

## The Effects of Rotating Magnetic Field on Growth Rate, Cell Metabolic Activity and Biofilm Formation by Staphylococcus Aureus and Escherichia Coli

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**This work presents results of the study which concerns the influence of the rotating magnetic field (RMF) on the growth rate, cell metabolic activity and ability to form biofilms by *E. coli* and *S. aureus*. Liquid cultures of the bacteria were exposed to the RMF (RMF frequency  $f = 1-50$  Hz, RMF magnetic induction  $B = 22-34$  mT, time of exposure  $t = 60$  min, temperature of incubation  $37$  °C). The present study indicates the exposition to the RMF, as compared to the unexposed controls causing an increase in the growth dynamics, cell metabolic activities and percentage of biofilm-forming bacteria, in both *S. aureus* and *E. coli* cultures. It was also found that the stimulating effects of the RMF exposition enhanced with its increasing frequencies and magnetic inductions.**

**Keywords :** rotating magnetic field, viability, growth dynamics, cell metabolic activity, biofilm formation, bacteria

### 1. Introduction

In the recent years, special attention has been focused on the influence of various types of electric fields (EFs), magnetic fields (MFs) and electromagnetic fields (EMFs) on the functional parameters and pathogenicity potentials of unicellular microorganisms (bacteria and yeast) [1-4]. Currently, there are abundant evidences that various types of EFs, MFs and EMFs affect functional processes in microorganisms and influence their pathogenicity potential. It was also proved that EFs, MFs and EMFs can influence the biological functions of different organisms which affect hormone secretion [5], enzyme activity [6], cell viability [7], cell proliferation [7, 8], transport of ions [9], cell metabolic activity [10] or synthesis and transcription of DNA [11]. The MFs and EMFs were also used in medicines as modulators of bacteria-host interaction during trials of developing alternative therapies [4, 12], and as potential agents to modulate the properties of microorganisms involved in biotechnological processes [13-16]. Possibilities of controlling the microbial viability and activity by using physical agents can be widely applicable to agriculture, food science and medicine [13, 17, 18].

However, studies on the influence of MFs for living organisms mainly concern the static magnetic field (SMF), whereas the research on the rotating magnetic field (RMF) has been limited and its effects on the microorganisms remain unexplored. Furthermore, due to the different nature of the SMF and the RMF, it can be expected that their effects on living organisms can also be different. It should be verified that the SMF does not vary over time or change slowly. Moreover, this kind of MF does not have frequency. In contrast, the RMF is a MF which changes direction, ideally, at a constant angular rate. The RMF arises as a result during the superposition on two or more alternating-current magnetic fields of identical frequency but are spatially displaced in phase with respect to one another. Therefore, the RMF changes over time and can be characterized by its frequency [19].

Nevertheless, it should be noticed that until now, the studies on the impact of SMF on the growth and activity of microorganisms have provided many useful information concerning possibilities of controlling the microbial cell's processes. It is agreed upon, that in the near future, this information can contribute to improve the effectiveness of treatment and prophylaxis of many serious bacterial infections [20-22]. It can be assumed that in the future the RMF could also be used as a relatively cheap and easy-to-use agent for modulating functional and pathogenic parameters of bacteria, and it could be applied to the medi-

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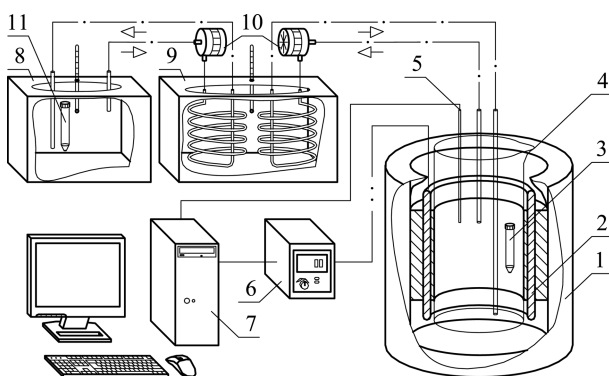
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cine, agriculture and food technology. Therefore, the aim of this study is to analyze the influences of RMF on the growth dynamics, cell metabolic activity and the ability to form biofilms by *E. coli* and *S. aureus*.

