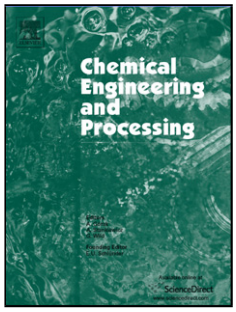

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The influence of low-frequency magnetic field regions on the *Saccharomyces cerevisiae* respiration and growth

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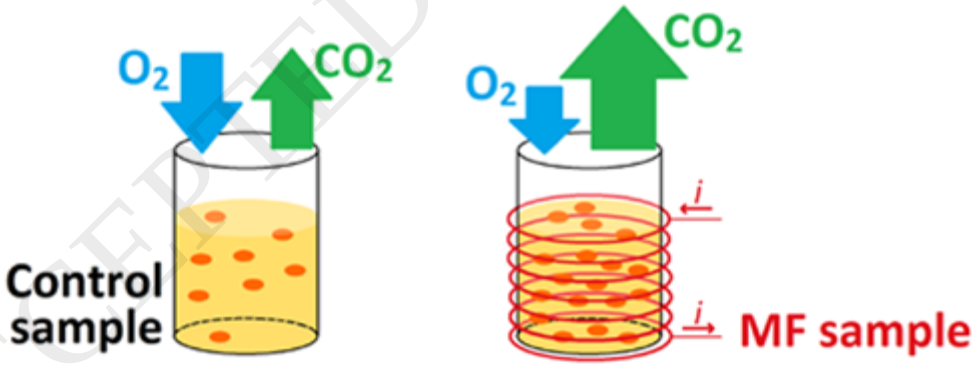
Graphical abstract



Highlights

1. Influence of low frequency magnetic field on yeast cells metabolism was examined.
2. Respiration activity, cell growth and glucose uptake were studied.
3. Regions (10-300) Hz, (10-100) Hz, (10-50) Hz and (50-100) Hz were investigated.
4. Statistical analysis indicates that 10 - 50 Hz region is the most bio-effective.
5. Frequencies 10 - 50 Hz stimulate cell growth and anaerobic metabolism.

Abstract

The influence of four low-frequency magnetic field (MF)[1] ranges 10 - 300 Hz, 10 - 100 Hz, 10 - 50 Hz and 50 - 100 Hz in scanning regime (all frequencies from selected range were scanned during 100s repetitively during 24h) on baker's yeast cells *Saccharomyces cerevisiae* was examined by continuous measurements of cumulative O_2 consumption and cumulative CO_2 production over 24 h with Micro-Oxymax® respirometer. Besides respiration activity, measurements of cell growth and glucose uptake were performed as well. Statistical analysis indicated that, among all investigated low-frequency MF ranges, range from 10 Hz to 50 Hz had the greatest influence to yeast cell respiration and cell growth. More precisely, for this region, paired two sample one-tail T-test showed statistically significant differences in cumulative O_2 consumption, cumulative CO_2 production and *S. cerevisiae* cell number. Moreover samples exposed to MF range from 10 Hz to 50 Hz showed the same behavior in all five replicates: lower cumulative O_2 consumption, higher cumulative CO_2 production and higher cell number compared to control sample. This could be important from the application aspect, in industry (food, feed, brewery etc.) and biotechnology, because changes in cells metabolism are not caused by chemical treatment.

[1]*Abbreviations*: MF, magnetic field; SMF, static magnetic field; CS, control sample; MFS, magnetic field exposed sample.

Keywords

Low-frequency magnetic field, *Saccharomyces cerevisiae*, cell respiration, oxygen consumption, carbon dioxide production, non-chemical cells treatment.

1. Introduction

Thanks to the great absorption of emitted microwave (MW) radiation in biological tissues with large content of water and electrolyte, it is not surprising that it may influence cell metabolism [1-,2,3,4,5,6]. Much more controversial is the influence of the magnetic field (MF) on biological systems because of their small relative magnetic permeability. Despite of this, over the years, many authors investigated the influence of static magnetic field (SMF) and low frequency MF on different prokaryotes and eukaryotes with more or less pronounced effects.

Fojt et al. [7] studied the influence of a 50 Hz MF effect, generated by a cylindrical coil (maximal effective current of 1.9 A) on the growth of *Escherichia coli*, *Leclerciaa decarboxylata* and *Staphylococcus aureus*. They concluded that the MF causes the decrease of colony forming units (CFU) in all exposed samples. Ji and coworkers [8] studied the biological effects of SMF, generated by Nd-Fe-B permanent magnets, on *E. coli*. Their results showed that the number of *E. coli* cells decreased with longer SMF exposure, which is in accordance with work of Fojt [7] and coworkers, while TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy) measurements showed obvious cell surface damage. Fan et al. [9] examined the synergic influence of the SMF (170 mT) with alkaline pH on *Enterococcus faecalis*. Moderate SMF showed slow but persistent antimicrobial action on *E. faecalis*, however when bacteria was exposed to alkali-SMF stress, the antimicrobial activity was significantly enhanced. Contrary to mentioned studies where experimental investigations were performed on different bacteria species, Deamici et al. [10] investigated influence of the SMF (25 mT) exposure (24 h or 1 h per day) on the growth of microalgae *Spirulina sp*. applied in both indoor and outdoor culture systems, as well as on chlorophyll content, medium consumption and protein profile. It was shown that SMF affected the metabolism of *Spirulina sp*. especially when it was grown outdoors in uncontrolled environmental conditions.

