56. savetovanje Srpskog hemijskog društva

KNJIGA RADOVA

56th Meeting of the Serbian Chemical Society

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Niš 7. i 8. juni 2019.
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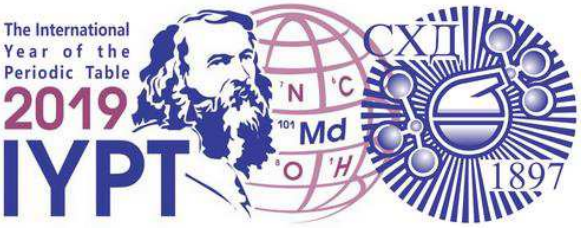

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The influence of the low frequency magnetic field with scan regime from 10 Hz to 50 Hz on *Saccharomyces cerevisiae* respiration

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Introduction

Over the years, due to the fast technological development, our environment has become more and more influenced by man-made low frequency electromagnetic fields (EMFs). Therefore, it is not surprising that this increasing exposure of the population in everyday life to EMFs has earned such a wide public interest. Recently, the effects of electric fields (EFs), magnetic fields (MFs) or EMFs on different microbes have become a very popular topic since the mentioned physical fields could potentially act as stress factors and thus affect the survival of the microbial cells as well as their metabolism and behavior.[1]

Many authors have investigated the influence of an MF on different eukaryote and prokaryote organisms. Among eucaryote organisms, yeast *Saccharomyces* cerevisiae[2-5] has become the most significant model to investigate the influence of static and a low frequency MF. This is mostly because *S. cerevisiae* has well-characterized metabolic and genetic properties but also because of their similarity in the molecular mechanisms of basic cellular processes with numerous eukaryotic species. On the other hand, the influence of a low frequency MF has also been investigated on *Enterococcus faecalis*[1], *Escherichia coli*[6]and other prokaryote organisms[7].

Considering the results available in the literature, a static magnetic field (SMF) or a 50 Hz low frequency MF were usually used to investigate effects on various microbial cells. Novak *et al.*[2] investigated the influence of the 50 Hz MF (10 mT) on the growth of *S. cerevisiae*. Based on the serial dilution method and measurements of the optical density at wavelengths of 570 and 620 nm, the authors concluded that an MF decreases the number of yeast cells, and slows down their growth.[2] Similarly to the work of Novak and coworkers, Ruiz-Gomez *et al.*[3] studied the influence of long-term exposure to static (0 Hz) and 50 Hz sinusoidal MF (0.35 mT and 2.45 mT) on the growth of *S. cerevisiae* by measuring the optical density of the suspension at 600 nm. In this study, a 50 Hz MF was induced by a pair of Helmholtz coils, while in the paper of Novak *et al.* a 50 Hz MF was induced in a cylindrical coil (0.35 mT and 2.45 mT). Contrary to Novak *et al.*, Ruiz-Gomez *et al.* concluded that neither a static nor 50 Hz sinusoidal MF could induce alterations in the growth of *S. cerevisiae*. These papers represent only one example among many others available in the literature with conflicting results of the bio-effects of the applied MFs. Possible reasons for this could be the use of different cell types, MF exposure protocols, intensities, frequencies and others. Besides static and 50 Hz MF examinations on the *S.*

cerevisiae cell growth by the optical density measurements, many authors also studied the effects of an MF on ethanolic fermentation[4] by *S. cerevisiae* as well as he effects of MF exposure on genome-wide gene expression[5].

