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ORIGINAL ARTICLE

The indirect influence of the electromagnetic field on the growth of *Bacillus subtilis*

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The change in the number of microorganisms during cultivation on nutrient media prepared using water, which is influenced by an electromagnetic field of low power, is shown. Field-exposed water was used to prepare liquid and solid culture media for the cultivation of Bacillus subtilis bacteria. Field water treatment was carried out at several frequencies in the range of 30-230 MHz. In all cases, when using water exposed to field exposure, significant suppression of the vital activity of bacteria is observed. The discovered effect is explained based on the idea of a change in the hydration size of ions due to field exposure and their activity in membrane processes.
Keywords: field effects, electromagnetic field, indirect biological effect of the HF field, Bacillus subtilis.

Introduction

The relevance of the study of the influence of electromagnetic fields on living objects is associated with a constant increase in the density of these fields and the expansion of their frequency range.

The sensitivity of biological objects to the effects of physical fields is presented in numerous publications (Betsky, 2004, Electromagnetic fields....., 1984). When studying field effects on living organisms, variable (low-frequency) electric fields, magnetic fields, and microwave radiation are most often used. The mechanism of field influence is also a subject of discussion in the literature and has several points of view. Perelmuter V.M. gives various models of the mechanisms of action of electromagnetic waves on biological objects: conformational changes in proteins in the aquatic environment under the influence of electromagnetic waves, increased hydrophobic interactions in the cell membrane, modulation of the activity of the membrane enzyme system, and, as a consequence, changes in cell metabolism. In experiments with microorganisms, V. M. Perelmuter noted both the stimulating and suppressing influence of radiation frequency on the growth of microbial cultures (Perelmuter, 2009). M. N. Astrakhantseva obtained similar results, evaluating the impact of radiation in the range of 53.77-54.57 GHz and 61.0-63.0 GHz on Bacillus subtilis bacteria (Astrakhantseva, 2006). It was found that at some frequencies of exposure, there is a significant activation of bacterial growth, while at others – suppression. The possibility of the indirect influence of the electromagnetic field on biological objects through the water environment is essential for assessing the field effect. Krynitsky P. P. notes that the influence of electromagnetic fields of extremely high frequencies of nonthermal intensity on the growth characteristics of Baker's yeast Saccharomyces cerevisiae 509 and microbial culture Bacillus subtilis D26 in a wide frequency range (from 56.2 to 61.2 GHz) can significantly increase the physiological activity of yeast and bacilli. The author notes the great importance of water in forming the response to electromagnetic influence in microorganisms (Krynitsky, 2017). Kovalenko O. I. experimentally confirms the assumption of a significant role of water in the perception of electromagnetic radiation by biological objects, which is essential in understanding the mechanism of their interaction with the electromagnetic field (Kovalenko, 2007). The paper shows the role of the structured state of the water environment in the management of vital processes that ensure the body's safety. The water environment is represented as an information-phase state, which actually translates the problem of safety and protection of the body in adverse and emergencies into a constructive channel of natural management of the state of the body, which will allow counteracting destructive factors. The specific matrix arrangement of water structural elements inherent in each organism turns out to be the initial information basis on which all life processes develop (Zenin, 1999).

The fact that water changes its physical and chemical properties due to the influence of physical fields is reflected in many articles. Thus, in the works (Khan, 2012, Shipunov, 2010), the results of changes in the reactivity of substances dissolved in water after exposure to a high-frequency field are presented. This allows us to make a reasonable assumption that the change in water properties due to field exposure is the cause of a specific response of biological objects to indirect field exposure and,

based on this, determine the direction of exposure: depression or activation. Considering the increasing density of artificial electromagnetic fields, it can be relevant to study their impact on biological objects as an independent environmental factor.
Considering the authors ' own experience, available technical capabilities, and data on field impact techniques, the frequency range of the RF field of 30-230 MHz was chosen. The literature does not contain data on the research results on the impact on fields of this range. The choice of the frequency range is also because it is the frequency of radio and television broadcasting and, therefore, the probability of such frequencies affecting biological objects is relatively high.
The purpose of the study was to establish the presence of an indirect influence of the electromagnetic field in the frequency range 30 ... 230 MHz on biological objects on the example of Bacillus subtilis VKPM B-12079. In this case, the field acted on water, which was later used to prepare media for cultured bacteria.