Besides mentioned investigations on prokaryotic organisms, yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) has become the most significant eukaryotic model to investigate effects of SMF and low-frequency MF, mostly because of the well-characterized metabolic and genetic properties but also due to their similarity in molecular mechanisms of basic cellular processes to various eukaryotic species [11]. Ruiz-Gomez et al. [11] studied the influence of long-term exposure to SMF and 50 Hz sinusoidal MF induced by a pair of Helmholtz coils (0.35 mT and 2.45 mT) on the growth of *S. cerevisiae.* Authors concluded that neither SMF nor 50 Hz sinusoidal MF induce alterations in the growth of *S. cerevisiae*. Anton-Leberre [12] and coworkers examined a wide variety of *S. cerevisiae* cellular processes such as: growth, cell viability, morphology, metabolic and fermentation activity after strong SMF (up to 16 T for 8h) and pulsed MF (up to 55 T) exposure and concluded that cellular activities were impaired in response to SMF or pulsed MF exposure which supports the work of Ruiz-Gomez. Novak et al. [13] investigated the influence of the 50 Hz MF on the growth of *S. cerevisae*, but in this case MF was induced in a cylindrical coil (10 mT). Contrary to the previous studies, it was concluded that 50 Hz MF decreases the number of yeast cells, and slows down their growth. On the other hand, Santos et al. [14] reported that SMF (8-16 h, 25.0-34.3 mT) can positively stimulate the

growth of *S. cerevisiae*. Mentioned papers represent only some examples with conflicting results of the bio-effects of the applied MFs. Potential reasons for that could be the use of different cell types, magnetic field exposure protocols, intensities, frequencies etc. Motta and coworkers [15] examined the influence of SMF (generated by NdFeBr magnets, 110 mT and 220 mT, during 24 h) on the *S. cerevisiae* metabolic activity by measuring CO_2 pressure, with mercury manometer hermetically connected to sample bottles, and pH of the culture medium before and after SMF exposure. Their results suggest that after a 12 h of incubation, 220 mT SMF enhances the biomass proliferation and cellular metabolic activity. Moreover, Motta et al. [16]also studied the effects of SMF (220 mT, exposure time 24 h) on ethanolic fermentation by *S. cerevisiae* measured by gas chromatography, and concluded that ethanol concentration was 3.4 times higher in MF treated cultures. Nakasono and coworkers [17] performed detailed study of the effect of 50 Hz MF exposure on genome-wide gene expression, of the yeast *S. cerevisiae*. Their results indicate that a 50 Hz MF below 300 mT did not act as a stress factor like heat shock or DNA damage which is in accordance with results from Anton-Leberre work obtained at much higher fields. Besides cell growth Santos et al. [14] investigated the effect of SMF (25.0-34.3 mT) exposure (8-16 h) on glutathione production by *S. cerevisiae* and concluded that application of SMF has a favorable alteration in glutathione concentration.

Even though many authors examined influences of the SMF and low-frequency MF on different microorganisms and biological systems (SMF effects are revised in ref [18])a unique explanation of the mechanism of their interaction still remains uncertain .[19] Although literature results are somewhat conflicting, investigations of this type can be very useful for potential non-chemical changing of the cells metabolism in biotechnology.

In our study, for the first time, based on the available literature, the influence of low-frequency MF on yeast cells *S. cerevisiae* was examined by measuring respiration activity with continuous multi gas detection, by high sensitivity Micro-Oxymax® respirometer. In addition, measurements of cell growth and glucose uptake were performed to better characterize MF effects. Contrary to the previously reported results, where cells were exposed to individual frequencies (the most usual one is 50 Hz) or SMF, in our study cells were sequentially exposed to various MF frequencies within the chosen scan range. Unfortunately, it is not possible to theoretically predict the most effective frequency on cells functioning. In addition, it is almost impossible to investigate biological effects of all frequencies in the range with the resolution of e.g. 0.01 Hz available in commercial function generators. Thus, the idea of our work was to find the frequency range which has the greatest effect on yeast cells. Since the most effective

frequencies on cells functioning might be distributed widely or narrowly in the low-frequency MF range, the largest MF range examined in our work (from 10 Hz to 300 Hz) was further divided into smaller ranges in order to identify frequency range containing the most effective frequencies. In our study we designed a special experimental setup to maintain the same bulk temperature in control and MF exposed samples.

2. Experimental

2.1. Yeast strain and experimental protocol

Commercially available baker's yeast (Alltech Serbia d.o.o., Senta, Serbia) was used.

Prior to the all experiments, *S. cerevisiae* was grown on the malt extract agar. The overnight culture was suspended in saline solution, and 200 µL of the cell suspension was transferred into Sabouraud dextrose broth (SBD). In order to prolong the log phase of cell division which will be monitored, SBD was diluted in ratio 1:1. Every experiment was performed in pair (CS and MFS) using the same SBD medium which was inoculated with the yeast cell suspension of the same cell density. The range of cell suspension density between experimental replicates was from 1.2×10^5 to 5.6×10^5 CFU/mL.