To the best of our knowledge, in one of our recently published studies, within 55th Meeting of the Serbian Chemical Society, in Novi Sad, Serbia, for the first time, a low frequency MF with frequency scan interval 10-1000 Hz was used rather than some particular frequency.[8] In our previously published paper[8] the influence of an MF on yeast cells was examined by measuring respiration activity with a powerful Micro-Oxymax® respirometer. The paired two sample one-tail T-test showed statistically important differences between the control sample and the sample exposed to a 10-1000 Hz MF for cumulative O_2 consumption which suggested that the applied scan regime of a low frequency MF could influence yeast cell respiration activity. However, inconsistency of the results was found in cumulative CO_2 production.[8] Considering most results available in the literature where a static or 50 Hz MF was examined, the potential explanation of why the cumulative CO_2 production was inconsistent could be that different frequencies have an opposite effect on respiration. Therefore, in this paper we narrowed the scan frequency interval down to 10-50 Hz. Besides, when 10-1000 Hz was studied, control and MF samples were not stirred. The lack of mechanical stirring, and relatively large CO_2 solubility, could lead to unequal CO_2 release from the solution which was supported only by the diffusion through the solution. Therefore, in order to obtain a better regularity for cumulative CO_2 production in this investigation both control and MF samples were stirred with the rate of 300 rpm.

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 the experiments, the diluted (1:1) Sabouraud dextrose broth (SBD) was inoculated with overnight culture suspension. All the experiments were performed in pair: control (CC) and magnetic field exposed cells (MFEC) and lasted 24 h (Fig 1). As 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 the samples. Also, both samples were mixed with a magnet and magnetic stirrer with stirring rate of 300 rpm.

A low frequency MF was generated inside of a Cu-coil which was wrapped around one bottle together with recirculation jacket. An arbitrary function generator was used to set up a scan regime interval from 10 Hz to 50 Hz and a scanning time interval of 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 the effective current during frequency scanning.

The respiration activity (cumulative O_2 consumption and cumulative CO_2 production) of CC and MFEC was continuously measured by a twelve-channel Micro-Oxymax® respirometer (Columbus Instruments, USA). All of the experiments were performed in two light-proof 5 mL glass bottles with 3 mL of the inoculated SBD medium. The constant temperature of (28.0 ± 0.1) °C was maintained by the thermostat (Julabo, F12 Refrigerated/Heating Circulator, Germany). The cumulative O_2 consumption and cumulative CO_2 production (mL)

were measured every 20 min during 24 h and the experiments were performed in five replicates.

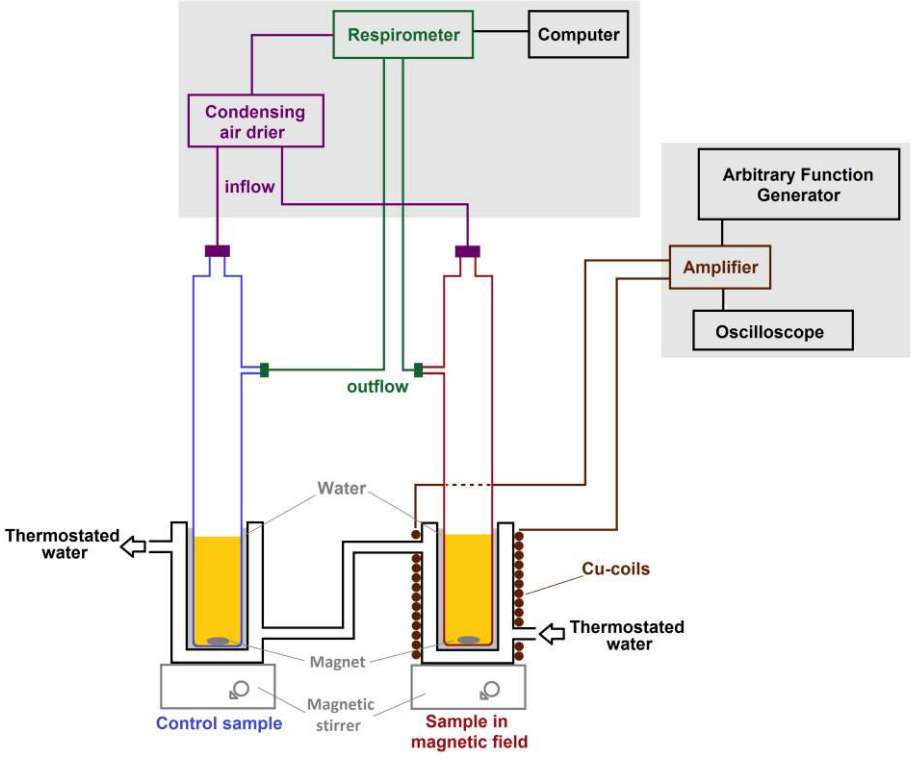

Figure 1. Schematic view of the experimental setup.