## 2. Experimental

The schematic diagram of the experimental set-up with the RMF generator is presented graphically in Fig. 1. The experimental set-up used in the current research was self-designed and adopted for cell culture studies. It was also used, with slight modifications, in the previously published work by Masiuk *et al.* [23], recording the influence of RMF on the expressions and intranuclear distributions of nucleolin in HL-60 and K-562 cells.

The experimental set-up consisted of the RMF generator made of a three-phase stator with an induction squirrel cage motor, and a glass container filled with the demineralized water that was a water bath incubator for the bacterial cultures placed inside. The glass container was axially aligned with the RMF generator and positioned symmetrically with respect to its lower and upper ends. The frequencies of the RMF were changed by using the transistorized inverter (Commander SK, APATOR, Poland). The incubation temperature during the exposure of the bacterial culture to the RMF was controlled by the thermostat (UTU-3, ZEAMiL, Poland), the cooling jacket and the circulating pump (WZ-250/BY, Ożarów Mazowiecki, Poland). This system was used to maintain constant water flow rate and to set the constant temperature of the water bath ( $37 \pm 0.5$  °C). The temperature fluctuations inside the glass container during the experiment were measured using the microprocessor temperature sensors (LM-61B, National Semiconductor Corporation, USA).

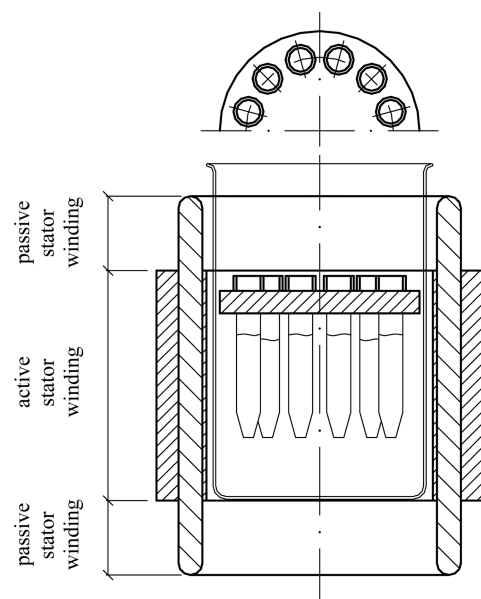


**Fig. 1.** Experimental set-up: 1 – cooling jacket, 2 – RMF generator, 3 – test tube, 4 – cylindrical glass vessel, 5 – microprocessor temperature sensor, 6 – a.c. transistorized inverter, 7 – personal computer, 8 – water bath, 9 – thermostat, 10 – circulating pumps, 11 – control tube.

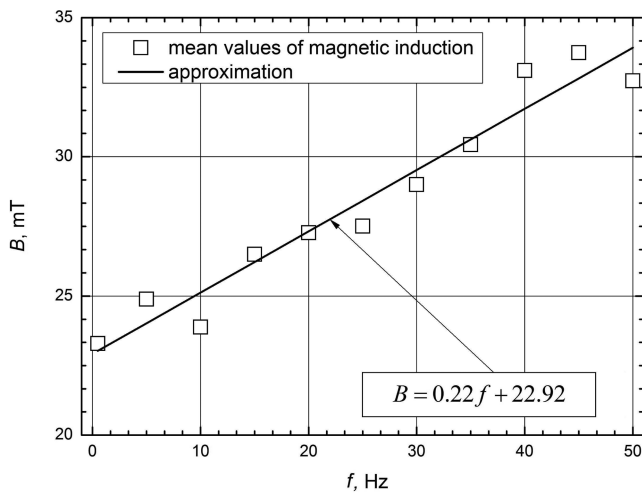
Bacteria were exposed to the RMF in 15 mL plastic tubes (Polypropylene Conical Centrifuge Tube, Becton Dickinson and Company, USA) filled with 10 mL of bacterial inoculum (described below, in the section “Microorganisms and culture conditions”). The test tubes with bacterial cultures were arranged in the RMF generator for allowing the same exposures to magnetic fields of the bacterial inoculum volume (see Fig. 2).