2.2. Magnetic field experiments

All experiments were performed in pair, in 5 mL specially designed glass bottles. One bottle served as a control sample (CS) while the other one was exposed to a low frequency magnetic field generated inside of the spiral Cu-coil or, in other words, it was used as a magnetic field exposed sample (MFS). To avoid undesirable temperature increase of sample cells, (because of the heating effect of the coil itself caused by current flow) sample bottle was installed in a glass water recirculation jacket around which Cu-coil is wrapped as it is shown in Fig. 1.

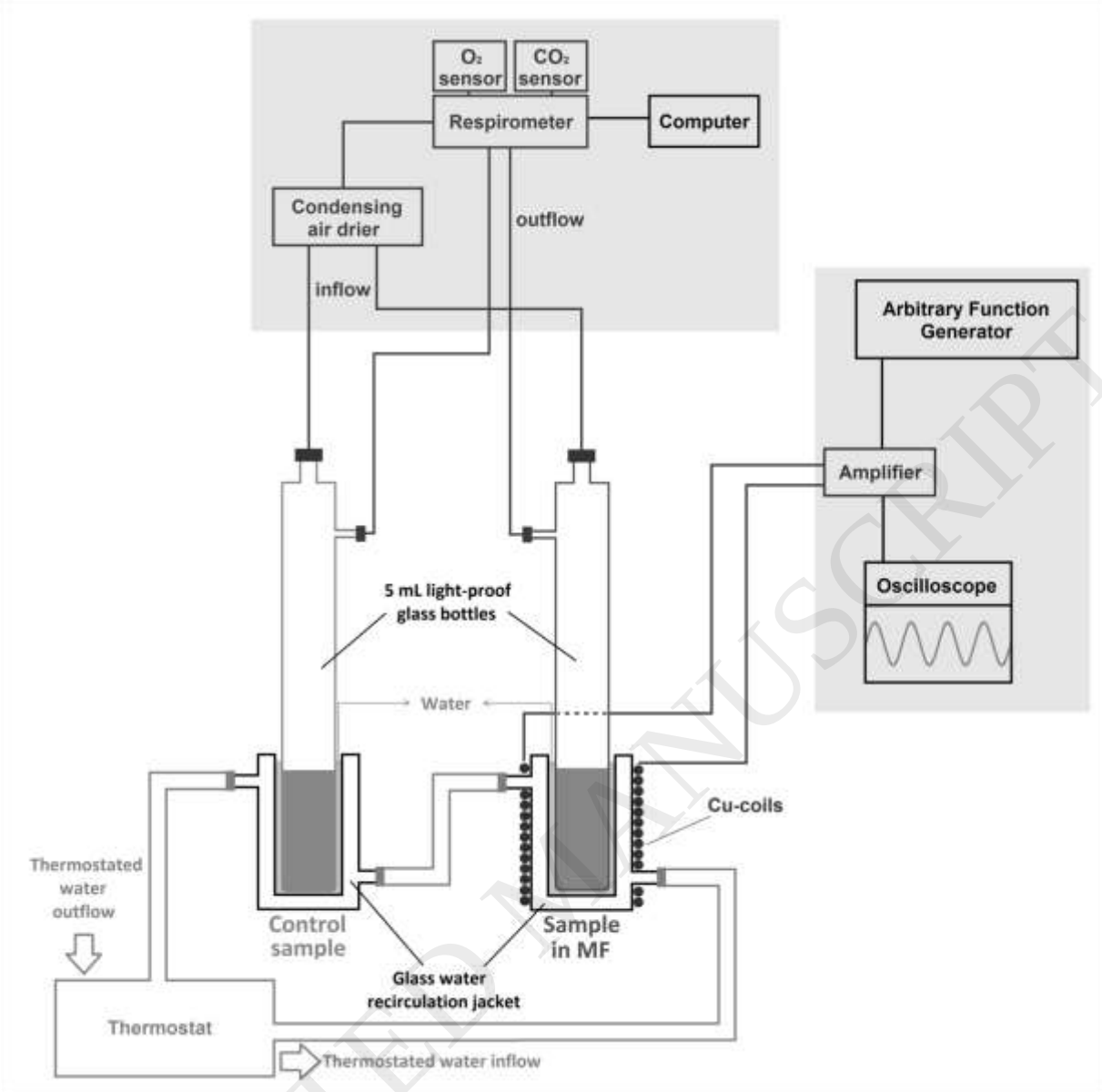


Fig. 1. Schematic view of the experimental setup.

(*double column fitting image*)

To provide good temperature exchange, narrow empty space between bottle and recirculation jacket was filled with deionized water. The bottle with CS was also installed in a glass water recirculation jacket. In order to avoid potential influence of the slightly heated water from recirculation jacket inside of a coil, both water recirculation jackets were serially connected to the circulation thermostat (Julabo, F12 Refrigerated/Heating Circulator, Julabo GmbH, Germany) as shown in Fig. 1. Such experimental design provided the same temperture in both reaction vessels (28.0 ± 0.1)°C as described below.

For temperature measurements, an ethanol-filled thermometer with 0.1 ^{o}C accuracy was used. This type of thermometer was used rather than mercury thermometer or conductor/semiconductor temperature sensors to avoid potential interactions with MF and incorrect temperature reading. Before the magnetic field was turned on, the same temperature was confirmed in both bottles. When the magnetic field was turned on, temperature was measured again in both CS and MFS vessels at 5^{th}, 15^{th}, 30^{th} and 60^{th} minute. In all measurements, in both control and sample exposed to MF, temperature was the same within the thermometer accuracy (28.0 ± 0.1)°C. It clearly shows that the power of the circulating thermostat was appropriate, for designed experimental configuration, to keep the same bulk temperature in both reaction vessels.