It is important to stress out that before all of the experiments, where the influence of an MF on yeast cells was examined, the experimental setup was tested to respirometer reading without an MF. In an ideal case, the cumulative O_2 consumption and cumulative CO_2 production should be the same in both sample vessels when an MF is turned off. However, all five test experiments without an MF showed that a small difference between two vessels exists. Therefore, in order to perform proper interpretation of the results when an MF is applied, these small differences were taken into account. From mean values (obtained from five replicates) a correction factor was calculated. For the cumulative O_2 consumption the correction factor is 1 and for the cumulative CO_2 production it is equal to 0.69. The obtained correction factors were used to adjust the values of the respirometer reading obtained in all MF experiments.

Results and discussion

In this paper the influence of the low frequency MF with scan regime 10-50 Hz on yeast cells respiration was examined. As mentioned in the Experimental part, the cumulative O_2 consumption and cumulative CO_2 production were monitored in CC and MFEC during 24 h. The changes of the cumulative O_2 consumption and cumulative CO_2 production in CC and MFEC over exposure time, obtained in all five repeated experiments, are given in Figure 2.

It can be noticed that in both the MFEC and CC cumulative the O_2 consumption decreases linearly over time, while the cumulative CO_2 production increases very slowly up to the 15th hour after which it immediately increases until it reaches saturation limit. Besides, Figure 2a. demonstrates that in all five replicates the cumulative O_2 consumption was slightly lower in MFEC in comparison to CC. However, the cumulative CO_2 production was higher in MFEC in four experiments while in only one experiment the cumulative CO_2 production showed somewhat lower values in MFEC (Figure 2b.).

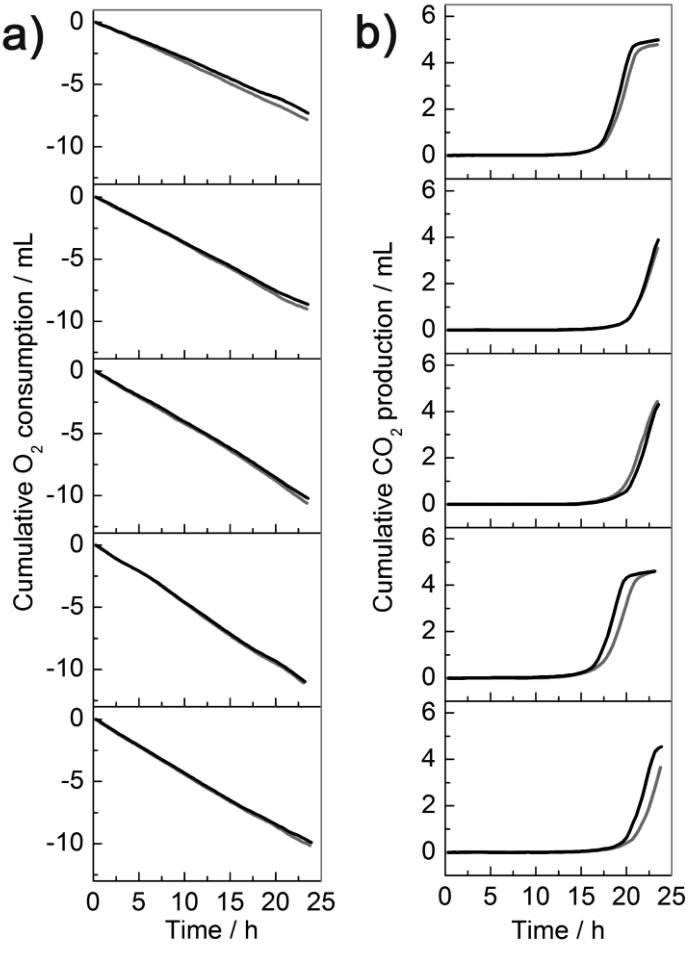

Figure 2. Experimentally obtained cumulative O_2 consumption in mL (a) and cumulative CO_2 production in mL (b) over 24 h for MF frequency range 10 - 50 Hz and samples stirring rate of 300 rpm. Gray curve corresponds to changes obtained in CC while the black curve represents changes of the O_2 and CO_2 in MFEC.