The RMF was generated by the stator supplied with the three-phase alternating current. In order to change the frequency ( $f$ ) of the RMF, the transistorized inverter was used. The values of magnetic induction ( $B$ ) inside the single tube with inoculum were detected by using Hall probe (Smart Magnetic Sensor-102, Asonik, Poland) connected to the personal computer. The measurements of magnetic induction at each selected RMF frequency:  $f = 1$  Hz,  $f = 5$  Hz,  $f = 25$  Hz,  $f = 50$  Hz were repeated several times, and the mean values of magnetic induction were calculated:  $B = 22$  mT,  $B = 25$  mT,  $B = 29$  mT,  $B = 34$  mT, respectively. As shown in the earlier study by Rakoczy and Masiuk [24], the magnetic induction was perpendicular to the surface of the glass container with the cell culture tubes placed along the axis of the RMF generator. The characteristics of the RMF generator for the frequency and the magnetic field are presented in Fig. 3.

As the controls, the same bacterial strains, incubated at the same time and under the same conditions as exposed samples but without exposures to the RMF were used. In order to exclude any exterior influences for results of the



**Fig. 2.** A graphical presentation showing the arrangement of the test tubes in the cylindrical glass container of the RMF generator.



**Fig. 3.** The graphical presentation for the relation between the values of the RMF frequency ( $f$ ) (equal to frequency of alternating current) and the mean values of magnetic induction ( $B$ ).

experimental set-ups with the RMF, the control system that included the RMF off control and the water bath control was being used.

The RMF off controls were incubated in the RMF generator when the RMF was switched off. The water bath controls were incubated in the water bath used to maintain the temperature of the test tubes in the RMF generator. As recorded, the fluctuations of the temperature during the incubation of controls were the same as during the exposition of bacteria to the RMF and were less than  $0.5\text{ }^{\circ}\text{C}$ . The water bath was placed two meters from the generator of the RMF, and it was measured by using a Hall probe (Smart Magnetic Sensor-102) as the source of the RMF did not influence the water bath controls during the experiment ( $B \leq 0.05\text{ mT}$ ).

Two reference strains of *E. coli* American Type Culture Collection (ATCC) 8739 and *S. aureus* ATCC 43300 were used. These species are characterized by various shapes (rods and cocci), different structures and chemical compositions of the cell wall (Gram-negative and Gram-positive bacteria). Bacteria were plated onto Columbia Agar with 5% sheep blood (Grasso, Poland) and cultivated for 24 hours at  $37\text{ }^{\circ}\text{C}$ . After the incubation, one colony forming unit (CFU) of each isolate was transferred into 10 mL of Tryptic Soy Broth (TSB, Oxoid, UK) and incubated for another 24 hours at  $37\text{ }^{\circ}\text{C}$  with shaking. In the next step, the cultures were diluted in TSB to obtain the same optical density (OD) which equals 0.2 at 540 nm for all bacterial inoculums. Obtained bacterial suspensions were mixed by using a vortex mixer and dispensed with 10 mL of volume into the 15 mL plastic tubes (Becton Dickinson and Co.).

The OD of bacterial cultures, which indirectly reflects cellular growth and number of bacteria (viable and dead), were measured at the wavelength of 540 nm in 96 well plates (Becton Dickinson and Co.), each well filled with 100  $\mu\text{L}$  sample of bacterial culture taken after 60 minutes of exposure to the RMF ( $f = 1\text{-}50\text{ Hz}$ ,  $B = 22\text{-}34\text{ mT}$ ).