Methods

Experimental technique
Preparation of water
In all studies, an axial-type capacitive cell was used, in which the object of influence-deionized water was placed. The cell was a cylindrical glass vessel with a capacity of 75 ml. Along the axis, a glass tube was soldered, in which the central electrode was placed. The second, on the outside, was a copper foil lining (Fig. 1). The electrodes are isolated from the solution by the glass walls of the vessel. The cell was connected to a source of high-frequency (HF) signal, which served as a generator G4-119A, to which the frequency of the RF field was set. For the frequencies of 210 and 230 MHz, a generator of their manufacture was used. The measurements showed that, depending on the frequency, the voltage on the electrodes varied between 11 and 18 V due to the different alignment of the cell and generators at different frequencies. Comparing the distance between the electrodes and the wavelength, it should be noted that the influence factor was the electric component of the electromagnetic field. Under the action of the RF field, the water sample was kept for 90 minutes each time at a specific frequency. This was based on my own numerous earlier studies.

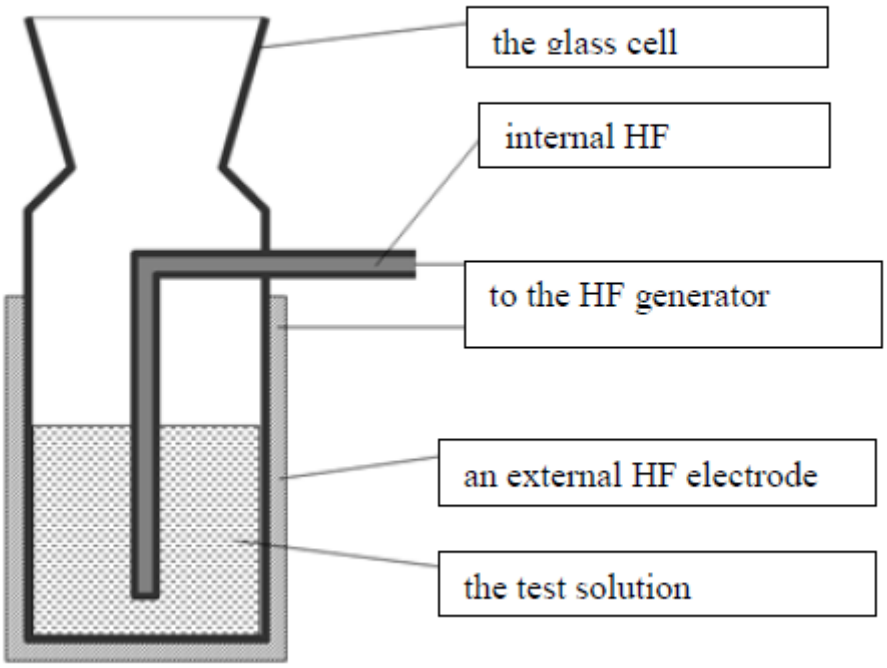

Fig. 1. Design cell

Deionized water, previously aged for a month to stabilize the parameters, was subjected to field exposure. This condition coincides with the data of some authors that the susceptibility of water to field effects depends on the time elapsed after its preparation (Hakobyan, 2005).
After field exposure, the water was used for microbiological experiments no earlier than a week later for more significant development of the effect.
Cultivation conditions
The Bacillus subtilis strain of VKPM B-12079 was used as the initial strain. The studied biological preparation is a powder of lyophilically dried bacterial spores, the number of which is not less than 5×109 CFU/g (CFU colony forming units).
For cultivation, a liquid medium of the following composition (g/L) was used: yeast extract – 5, peptone – 15, sodium chloride – 5, water – up to 1 l (pH 6.8–7.0). Replanting was performed on a dense medium of the following composition (g/L): agar-agar –

18, yeast extract – 5, peptone – 15, sodium chloride – 5, distilled water – up to 1 l (pH 6.8–7.0). Sterilization of media at a temperature of 115 ° C and a pressure of 1.1 bar for 30 minutes.

To determine the quantitative indicators of the intensity of bacterial growth, the standard method of ten-fold dilution was used. A suspension was prepared from the spores: 5 g of spores were added to a 50 ml Erlenmeyer flask containing 50 ml of liquid medium. Incubation was carried out in flasks in the shaker incubator "Innova 44" (NewBrunswick, USA), the number of revolutions was 250 min-1 (eccentricity 5 cm), temperature 37 °C, time 24 hours. Then they were transplanted to a dense nutrient medium, and the cups with the crops were placed in a thermostat at 37 °C for 24 hours. After thermostating, the number of growing colonies was calculated. The number of colonies was calculated visually using the Skan 100 colony counter.