The MF was generated inside of the spiral Cu-coil. Arbitrary function generator (GwInstek AFG-2105, Good Will Instrument Co., Ltd, Taiwan) was used to set up a desired frequency range and a scan interval as well. In this paper we examined influence of four different low-frequency scan regimes: 10-300 Hz, 10-100 Hz, 10-50 Hz and 50-100 Hz. In all experiments all frequencies from selected range were scanned (from lowest to highest) during 100 s and total time of exposition was 24 h. Due to the frequency dependence of coil inductance, maximal current through the coil is maintained at 2.00 A (at the lowest frequency of the examined range) in all experiments and provided by home-made signal amplifier of 100 W (based on STMicroelectronics IC TDA7293) Maximal effective current through the coil of 2.00 A corresponds to magnetic induction of 33 mT, which was determined by gaussmeter LakeShore Model 410. Besides, for minimal effective current of 1.54 A magnetic induction is 25 mT. Finally, oscilloscope was used to control and monitor effective current during frequencies scanning.

2.3. Respiration measurement

Respiration activity of *S. cerevisiae* was measured by a twelve-channel Micro-Oxymax® respirometer (Columbus Instruments, Columbus, USA) in closed mode. The experiments were thermostated with water inflow at (28.0 ± 0.1)°C as schematically represented in Fig 1. Two specially designed light-proof 5 mL glass bottles (CS and MFS) contained per 3 mL of inoculated SBD medium and were circularly connected to O_2 paramagnetic sensor and CO_2 infrared sensor. Cell respiration was measured every 20 min during 24 h. Cumulative O_2

consumption and cumulative CO_2 production (in mL) were determined. All experiments were performed in five replicates.

2.4. Glucose determination

After separation of yeast biomass by centrifugation, the glucose content in the supernatant was determined using the Megazyme D-Glucose (GOPOD) reagent kit. This enzymatic assay is highly specific and accurate, within concentration range of measurement, avoiding unwanted interfering/artefactual reactions with other components of the cell metabolism.

2.5. Determination of cell number

Number of *S. cerevisiae* cells after 24 h in magnetic field was determined by serial dilution technique. Both control samples and samples before and after exposition in magnetic field were used for comparing growth on malt agar plates after 24 h incubation time at 28 °C.

2.6. Statistical analysis

All obtained results from respiration, glucose and serial dilution method measurements were statistically considered by paired two sample one tail T-test with 0.95 confidence level. For respiration measurements calculated correction factors (see Section Results and discussion) were taken into consideration for determination of the statistically significant differences between CS and MFS.

3. Results and discussion

Before all experiments, where the influence of MF on yeast cells was examined, our entire experimental setup was tested to respirometer reading without magnetic field. In this test experiment, in ideal case, it would be expected that cumulative O_2 consumption and cumulative CO_2 production are exactly the same in both vessels. However, all three test experiments without magnetic field showed that a small difference between two vessels exists. Therefore,

in order to enable correct interpretation of the results when MF is applied this small differences must be taken into account providing appropriate correction factors. From mean values (obtained from three test experiments) a correction factor was calculated. For cumulative O_2 consumption correction factor is 1.07 and for cumulative CO_2 production is equal to 1.14. Obtained correction factors were used to adjust values of reading obtained in all MF experiments.

Influence of MF scan regimes in four frequency ranges (10 - 300 Hz, 10 - 100 Hz, 10 - 50 Hz and 50 - 100 Hz) on yeast cell respiration were monitored in both CS and MFS during 24 h. In all performed experiments the same bulk temperature was maintained in both reaction vessels. Fig. 2 illustrates cumulative O_2 consumption and cumulative CO_2 production changes over exposure time of CS and MFS, traced by Micro-Oxymax respirometer. At Fig. 2 only one representative experiment (out of total five) per each frequency range is given. As it can be seen from Fig. 2, differences in cumulative CO_2 production between CS and MFS up tp 10th hour at all investigated frequency ranges, are negligible. After 10^{th} hour those differences begin to grow and are the highest at the end of exposure. In order to equally compare all obtained results from respirometer for all frequency ranges, comparison between CS and MFS were made at 23^{rd} hour. In order to obtain better statistical treatment of results all frequency ranges are investigated in five replicates.