The absorbance readings were made by the ELx800 microplate reader (ELx800 Universal Microplate Reader, Biotek Instruments Inc., USA). The results are shown as raw OD values or expressed as an index of proliferation, and calculated by the formula: Index of proliferation =  $(\text{OD sample} - \text{OD background}) / (\text{OD control} - \text{OD background})$ , where OD is optical density.

The MTT test, which determines the metabolic activities of bacteria, was done as previously described by Wang *et al.* [25], and originally described by Mosmann [26], with slight modifications. Briefly, the bacteria were cultivated and exposed to the RMF ( $f = 1\text{-}50\text{ Hz}$ ,  $B = 22\text{-}34\text{ mT}$ ) for 60 minutes. Upon the exposition, 100  $\mu\text{L}$  of bacterial cultures were transferred to 96 well plate (Becton Dickinson and Co.), where 10  $\mu\text{L}$  of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (3 mg/mL in PBS, Sigma-Aldrich, Germany) was added to the wells, and then, the plates were incubated for 15 minutes at  $37\text{ }^{\circ}\text{C}$ . In the next step, 100  $\mu\text{L}$  of isopropanol (Sigma-Aldrich) was added to each well, and the plates were vigorously shaken. The amount of MTT formazan formed during the incubation was measured with the ELx800 Universal Microplate Reader at a wavelength of 540 nm and reference wavelength of 630 nm. As blanks, the pure TSB (Oxoid) was used. The results are shown as an index of cell metabolic activity calculated by the same formula as described above.

The qualitative assessment of biofilm formation was determined as previously described by Peeters *et al.* [27] with minor modification. Two hundred microliters of bacteria culture after 60 minutes of exposure to the RMF ( $f = 1\text{-}50\text{ Hz}$ ,  $B = 22\text{-}34\text{ mT}$ ) were transferred into 96 well plates (Becton Dickinson and Co.) and incubated for 24 hours at  $37\text{ }^{\circ}\text{C}$ . Next, the medium was gently removed and the microtiter plate wells were washed three times with 200  $\mu\text{L}$  of PBS (0.1 M, pH 7.4, Sigma-Aldrich) and stained with 200  $\mu\text{L}$  of 0.4% crystal violet (Sigma-Aldrich). Excess stain was removed, the wells were air-dried for 15 minutes and the dye bounded to the adherent cells was solubilized with 200  $\mu\text{L}$  of 33% acetic acid (Chempur, Poland). The OD of each solubilized liquid was measured against the OD of blank at wavelength of 620 nm, by using a microplate reader (Infinite 200 PRO NanoQuant, Tecan, Switzerland). The results are shown as an index of biofilm formation as calculated by the same formula de-

scribed above.

The data gathered in this study are presented as the means  $\pm$  standard errors of the means (SEM) calculated from the three repetitions of the experiment. The statistical significance of the differences between exposed and controls were analyzed by Student's *t* test. Statistical differences between results obtained after exposing to the RMF with different frequencies were determined by one-way analysis of variance (ANOVA). All the analyses were considered statistically significant when the P-value was less than 0.05. The statistical analyses were conducted with GraphPad Prism 5.0 (La Jolla, USA) and Statistica 9.0 (StatSoft, Poland).

### 3. Results and Discussion

In this study, attention has been focused on the effects of exposing *E. coli* and *S. aureus* to the RMF according to their growth, cell metabolic activity and ability to form biofilms. By a thorough review on the available literature, the research concerning the influences of the RMF on microorganisms including *E. coli* and *S. aureus* have not yet been performed. Therefore, the present study can be considered a novel and critical discussion of the obtained results is limited due to the lack of adequate literature. The analysis on present results can only be obtained by the studies on other types of MFs (mainly SMFs) and EMFs.