Three groups of experiments were conducted in parallel to study the indirect influence of the RF field on the activity of microorganisms:

1. Vppv-based Nutritional broth, vppv-based nutritional agar.
2. Nutritional broth based on deionized water, nutritional agar based on VPPV.
3. Nutritional broth based on VPPV, nutritional agar based on deionized water.

Control: the nutrient broth and nutrient agar are prepared based on deionized water.

All experiments were performed in three dimensions and statistically processed. Below we presented the values of the number of microorganisms obtained for different uses of water exposed to field action (Table 1).

Results

Table 1. Changes in the number of microorganisms depending on the use of VPPV in nutrient media.

Experiment	The frequency of the field-effect, MHz	Number of colonies, CFU / ml, $n \times 10^{-12}$
1.VPPV-based Nutrient broth, VPPV-based nutrient agar	30	50±10
	90	90±10
	110	120±30
	170	100±30
	210	90±10
	230	180±30
2 Nutritional broth based on deionized water, nutritional agar based on VPPV.	30	50±10
	90	100±10
	110	150±30
	170	90±10
	210	100±20
	230	210±40
3. Nutritional broth based on VPPV, nutritional agar based on deionized water.	30	70±10
	90	50±0
	110	90±10
	170	70±10
	210	90±10
	230	90±20
Control: nutrient broth and nutrient agar are prepared based on deionized water.	0	300± 50

Analysis of the results shown in the table shows a significant decrease in the number of microorganisms during cultivation in water based on VPPV, both in the case of its presence in the broth and in the agarized medium, relative to the control sample. The synergistic effect of simultaneous use of VPPV on broth and agar did not appear. The most substantial effect was observed in using treated water for cooking broth and for all frequencies. The tendency to decrease the efficiency of the field effect with an increase in the frequency of the RF field is quite clear. It should be noted that the results of the first two experiments are similar, and the maximum decrease in numbers is observed when using VPPV in the first stage of cultivation when the spore germination of the bacterium occurs. Probably, VPPV in the broth reduces the possibility of spores germinating, as demonstrated by the third experiment.

Discussions

All nutrient media used in the experiment were subjected to autoclaving sterilization at a temperature of 115 ° C. It would seem that the high temperature should "erase" the result of field exposure. This should also be facilitated by the introduction of various components in the broth and agar. However, this did not happen. Consequently, changes in the properties of cultivation media have deeper causes. We can offer the following explanation. Activation or deactivation of the growth of a living organism, particularly bacteria, is caused by a change in the speed of delivery of necessary elements inside, through the membrane or wall of the spore. The publications of Artemov D. G show that the direct effect of microwave radiation on the cell changes the size of membrane pores (Artemova, 2013). Kazak E. V also points to the direct influence of an alternating magnetic field on ion transport (Kazak, 2003). However, explanations and mechanisms of indirect effects of RF fields or other fields (magnetic, electromagnetic, electric) were not found. Based on the data given above that changes in cell membrane behavior and ion transport characteristics are the most apparent causes of field effects, we assume that the indirect influence of the RF field is related to changes in ion flow through the cell membrane. Antonov V. F. presents classical data on the ion radii of monovalent ions and explains the specificity of cell membrane permeability for potassium ions compared to other ions (Antonov, 1998). The main active factor is not the size of the crystallographic radius of the ion but its size in the hydrate shell.

Moreover, it can be not only the first but also a more distant shell. The fact that external fields, in particular magnetic fields, change the hydration state of ions in solution was shown by V. S. Dukhanin (Dukhanin, 1973). In our work on the dissolution of salts in magnetized water, there were noticeable changes in the value of the thermal effect (Shipunov, 2005), which is undoubtedly due to the hydration component of the thermal effect. The results of thermogravimetric studies of aquacomplexes of several metals are even more convincing concerning changes in the strength of hydration bonds. Thus, in the paper (Chashchevaya, 2014), the use of water subjected to field treatment for growing crystals of cobalt aquacomplexes changes not only the energy of ion dehydration, but also the energy of the ion dehydration the mechanism of this process. This proves that the impact of the RF field is quite capable of changing the structural organization of water and its ability to hydrate processes. Given the sufficient evidence for the existence of molecules with various degrees of association in liquid water, including single molecules (Penkov, 2013), it can be assumed that the monomolecular water – structured water equilibrium is shifted as a result of the RF electromagnetic field. As a result, the average water capacity for hydration processes changes, the hydration radius of ions changes, resulting in a change in the rate of ion diffusion through the membrane channels. This can lead to inhibition of the biological processes noted in the experiment.

Conclusions

Thus, as a result of the study, it was found that artificial coherent electromagnetic fields of low-power radio frequency range have an indirect depressing effect on the development of microorganisms.

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