



KAPITEL 11 / CHAPTER 11 ¹¹
**THE ROLE OF OXYGEN IN THE MODIFICATION PROCESS OF STATE
FUNCTIONALS OF WHEAT SEEDS AND LACTOBACTERIA BY AN
ELECTROMAGNETIC FIELD**

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Introduction.

Currently, one of the important tasks is the development and implementation of environmentally friendly technologies in various sectors of economic activity. In particular, attention is paid to the intensification of the cultivation of agricultural crops, including the improvement of methods of pre-sowing seed treatment in order to increase their germination and productivity. In medicine, the issues of increasing the effectiveness of existing drugs, as well as the creation of alternative means to antibiotics for the treatment and prevention of infectious processes based on improved probiotic strains, remain relevant.

One of the universal factors that can influence the functional performance of biological objects of different classes is low-intensity EMF. There are experimental and clinical evidence of the effectiveness of the effect of specially organized EMFs on different classes of living organisms, including humans [1–4].

Existing models of the EMF interaction with the matter take into account the lines of resonant absorption of gases, the excitation of which triggers a chain of physicochemical processes that ultimately lead to functional changes in the organism.

In this regard, the question arises about the role of some gases on the life processes of biological objects and the possibility of their activation through the effect of low-intensity EMFs on the corresponding absorption lines.

Oxygen is the main biogenic element that is part of the molecules of all the most important substances that provide the structure and function of cells: proteins, nucleic acids, carbohydrates, lipids, as well as many low-molecular compounds. In percentage terms, the oxygen content in each plant or animal is much higher than the content of other elements (on average from 25 to 65 %). It enters living organisms in free and

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bound form (with water) during the process of respiration or biological oxidation, which is a more accurate formulation, since it characterizes the main reaction, and not the «devices» for its implementation. The process of biological oxidation, like all intracellular reactions that occur using nutrients as energy sources, is exergonic, i.e. with the release of energy [5, 6].

Biological oxidation, regardless of whether it occurs in the human body, plant tissues or in a bacterial cell, is a modification of chemical reactions of one of two types: 1) direct oxidation – obtaining energy as a result of the direct oxidation of various substrates by free atmospheric oxygen: molecular hydrogen, oxide carbon and sulfur, using oxidase enzymes, 2) indirect oxidation by dehydrogenation – the process of enzymatic removal of hydrogen from nutrient molecules (or substrates). The enzymes involved in these reactions are called dehydrogenases. When hydrogen is removed, an electron (energy) is released and becomes available for the cell. One of the dehydrogenases, the coenzyme diphosphopyridine nucleotide, contains the vitamin nicotine amide, or niacin, as its main component. Another dehydrogenase, the coenzyme flavin adenine dinucleotide, contains the vitamin riboflavin (vitamin B_2). Hence there is the need for these vitamins in food (or nutrient media) for those organisms that are not able to synthesize these compounds for their needs (humans and some bacteria).

The result of both processes is the same one, since in both cases, due to the transfer of electrons, the energy necessary for the cells is released. Consequently, the basis of all biological processes is electron transfer. The loss of an electron leads to oxidation, the addition of an electron to reduction. Since electrons cannot remain in a free state, every oxidation is accompanied by a reduction.

The presence of oxygen in the atmosphere significantly determined the nature of biological evolution, as a result of which aerobic, i.e., proceeding with the participation of free O_2 , and anaerobic – without the participation of O_2 , types of metabolism were formed. The use of oxygen, which has a high redox potential, as the final electron acceptor in the chain of respiratory enzymes has led to the emergence of a biochemical respiration mechanism that provides energy to aerobic organisms and is energetically



more efficient than the anaerobic type [7].

In the aerobic type of respiration, molecular oxygen acts as a hydrogen acceptor, in the anaerobic one the hydrogen acceptor is not oxygen, but inorganic compounds – nitrate or sulfate.

In a living cell, the hydrogen of the substrate is transferred from the molecule of one hydrogen acceptor to the molecule of another acceptor, as if along a conveyor belt. As a result, hydrogen is transferred to a final acceptor located outside the cell, for example, oxygen (forming H_2O or H_2O_2), sulfur (forming H_2S) or CO_2 (forming CH_4). The nature of the final hydrogen acceptor is determined by the set of enzymes existing in the cell and is a constant and distinctive feature of cells of various types (aerobic or anaerobic) [6].

Aerobic respiration is typical for seeds. Anaerobic (intramolecular) respiration is concomitant at certain stages. Under unfavorable conditions, anaerobic respiration can become the main one. The difference between these two types of respiration is the final product that comes out. Aerobic respiration releases CO_2 and H_2O , and anaerobic respiration releases CO_2 and C_2H_5OH . Since in both cases the reactions occur with the release of carbon dioxide, the intensity of respiration is judged by the ratio of the volume of carbon dioxide released to the absorbed oxygen: CO_2/O_2 – respiratory coefficient. In seeds of cereal crops and seeds of other plants that have a lot of starch, it is close to 1. Under conditions of oxygen starvation, ethyl alcohol accumulates in plant seeds, which can lead to their poisoning with loss of germination [8].