The selected to the current research bacterial species, *S. aureus* and *E. coli* were the most frequently chosen for different experiments on the effect of SMFs on microorganisms (see Table 1). These species are characterized by various shapes (rods, cocci), structures and chemical compositions of the cell wall (Gram-negative and Gram-positive bacteria), which, according to various authors, could be crucial for observing the effects of exposure to MFs [40, 42]. The choice of frequencies analyzed and exposition time are also based on the previously published studies [1, 35]. The control system was used to exclude any exterior influences for the results obtained by the experimental set-ups with the RMF switched on and off and during the incubation in the water bath. The results of growth rate, cell metabolic activity and biofilm formation obtained from control bacterial cultures incubated in the RMF generator with the RMF switched off and in the water bath did not show any statistically significant differences (Tables 2 and 3). Therefore, in order to carry out all experiments with exposed and control samples at the same time and under identical conditions, we decided to use the water bath control throughout the experiment.

Investigations on the present study showed statistically

significant increases in the growth rate (based on the results of OD) of exposed *E. coli* and *S. aureus* along with increased frequency and magnetic induction of the RMF as compared to the water bath controls (see Fig. 4). Based on the proliferation index, calculated as a ratio of OD of exposed sample and water bath control, it was found that proliferation of *S. aureus* after exposing to the RMF of  $f=5$  Hz ( $B=25$  mT),  $f=25$  Hz ( $B=29$  mT) and  $f=50$  Hz ( $B=34$  mT) was significantly higher as compared to the water bath control. Additionally, proliferation of *S. aureus* after exposing to the RMF of  $f=25$  Hz ( $B=29$  mT) and  $f=50$  Hz ( $B=34$  mT) was also significantly higher as compared with RMF of  $f=1$  Hz ( $B=22$  mT). Whereas, the results obtained in *S. aureus* cultures being exposed to the RMF of  $f=1$  Hz ( $B=22$  mT) did not differ significantly from the water bath controls (see Table 2). In contrast, in the *E. coli* cultures, even exposure to the RMF of  $f=1$  Hz ( $B=22$  mT) caused a statistically significant increase in proliferation as compared to the water bath controls. However, the statistical significant differences in proliferation after exposition to the RMF in the range between  $f=1$ -50 Hz ( $B=22$ -34 mT) were not found (see Table 3). It can also be noticed that the higher proliferation rate, as the result of exposition to the RMF, was found in *E. coli* cultures as compared to *S. aureus*.

In order to explore the effects of RMF on the bacterial cell metabolic activity, the MTT assay was performed. The MTT test is based on the reduction of the yellow MTT dye by dehydrogenase in living cells (including most bacteria) to purple MTT formazan which can be solubilized and quantified by spectrophotometric measurements. The results obtained in this test are expressed as an index of cell metabolic activity and presented in Table 2 (*S. aureus*) and Table 3 (*E. coli*). As a result from 1 hour exposition of *E. coli* and *S. aureus* to the RMF of  $f=1$  Hz and  $B=22$  mT, an increase in the cell metabolic activity as compared to the water bath control was found. However, after exposures to the RMF of the  $f=1$  Hz ( $B=22$ ) mT the cell metabolic activity was significantly lower than after exposing to the RMF of  $f=25$  Hz ( $B=29$  mT). In turn, the results of cell metabolic activity obtained after exposure to the RMF of  $f=5$  Hz ( $B=25$  mT),  $f=25$  Hz ( $B=29$  mT) and also  $f=50$  Hz ( $B=34$  mT) did not differ significantly. It can also be noticed that higher cell metabolic activities, as the result of exposition to the RMF, were found in *E. coli* cultures as compared to *S. aureus*.

In the studies of biofilm formation, it was found, that 1 hour of exposure to the RMF of  $f=1$  Hz ( $B=22$ ) mT and  $f=5$  Hz ( $B=25$  mT) did not cause statistical significant changes associated with biofilm formations by *S.*

**Table 1.** Comparison of experimental results for the influence of different magnetic fields on parameters of bacteria tested in the current study.

