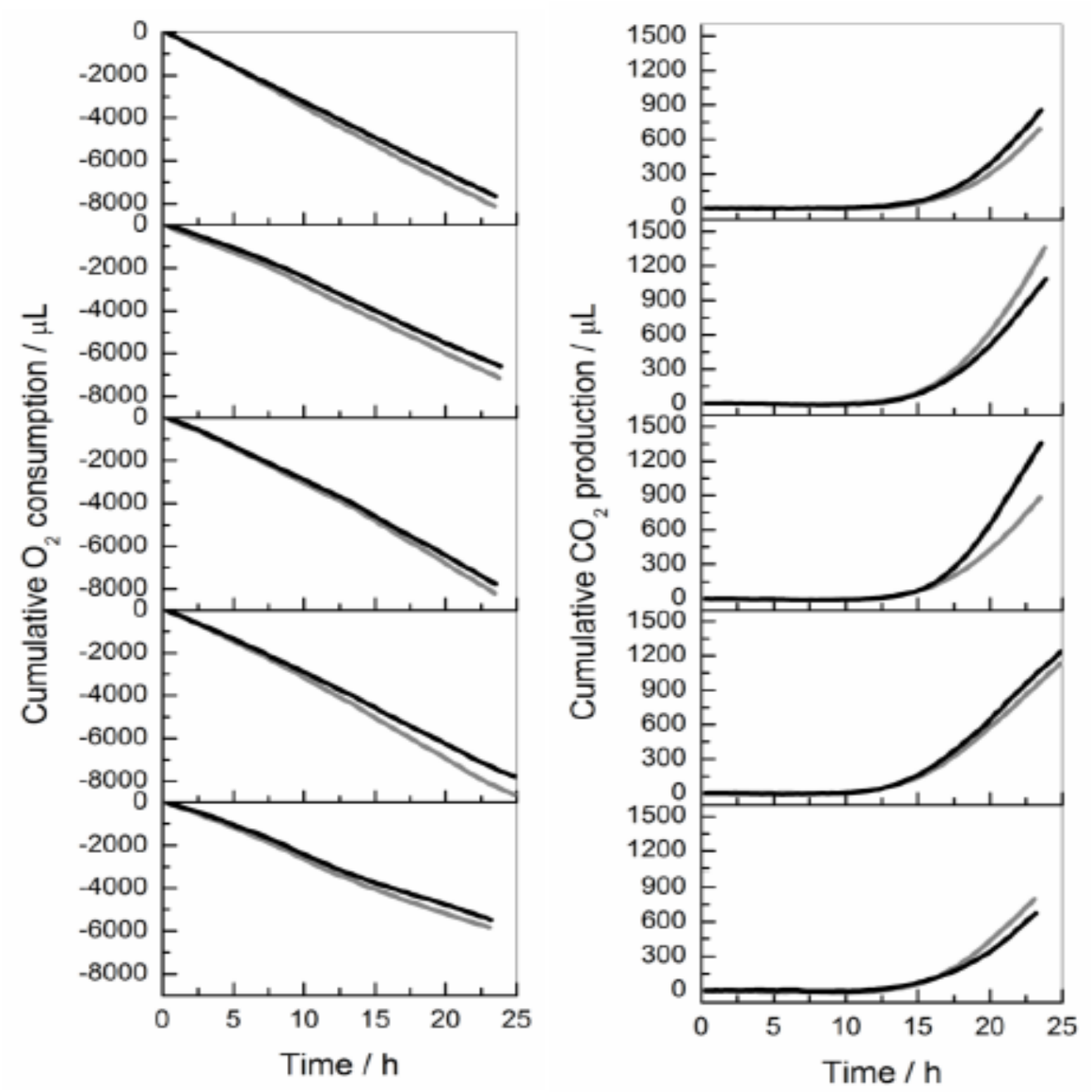

Figure 2. *Experimentally obtained cumulative O_2 consumption in µL (left side of the figure) and cumulative CO_2 production in µL (right side) over 24 h for magnetic field frequency range 10 – 1000 Hz. Gray curve corresponds to changes in CC while the black curve represents changes of the O_2 and CO_2 in MFEC.*

As it can be seen from Figure 2, up to 15th hour, the differences in cumulative O_2 consumption and cumulative CO_2 production between CC and MFEC at investigated frequency range are negligible. After 15th hour those differences begin to grow and are the biggest at the end of the magnetic field exposure. For examined magnetic field frequency

range from 10 to 1000 Hz all five repeated experiments showed that cumulative O_2 consumption in MFEC was lower in comparison to CC. However, for the same frequency range behavior in cumulative CO_2 production differed. Namely, in three experiments it was obtained that cumulative CO_2 production in MFEC was higher in comparison to CC, while in other two repeated experiments for the same experimental conditions cumulative CO_2 production in magnetic field sample was lower.