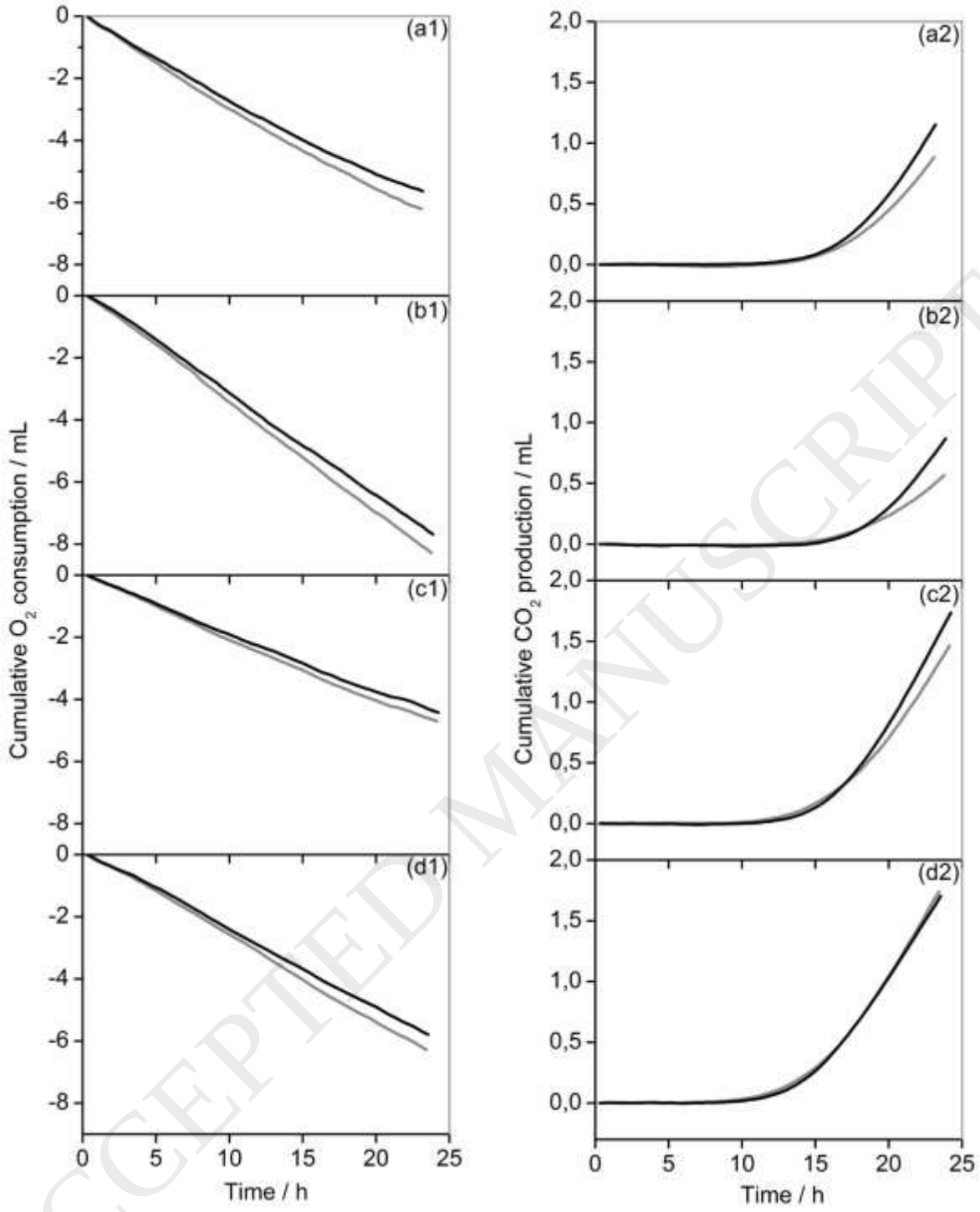


Fig. 2. Cumulative O_2 consumption in mL (a1, b1, c1, d1) and cumulative CO_2 production in mL (a2, b2, c2, d2) over 24h for one representative replicate per different frequency ranges (10-300 Hz (a1, a2), 10-100 Hz (b1, b2), 10-50 Hz (c1, c2) and 50-100 Hz (d1, d2)). Gray curve corresponds to changes in CS while black curve represents O_2 and CO_2 changes in MFS. (*double column fitting image*)

The values of cumulative O_2 consumption and cumulative CO_2 production in CS and MFS at 23 h of exposure, in all five experiments per each examined frequency range, are shown in Table 1. Values of cumulative O_2 consumption are negative since oxygen at the beginning of the experiment (of 21% in air) is taken as reference (0 mL).

Table 1 Cumulative O_2 consumption and cumulative CO_2 production in CS and MFS at 23rd hour of MF exposure for all experiments and all examined MF ranges.

Frequency range / Hz	Cumulative O_2 consumption / mL		Cumulative CO_2 production / mL	
	MFS	CS	MFS	CS
10 - 300	-5.875	-6.601	0.660	1.242
	-3.385	-4.560	1.000	1.750
	-5.637	-6.631	1.152	1.007
	-6.192	-7.068	1.049	0.747
	-5.064	-5.900	1.654	0.903
10 - 100	-4.610	-5.394	0.911	1.783
	-4.350	-4.310	1.013	0.570
	-3.763	-4.682	1.734	0.916
	-7.432	-8.616	0.752	0.561
	-4.495	-5.170	0.729	0.580
10 - 50	-2.534	-3.206	0.694	0.571
	-2.701	-2.843	0.710	0.492
	-2.453	-2.994	1.226	0.645
	-4.055	-4.789	0.939	0.937
	-4.221	-4.848	1.500	1.432
50 - 100	-3.067	-3.621	1.040	1.217
	-5.657	-5.410	1.026	1.160
	-5.743	-6.450	1.196	0.610
	-5.708	-6.616	1.633	1.905
	-7.077	-6.380	1.508	1.957

For frequency range from 10 to 300 Hz cumulative O_2 consumption in MFS was lower in comparison to CS in all five replicates. However, for the same frequency range, behavior in cumulative CO_2 production differed, as two replicates showed lower cumulative CO_2 production in MFS and the other three showed opposite behavior. Similar inconsistency was found for frequency range from 50 to 100 Hz in cumulative O_2 consumption behavior as well as in cumulative CO_2 production. From this point of view the the most consistent results were

obtained in experiments where the range from 10 to 50 Hz was applied, as well as from 10 to 100 Hz in which only one experiment showed different behavior.