Bacteria	Characteristic of applied magnetic field	Conclusions of the biological effects	Ref.
<i>E. coli</i>	SMF; 100 mT; exposure time 30, 60, 120, 240 min	the MF exposure for 30 min had no effect on bacterial cell density; the longer MF exposure (120, 240 min) caused growth inhibition	[28]
	SMF; 7-11 Hz; exposure time 220 min	the MF had no effect on DNA damage; the MF had stimulating effect on cell growth	[29]
	SMF; 5, 17 and 50 mT; exposure time 1, 2, 3 and 4 h	the MF negatively influences the growth; the MF caused an increase in dehydrogenase activity and higher intracellular ATP concentrations	[30]
	SMF; 10 mT; 50 Hz; exposure time 1h	the MF had no effect on bacterial morphology	[1]
	SMF; 2 mT; 50 Hz; exposure time 6, 16 h	the MF caused a decrease in the cell growth	[31]
	SMF; 5.2-6.1 T; exposure time 30 h	the MF increased suppression of cell death; the MF stimulated growth	[32]
	SMF; 50 Hz; 1 mT; exposure time 8 min, 2, 5 and 15 h	the MF had no effect on cell viability regardless of exposure time	[33]
	SMF; 5.2-6.1 T; exposure time 12 h	the MF caused a suppressive effect on the cell death rate	[34]
	SMF; 45-3500 mT; exposure time 60 min	the MF caused a decrease in the number of CFU; the MF influenced on cell surface damage	[35]
	SMF; 300 mT; exposure time 50 h	the MF had no effect on the growth; the MF stimulated transposition activity	[36]
	SMF; 2.7-10 mT; 50 Hz; exposure time 0-12 min	the MF affected the bacteria <i>E. coli</i> ; the MF was not bacteriostatic; the MF had no effect on the metabolism of the bacteria; the MF killed a part of bacteria exposed	[37]
	SMF; 5, 10 and 13 T; exposure time 24 h	the MF had no effect on mutation frequency in thymine synthesis genes	[38]
	<i>E. coli</i> ; <i>S. aureus</i>	SMF; 10 mT; 50 Hz; exposure time < 30 min	the MF caused a decrease in the cell viability; the MF caused a decrease in CFU
DCMF; 0.5-4 T; exposure time 30-120 min		the MF had no influence on growth	[21]
SMF; 30-100 mT; exposure time 30 h		the MF had no effect on growth	[22]
homogeneous SMF (400 mT); inhomogeneous SMF; (1.2-47.7 T); exposure time 10, 30, 50, 1440 min		the MF had no effect on growth	[39]
MF; up to 10 mT; 50 Hz; exposure time up to 24 min		the MF caused a decrease in optical densities of bacterial cultures	[40]
SMF (DCMF); 10 T; exposure time 5-60 min		the MF altered the components and structure of nucleic acid, protein, and fatty acids	[37]
<i>S. aureus</i>	SMF; 50-20.000 Gauss	the MF had no effect on growth, when the field strength increased there were a slight growth inhibition	[41]
	EMF + AC MF; 20 Hz; 5 mT, 470 mV/c; exposure time 24 h	the MF improved activity of antibiotics against planktonically grown bacteria	[2]
	EMF; 20 Hz; 5 mT; exposure time 24 h	the MF had no effect on growth on gel-like medium; the MF caused a decrease in growth in fluid medium	[4]

AC – alternating current; ACMF – alternating current magnetic field; DC – direct current; DCMF – direct current magnetic field; EF – electric field; EMF – electromagnetic field; MF – magnetic field; SMF – static magnetic field.

*aureus* (Table 2) and *E. coli* (Table 3). While the RMF of  $f = 25$  Hz ( $B = 29$  mT) and  $f = 50$  Hz ( $B = 34$  mT) significantly enhanced biofilm formations in comparison to the water bath controls. However, statistically significant differences between the results obtained after exposure to

the RMF of  $f = 25$  Hz ( $B = 29$  mT) and  $f = 50$  Hz ( $B = 34$  mT) were not found.