The obtained results were statistically analyzed using the paired two sample one tail T-test, and the cumulative values for both O_2 and CO_2 in the 20th h were used. It should be emphasized that the mentioned calculated normative factors were taken into

consideration during the determination of statistical differences. As is well-known, the paired two sample one-tail T-test considers whether observed differences between two samples are significant or whether they could be explained just by random variations. By the most commonly used criteria, if the calculated probability is equal or smaller than 5 %, the differences are considered statistically significant. The calculated probabilities for the cumulative O_2 consumption and cumulative CO_2 production are 2 % and 5 % respectively. In other words, the paired two sample one-tail T-test showed that for both measured system parameters the differences between MFEC and CC are statistically significant.

In comparison to our previously published results, where a 10-1000 Hz MF was examined, here the paired two sample one-tail T-test showed statistically important differences for both the cumulative O_2 consumption and cumulative CO_2 production. Also, when a 10-50 Hz MF was applied, only one experiment showed a bit lower cumulative CO_2 production MFEC in comparison to CC, while in the case of 10-1000 Hz a complete inconsistency was found. Interestingly, all other performed experiments showed that the lower cumulative O_2 consumption is followed by the higher cumulative CO_2 production in the sample exposed to a 10-50 Hz MF in comparison to CC. This behaviour may indicate that a low frequency MF for chosen scan region from 10 to 50 Hz may favorize anaerobic cells metabolism. However, in order to confirm whether a low frequency MF from 10-50 Hz favorizes anaerobic methabolism, it is important to take into account other important parameters of the system such as cells growth, glucose uptake and ethanol production.

As we assumed in our previous paper, the narrowing frequency scan interval emphasized better effects of the MF on yeast cell respiration. However, further investigation is needed in order to find a scan range which covers more "bio-effective" frequencies. We believe that our obtained results are very promising and that they represent a good basis for further investigation in this field.

Conclusion

The examined MF with a constant low frequency scan regime from 10 Hz to 50 Hz in all five repeated experiments showed the lower cumulative O_2 consumption of cells exposed to the MF and a better regularity for the cumulative CO_2 production was obtained. Also, the applied paired two sample one-tail T-test showed statistically important differences for both the cumulative O_2 consumption and cumulative CO_2 production between control cells and the MF exposed cells. The obtained results strongly suggest that a 10-50 Hz MF influences cell respiration. Even though the presented results are promising, further investigation should cover other important properties of the system, besides respiration, such as cell growth, glucose consumption and ethanol production. Additionally, other MF frequency scan intervals should be analyzed so that the scan interval containing the most bio-effective frequencies could be determined.

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Uticaj niskofrekventnog magnetnog polja (10-50 Hz) na respiracionu aktivnost ćelija kvasca *Saccharomyces cerevisiae*

Ispitivanje uticaja električnog, magnetnog i elektromagnetnog polja na mikroorganizme je veoma aktuelni predmet istrazivanja, jer ova fizička polja potencijalno deluju kao faktori stresa i tako utiču na mikrobni metabolizam, ponašanje i preživljavanje. U ovom radu ispitivan je uticaj niskofrekventnog magnetnog polja (MP) sa konstantnim intervalom skeniranja od 10 do 50 Hz na respiraciju ćelija kvasca, S. cerevisiae. Eksperiment je rađen u pet ponavljanja i praćen Micro-Oxymax® respirometrom. Kumulativna potrošnja kiseonika je bila manja kod ćelija izloženih MP u svih pet ponavljanja, dok je produkcija CO_2 bila nekonzistentna. Međutim, ove razlike u potrošnji O_2 i produkciji CO_2 su statistički značajne. Iako su dodatna ispitivanja neophodna, dobijeni rezultati ovih inicijalnih eksperimenata predstavljaju dobru osnovu za dalja istraživanja u ovoj oblasti.

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