Besides respiration, for all examined frequency regimes, glucose uptake and number of yeasts cells were determined. The average values of the remaining glucose in CS and MFS after 24h of exposure are given in Fig. 3. At the beginning of the experiment, both CS and MFS had the same glucose concentration (10 g/L). Calculated standard errors of the mean values (from five experiments) of the remaining glucose in CS and MFS are showed as well.

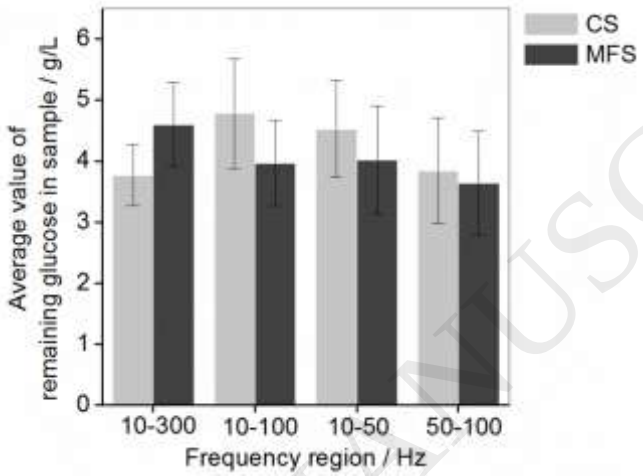


Fig. 3. Average values (from five experiments) of the remaining glucose in CS and MFS, for all investigated frequency regions, with marked standard errors of the mean values.
(*single column fitting image*)

As it can be seen from Fig. 3 the average value of the remaining glucose was slightly higher in MFS than in CS for frequency range 10-300 Hz. Contrary to that, when other examined regions were applied, yeast cells consumed more glucose in MF than in CS. Number of yeasts cells at the end of the experiment was determined by the serial dilution method since it counts only viable cells. Obtained mean values of the yeasts cell number and standard errors, calculated from five experiments are shown in Fig. 4.

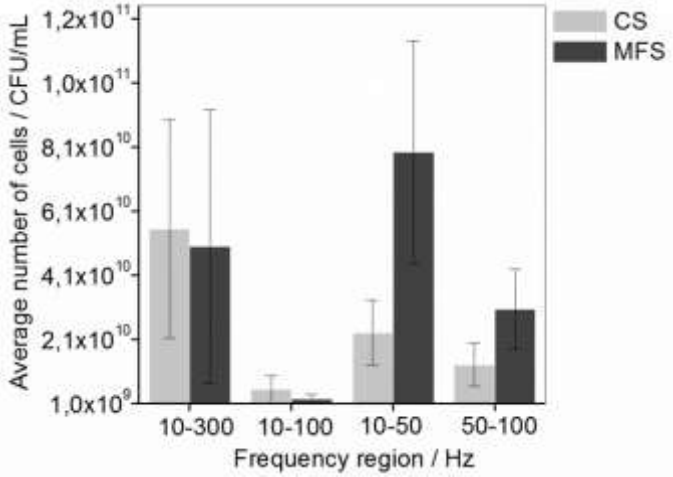


Fig. 4. Average number of yeast cells (CFU/mL) in CS and MFS, for all examined frequency regions, with marked standard errors of the mean values (from five experiments).
(*single column fitting image*)

Interestingly when frequency regions 10-300 Hz and 10-100 Hz were applied, number of cells in those regions can be regarded unchanged taking into account large standard deviations of results. On the other side differences between cell numbers in regions 10-50 Hz and 50-100 Hz are larger than standard deviations indicating more significant effects of MF.

Large statistical errors of the results shown in Figs. 3 and 4 are the consequence of comparing five replicated experiments with slightly different initial number of cells. Although, in all paired (CS and MFS) experiments the same yeast cell suspension was used, number of cells between experimental replicates varied from 1.2×10^5 to 5.6×10^5 CFU/mL. Due to this, it is better to use a different type of statistical analysis to determine effects of the investigated frequency regions. For this reason a paired two sample one-tail T-test was used. The statistical results for all examined frequency ranges and all tested parameters are shown in Table 2.

Table 2 Results from paired two sample one-tail T-test with the 95 % confidence level (The results that were considered significant were marked in grey).

Frequency range / Hz	P_{sample} / %			
	Cumulative O_2 consumption	Cumulative CO_2 production	Glucose uptake	Number of cells
10-300	0.01	46	22.5	30.5
10-100	1	31	5.7	21.9
10-50	0.33	6	11.2	6.5
50-100	23	32	30.3	13.2

Paired two sample one-tail T-test considers whether observed differences between data are significant or they may be merely explained by random variations. Assuming that differences *d* between paired samples follow normal distribution, significant deviation between samples may be determined by examining the position of the characteristic value:

$$t_{sample} = \frac{\bar{d}\sqrt{n}}{\sigma}$$

($\bar{d}$ represents mean value of differences, n degree of freedom and σ standard deviation of data) relatively to the distribution function.