Obtained results were statistically considered by a paired two sample one tail T-test. Results from the T-test showed that statistically important differences between CC and MFEC exist for cumulative O_2 consumption, but do not exist for cumulative CO_2 production.

Even though evident inconsistency was found in cumulative CO_2 production, we believe that obtained results still represent a good basis for further investigations in this field. Namely, paired two sample one-tail T-test showed statistically important differences for cumulative O_2 consumption which suggests that applied low frequency magnetic field could influence yeast cell respiration activity. The inconsistency in cumulative CO_2 production could be influenced by the lack of sample stirring and differences in the initial cell number between replicates. Therefore, it is important for the future investigations, besides respiration activity, to take into account other important parameters of the system such as cells growth, glucose uptake and ethanol production. On the other hand, if we consider most results available in the literature where a static or 50 Hz magnetic field was examined[1,3,4,5], than the potential reason for the inconsistency in cumulative CO_2 production could indicate that different frequencies could have an opposite effect on respiration. This would lead to the combination of positive and negative effects during the exposure time which could explain the absence of statistically significant differences. So, further investigations should consider shorter frequency intervals up to 1 kHz. By narrowing the frequency range, we could more precisely isolate positive or negative effects on yeast cells.

Conclusion

Obtained results represent a good basis for the following investigations in this area. Examined magnetic field with constant low frequency scan regime from 10 to 1000 Hz in all five repeated experiments showed lower cumulative O_2 consumption of cells exposed to magnetic field. Also applied paired two sample one-tail T-test showed statistically important differences for cumulative O_2 consumption between control cells and magnetic field exposed cells. In order to ascertain the effects of magnetic field on the CO_2 production, further experiments should be conducted.

***Acknowledgement**: This research was financially supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, through Projects No. III 43004 and OI 172015.*

Uticaj niskofrekventnog magnetnog polja (10-1000 Hz) na respiracionu aktivnost ćelija kvasca *Saccharomyces cerevisiae*

Veoma popularna tema današnjice je ispitivanje električnog, magnetnog i elektromagnetnog polja na različite mikroorganizme, jer pomenuta fizička polja potencijalno deluju kao faktori stresa i tako utiču na njihovo preživljavanje, ponašanje i metabolizam. U ovom radu ispitivan je uticaj niskofrekventnog magnetnog polja sa

konstantnim intervalom skeniranja od 10 do 1000 Hz na respiraciju ćelija kvasca, Saccharomyces cerevisiae. Eksperimenti su rađeni u pet ponavljanja i kumulativna potrošnja O_2 i produkcija CO_2 praćena je pomoću Micro-Oxymax® respirometra. U svih pet ponavljanja, ćelije koje su bile izložene magnetnom polju pokazale su manju kumulativnu potrošnju kiseonika u poređenju sa uzorcima van magnetnog polja i nekonzistentnu produkciju CO_2. Rezultati su obrađeni uporednim jednosmernim T-testom, koji je pokazao da postoje statistički značajne razlike u kumulativnoj potrošnji O_2 između kontrolnih ćelija i onih izloženih magnetnom polju, što nije slučaj sa kumulativnom produkcijom CO_2. Iako su dodatna ispitivanja neophodna da se objasni nekonzistentnost produkcije CO_2, dobijeni rezultati ovih inicijalnih eksperimenata predstavljaju dobru osnovu za dalja istraživanja u ovoj oblasti.

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Influence of the low frequency 10-1000 Hz magnetic field on *Saccharomyces cerevisiae* respiration activity

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Marija Lješević, Branka Lončarević, Ilana Nuša Bubanja*, Vladimir Beškoski**, Gordana Gojgić-Cvijović, Zoran Veličkić*** and Dragomir Stanisavljev*

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Introduction

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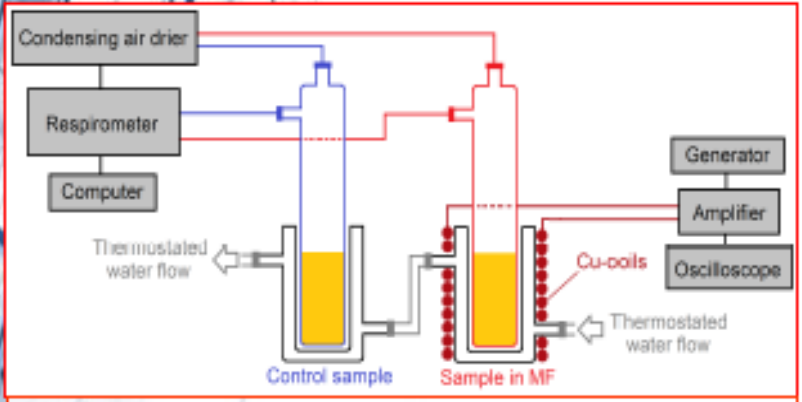