Generally, based on literature review, it can be noticed that the study with the use of SMFs and EMFs did not show any effects or point to inhibitory influences on the

**Table 2.** The effects of 1 h exposure to the RMF with indicated frequencies on the selected parameters of *S. aureus*.

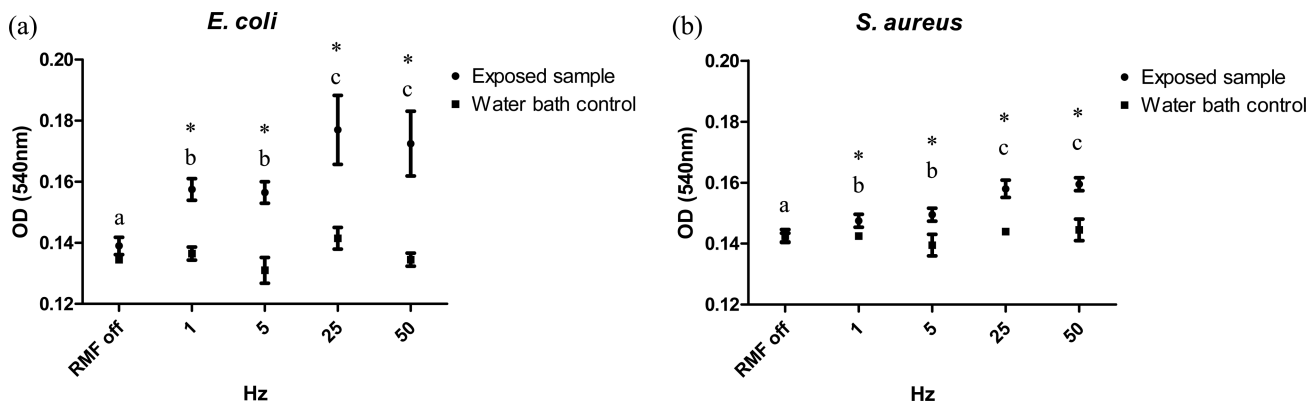
<i>S. aureus</i>	Frequency and magnetic induction of the RMF					WB control
	RMF off	1 Hz 22 mT	5 Hz 25 mT	25 Hz 29 mT	50 Hz 34 mT	
Proliferation	1.003 <sup>a</sup> ± 0.006	1.037 <sup>ab</sup> ± 0.017	1.081 <sup>bc</sup> ± 0.008	1.111 <sup>c</sup> ± 0.012	1.122 <sup>c</sup> ± 0.004	1.00 <sup>a</sup> -
Cell metabolic activity	1.032 <sup>a</sup> ± 0.014	1.169 <sup>b</sup> ± 0.012	1.267 <sup>c</sup> ± 0.005	1.266 <sup>c</sup> ± 0.011	1.263 <sup>c</sup> ± 0.009	1.00 <sup>a</sup> -
Biofilm formation	0.964 <sup>a</sup> ± 0.067	1.005 <sup>a</sup> ± 0.048	1.065 <sup>a</sup> ± 0.033	1.350 <sup>b</sup> ± 0.046	1.578 <sup>b</sup> ± 0.087	1.00 <sup>a</sup> -

Mean values represented with various letters (a/b/c) are significantly different ( $P < 0.05$ ). WB control – water bath control.