If t_{sample} is positioned close to the 0 (*i.e.* $\bar{d} = 0$) there is no significant differences between paired data sets. How close t_{sample} is positioned to the tail of the distribution is determined by the probability of finding data right to t_{sample}. If the probability P_{sample} is smaller from the probability P_{crit} (related to some critical value t_{crit}), than differences are considered as significant. Usually as a criteria of significance $P_{crit} = 0.05$ (or 5 %) is chosen, meaning that the probability of obtaining lager values by chance is only 1 out of 20 experiments. Accordingly, if $P_{sample} < P_{crit}$ differences may be considered as significant since the probability of obtaining larger differences by chance is less than 1 experiment out of 20. At the same time, P_{sample} of 6 % means that the probability of obtaining larger differences between data by chance is 1 experiment out of 17 which is still a very small probability, especially for biochemical systems, and could be considered as statistically significant.

To summarize, range from 10-300 Hz showed statistically significant differences in only one measured system parameter (cumulative O_2 consumption), range 10-100 Hz had statistically significant differences in two (cumulative O_2 consumption and glucose uptake), while 10-50 Hz had statistically significant differences in all measured parameters except glucose uptake. Frequency ranges 10-300 Hz and 10-100 Hz both cover this most effective 10-50 Hz range but are less significant than 10-50 Hz range was. This may be explained by the fact that exposure time to particular "effective" frequency from the range is very important parameter. Frequency range 50-100 Hz is the least effective since it didn't show statistically significant differences in any of the considered parameters.

Moreover, range 10-50 Hz had the same trend in all five replicates: lower cumulative O_2 consumption, higher cumulative CO_2 production and higher cell number in MFS (Table 1).

Higher cumulative CO_2 production in MFS compared to CS is in good agreement with results of Motta and coworkers [15]. Contrary to the work of Motta et al. [15] in our study samples were in aerated conditions and instead of 220 mT SMF, weaker MF (maximal field 33 mT) in the frequency range 10-50 Hz MF was used. Our results suggest that 10-50 Hz MF slightly motivates cell growth which can explain higher cumulative CO_2 production. If this is the only explanation of MF effects, then higher cumulative O_2 consumption may be expected as well. However, we observed lower cumulative O_2 consumption. Yeasts can carry out two opposing modes of metabolism, both respiration and fermentation, depending on the oxygen concentration [20]. Since both samples (CS and MFS) were inoculated with the yeast suspension of approximately the same cell density and were placed in the same conditions with the same volume of air, one possible explanation could be that MF of 10-50 Hz stimulates anaerobic metabolism (fermentation). If this was true MF range would be very important from the application aspect because during anaerobic regime cells produce an important energy product of nowadays, ethanol. Unfortunately, we were unable to confirm this experimentally since concentration of ethanol in both CS and MFS were below GC (Varian CP 3400 with PID/ECD detector and Perkin Elmer HS-400 Headspace autosampler) detection limits of 1ppm. Low ethanol concentration is the consequence of working in aerobic conditions and with the very dilute cell medium, which was used in order to achieve the best conditions for cell growth during 24 h of MF exposure. Besides, in our experiments baker's yeast was used which doesn't produce ethanol as much as e.g. brewer's. Therefore, in future experiments influence of the frequency scan regime 10-50 Hz should be examined on brewer's yeast with less dilute cell medium in anaerobic conditions.

Considering statistical significance, frequency scan regime of 10-50 Hz had the greatest influence on yeast cells. It is interesting that region 10-100 Hz had less biological effects despite of the fact that 50 % of its frequencies are shared with the most effective 10-50 Hz region. Also region 10-300 Hz had even less biological effects since it contains only about 15 % frequencies from the most effective region of 10-50 Hz. This indicates two important implications. One is that exposure time is important experimental parameter since exposure to "interesting" frequencies of the 10-50 Hz is lower in other investigated regions. The second is that even region of 10-50 Hz may contain narrower regions with even more emphasized effects. Exposure to only those specific frequencies could facilitate non-chemical forcing of cells to function in desired metabolic regime.

4. Conclusion

Influence of MF scan regimes in four frequency ranges (10-300 Hz, 10-100 Hz, 10-50 Hz and 50-100 Hz) on *S. cerevisiae* respiration activity, glucose uptake and cell growths was examined. Cumulative O_2 consumption and cumulative CO_2 production were continuously followed with Micro-Oxymax® respirometer in both CS and MFS during 24 h of experiment. For the determination of glucose content in the supernatant, highly specific and accurate GOPOD reagent kit was used, while the number of *S. cerevisiae* cells was obtained by serial dilution method. All results were statistically analysed by paired two sample one tail T-test with 0.95 confidence level.

Taking into consideration statistical analysis and results consistency for all five replicates it can be concluded that the greatest influence on yeast cells respiration activity and cell growth has the 10-50 Hz MF range. For this region statistically significant differences between CS and MFS for three examined parameters (cumulative O_2 consumption, cumulative CO_2 production, cell number) were found. In addition, lower cumulative O_2 consumption, higher cumulative CO_2 production and higher cell number in MFS are consistently preserved in all five replicates. Obtained results strongly suggest that 10-50 Hz MF stimulates cell growth, and possibly anaerobic metabolism due to the fact that higher cumulative CO_2 production is accompanied by lower cumulative O_2 consumption.