Figure 1. Schematic view of the experimental setup

Results

Cumulative O_2 consumption and cumulative CO_2 production were monitored in control (CC) and magnetic field exposed cells (MFEC) during 24 h. Respiration was measured using Micro-Oxymax® respirometer. The results are shown in Figure 2.

- Differences in cumulative O_2 consumption and cumulative CO_2 production between CC and MFEC are negligible up to 15th hour, after which they start to grow and are biggest at the end of exposure
- Cumulative O_2 consumption in MFEC was lower in comparison to CC, and this difference was statistically significant according to the paired two sample one tail T-test
- Differences in cumulative CO_2 production weren't statistically significant; in three replicates CO_2 production was higher in MFEC, and in two in CC
- Lack of sample stirring and differences in the initial cell number between replicates could explain the inconsistency in cumulative CO_2 production, as well as an opposite effect of different frequencies.

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Aim

In this study the influence of magnetic field on yeast cells is examined by measuring respiration activity. This is the first experimental report of the changes in respiration influenced by magnetic field which was measured by Micro-Oxymax® respirometer, to the best of our knowledge. Also, contrary to the previously performed studies, in this study the low frequency magnetic field with constant frequency scan regime from 10 to 1000 Hz was used.

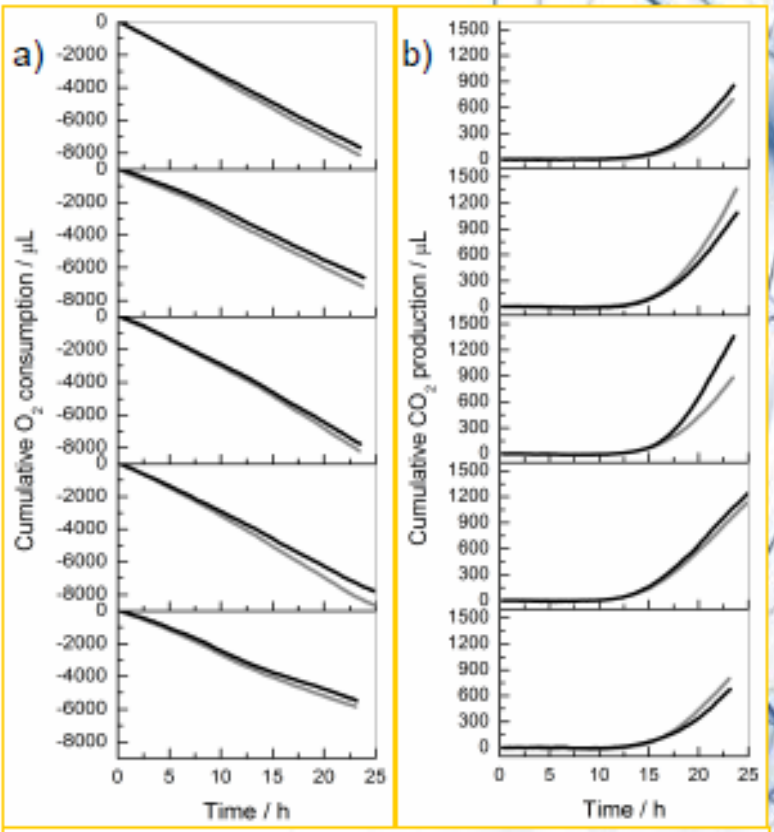

Figure 2. Cumulative O_2 consumption in µL (a) and cumulative CO_2 production in µL (b) over 24 h for magnetic field frequency range 10 - 1000 Hz. Gray curve corresponds to changes in CC while the black curve represents changes of the O_2 and CO_2 in MFEC

Conclusion

Obtained results represent a good basis for the following investigations in this area. Examined magnetic field with constant low frequency scan regime from 10 to 1000 Hz in all five repeated experiments showed lower cumulative O_2 consumption of cells exposed to magnetic field. Also applied paired two sample one-tail T-test showed statistically important differences for cumulative O_2 consumption between control cells and magnetic field exposed cells. In order to ascertain the effects of magnetic field on the CO_2 production, further experiments should be conducted.

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