**Table 3.** The effects of 1 h exposure to the RMF with indicated frequencies on the selected parameters of *E. coli*.

<i>E. coli</i>	Frequency and magnetic induction of the RMF					WB control
	RMF off	1 Hz 22 mT	5 Hz 25 mT	25 Hz 29 mT	50 Hz 34 mT	
Proliferation	1.009 <sup>a</sup> ± 0.011	1.160 <sup>b</sup> ± 0.004	1.193 <sup>b</sup> ± 0.004	1.268 <sup>b</sup> ± 0.021	1.295 <sup>b</sup> ± 0.026	1.00 <sup>a</sup> -
Cell metabolic activity	1.035 <sup>a</sup> ± 0.015	1.252 <sup>b</sup> ± 0.024	1.459 <sup>c</sup> ± 0.024	1.414 <sup>c</sup> ± 0.013	1.452 <sup>c</sup> ± 0.017	1.00 <sup>a</sup> -
Biofilm formation	1.036 <sup>a</sup> ± 0.086	1.009 <sup>a</sup> ± 0.052	0.972 <sup>a</sup> ± 0.021	1.603 <sup>b</sup> ± 0.092	1.365 <sup>b</sup> ± 0.032	1.00 <sup>a</sup> -

Mean values represented with various letters (a/b/c) are significantly different ( $P < 0.05$ ). WB control – water bath control.



**Fig. 4.** The influence of the RMF with indicated frequencies on the growth of *E. coli* (a) and *S. aureus* (b). Liquid cultures of the bacteria were exposed to the RMF of  $f = 1-50$  Hz ( $B = 22-34$  mT) during 1 hour incubation at 37 °C. Mean values with different letters (a/b/c) are significantly different. \* – statistically significant differences between exposed sample and water bath control ( $P < 0.05$ ).

proliferation, cell metabolic activity and biofilm formation by bacteria as discussed in the current research (see Table 1). The available literature indicates that the observed effects depend mainly on the bacterial species or their cell shapes and conditions of exposition (characteristic of the MF and time). In contrast, investigations by the present study showed that exposures to the RMF had stimulatory influences on the growth, cell metabolic activity and ability to form biofilm by both the *E. coli* and *S. aureus*. It was

also found that the observed effects varied with frequency and magnetic induction of the generated RMF. Such findings agreed with previous observation by Masiuk *et al.* [23] for the influences of RMF on the HL-60 and K-562 cells (eukaryotic cells), where the short-term exposures to the RMF had stimulatory effects on the expressions and intranuclear distributions of nucleolin in these cells, which at least partly confirms the results of our study. Therefore, by taking into consideration the findings from

Masiuk *et al.* [23] and also the results obtained in the current study, it could be expected that the RMF has a stimulating effect on the eukaryotic and prokaryotic cells. Nonetheless, researches on the RMF have only been carried out with *E. coli* and *S. aureus* (this study) and HL-60 and K-562 cells [23], and until now there has been no further evidence confirming or supporting the assumption. It can only be assumed that stimulatory influences of the exposure to RMF for the growth, cell metabolic activity and ability to form biofilm by *E. coli* and *S. aureus* is connected with the effects exerted by the RMF on bio-liquids [43]. As shown by Hunt *et al.* [44], the RMF can cause mixing of the bio-liquids at microlevels. Such microscopic mixing can influence the transfer process between the cell surface and the liquid phase, affecting the cell transport mechanism. Moreover, the RMF can cause relative motions of the medium and the magnetic flux lines [45].

#### 4. Conclusions

In conclusion, the results obtained in this study revealed that the RMF, contrasting with other studies on MFs and EMFs, can stimulate the growth dynamics, cell metabolic activity and the ability to form biofilms by *E. coli* and *S. aureus*. However, higher proliferation rate and cell metabolic activity, as the result of exposition to the RMF, was found in *E. coli* cultures as compared to *S. aureus*. It was also demonstrated that the stimulating effects of the RMF exposition enhanced with its increasing frequencies and magnetic inductions. Based on the obtained results for the stimulation of growth rate and cell metabolic activity, it can be expected that the RMF also influences the pathogenicity of microorganisms.

Further studies are in progress to extend the current state of knowledge for the influence of RMF on microorganisms, including other parameters of exposition and different species of bacteria. Moreover, the current strategy will be adapted to the yeast *Saccharomyces* spp. and *Candida* spp. as the eukaryotic model systems.

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