Our results show that wider regions, containing smaller fractions of the most effective frequencies (10-50 Hz), have progressively less biological effects. Thus, it is possible that even the region 10-50 Hz could be divided into more narrow biologically active ranges. Exposure to these specific MF frequencies could be important for larger scale biotechnology applications which will be examined in further investigations.

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References

[1] S. Banik, S. Bandyopadhyay, S. Ganguly, Bioeffects of microwave-a brief review, Bioresour. Technol. 87 (2003) 155–159.

[2] A.V. Vorst, A. Rosen, Y. Kotsuka RF/microwave interaction with biological tissues, 1st edn. Wiley-IEEE Press, Hoboken, 2006

[3] I. Vrhovac, R. Hrascan, J. Franekic, Effect of 905 MHz microwave radiation on colony growth of the yeast *Saccharomyces cerevisiae* strains FF18733, FF1481 and D7, Radiol. Oncol. 44 (2) (2010) 131-134.

[4] Y. Shamis, R. Croft, A. Taube, R.J. Crawford, E.P. Ivanova, Review of the specific effects of microwave radiation on bacterial cells, Appl. Microbiol. Biotechnol. 96 (2012) 319–325.

[5] D. Stanisavljev, G. Gojgić‐Cvijović, I.N. Bubanja, Scrutinizing microwave effects on glucose uptake in yeast cells, Eur. Biophys. J. 46(1) (2017) 25-31.

[6] I. Calinescu, A. Vlaicu, P. Chipurici, D. Ighigeanu, V. Lavric, Alcoholic fermentation in the presence of microwaves, Chem. Eng. Process. 126 (2018) 16-22.

[7] L. Fojt, L. Strašák, V. Vetterl, J. Šmarda, Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*, Bioelectrochemistry 63(1-2) (2004) 337-341.

[8] W. Ji, H. Huang, A. Deng, C. Pan, Effects of static magnetic fields on *Escherichia coli*, Micron 40(8) (2009) 894-898.

[9] W. Fan, Z. Huang, B. Fan, Effects of prolonged exposure to moderate static magnetic field and its synergistic effects with alkaline pH on *Enterococcus faecalis*, Microb. Pathogenesis 115 (2018) 117-122.

[10] K.M. Deamici, L.O. Santos, J.A.V. Costa, Magnetic field action on outdoor and indoor cultures of *Spirulina*: Evaluation of growth, medium consumption and protein profile, Bioresource Technol. 249 (2018) 168-174.

[11] M.J. Ruiz-Gomez, M.I. Prieto-Barcia, E. Ristori-Bogajo, M. Martınez-Morillo, Static and 50 Hz magnetic fields of 0.35 and 2.45 mT have no effect on the growth of *Saccharomyces cerevisiae*, Bioelectrochemistry 64 (2004) 151-155.

[12] V. Anton-Leberre, E. Haanappel, N. Marsaud, L. Trouilh, L. Benbadis, H. Boucherie, S. Massou, J.M. Francois, Exposure to High Static or Pulsed Magnetic Fields Does Not Affect Cellular Processes in the Yeast *Saccharomyces cerevisiae*, Bioelectromagnetics 31 (2010) 28-38.

[13] J. Novák, L. Strašák, L. Fojt, I. Slaninová, V. Vetterl, Effects of low-frequency magnetic fields on the viability of yeast *Saccharomyces cerevisiae*, Bioelectrochemistry 70 (2007) 115-121.

[14] L.O. Santos, R.M. Alegre, C. Garcia-Diego, J. Cuellar, Effects of magnetic fields on biomass and glutathione production by the yeast *Saccharomyces cerevisiae*, Process Biochem. 45 (2010) 1362-1367.

[15] M.A. Motta, E.J.N. Montenegro, T.L.M. Stamford, A.R. Silva and F.R. Silva, Changes in *Saccharomyces cerevisiae* Development Induced by Magnetic Fields, Biotechnol. Prog. 17 (2001) 970-973.

[16] M.A. da Motta, J.B.F. Muniz, A. Schuler and M. da Motta, Static Magnetic Fields Enhancement of *Saccharomyces cerevisae* Ethanolic Fermentation, Biotechnol. Prog. 20 (2004) 393-396.

[17] S. Nakasono, C. Laramee, H. Saiki, K.J. McLeod, Effect of Power-Frequency Magnetic Fields on Genome-Scale Gene Expression in *Saccharomyces cerevisiae*, Radiat. Res. 160 (2003) 25-37.

[18] W.W.C. Albuquerque, R.M.P.B. Costa, T. de Salazar e Fernandes and A.L.F. Porto, Evidences of the static magnetic field influence on cellular systems, Prog. Biophys. Mol. Bio. 121 (2016) 16-28.

[19] R. Matthes, P. Vecchia, A. F. McKinlay, B. Veyret, J. H. Bernhardt, Exposure to Static and Low Frequency Electromagnetic Fields, Biological Effects and Health Consequences (0-100 kHz), International Commision on Non-Ionizing Radiation Protection, Munich, Germany, 2003.

[20] M. T. Madigan, J. M. Martinko, D. A. Stahl, D. P. Clark, Brock biology of microorganisms, Pearson Education Inc, San Francisco, CA, 2012