The presence of oxygen is one of the main factors determining seed germination. Thanks to cell respiration, energy is provided too many interconnected processes – the breakdown of nutrients, their transformation, transport and the formation of new substances from them that go towards building cells and organs [9].

The highest intensity of oxygen absorption by seeds occurs in the first stages of germination after they are soaked. The intensity of the hexose monophosphate pathway, and then glycolysis, increases especially strongly. Increasing respiration intensity is accompanied by an increasing the accumulation of adenosine triphosphate (ATP), which is, in turn, a necessary condition for metabolic processes. After 10–



12 hours from the beginning of swelling, mitochondria are rapidly growing and differentiating. Some of the mitochondria that were degraded during seed maturation are reactivated. Subsequently, after 24 hours, mitochondria fission occurs and their number increases sharply. The process of oxidative phosphorylation intensifies and becomes the main source of ATP accumulation. The compounds, formed as a result of decomposition, flow into the axial part of the embryo, where they are partially consumed during respiration, and partially for the construction of substances necessary for the growth of new cells and organs (proteins and nucleic acids, components of cell membranes: cellulose, pectin substances, as well as various lipids, which are part of the membranes). At this germination phase, DNA (deoxyribonucleic acid), RNA (ribonucleic acid) are synthesized, and phytohormones are also formed that regulate the growth of the embryo (embryogenesis). Thus, the germination phase is characterized by the sprout being fed with ready-made organic substances found in the endosperm or cotyledons. When the first green leaves appear and photosynthesis begins, germination ends and the plant enters the next – juvenile phase [10].

The quantity of oxygen required varies greatly among different plants. For example, rice seeds germinate underwater with very little dissolved oxygen. Most agricultural seeds need plenty of air and do not germinate under water [11].

As for microorganisms, they are characterized by aerobic, anaerobic and mixed types of respiration. Thus, many bacteria can exist in aerobic and anaerobic conditions. Such microorganisms are called facultative (optional) anaerobes. For example, staphylococci, *Escherichia coli* and other facultative anaerobes have a full set of respiratory enzymes that ensure their existence in oxygen and oxygen-free environments. Facultative anaerobes have nitrate respiration, when the oxidation of organic compounds produces nitrate (a hydrogen acceptor), which is reduced to molecular nitrogen and ammonia.

There are also obligate (obligatory) anaerobes, which can only exist in strictly anaerobic conditions. Among the pathogenic ones are the causative agents of tetanus, gas gangrene, and botulism. Obligate anaerobes, when oxidizing organic compounds, form sulfate, which is reduced to hydrogen sulfide, therefore obligate respiration is also



called sulfate one [6].

To neutralize toxic forms of oxygen, microorganisms that can exist in its atmosphere have protective mechanisms. In obligate aerobes and facultative anaerobes, the accumulation of the oxygen radical O_2 is prevented by the enzyme superoxide dismutase, which breaks down the oxygen radical into hydrogen peroxide and molecular oxygen. Hydrogen peroxide in these bacteria is decomposed by catalase into water and molecular oxygen. The growth of obligate anaerobes stops in the presence of oxygen. This is due to the fact that life in aerobic conditions leads to the fact that the final product of the oxidation of organic compounds is hydrogen peroxide, and since anaerobes do not produce the enzyme catalase, which breaks down hydrogen peroxide, it accumulates and has a toxic effect on anaerobic bacteria [11].

The universal carrier of chemical energy in processes with releasing energy is ATP. The formation of ATP energy is observed among other things during fermentation. The peculiarity of fermentation is that organic compounds simultaneously serve as electron donors (during their oxidation) and acceptors (during their reduction). Fermentation occurs in the absence of oxygen, under strictly anaerobic conditions. The main compounds of fermentation are carbohydrates. The alcoholic, lactic acid (homofermentative and heterofermentative), acetic acid, butyric acid and other types of fermentation are distinguished depending on the participation of a particular microbe and the final products of carbohydrate breakdown. The release of energy during anaerobic processes is much less; for example, during the fermentation of glucose by yeast, alcohol is formed and only 31,2 kcal. Alcoholic fermentation occurs mainly in yeast. The final products are ethanol and CO_2 . Glucose fermentation occurs under anaerobic conditions. With the access of oxygen, the fermentation process weakens and respiration takes its place.

Lactic acid bacteria are aerotolerant, i.e. they do not use oxygen to obtain energy, but can exist in its atmosphere. Basically, lactobacilli obtain energy through heterofermentative lactic acid fermentation [11].

It is necessary in laboratory studies of this group of bacteria to take into account the gas composition of the incubation atmosphere as one of the important parameters



for the development of microorganisms. It is known that in the biological niches of the human body *in vivo*, the conditions for cultivating bacteria differ significantly when they are extracted *in vitro*. The priority for them is microaerophilic conditions – with a reduced oxygen content. The atmosphere of reduced partial pressure of oxygen and increased carbon dioxide content, to a certain extent, reproduces the living conditions of lactobacilli *in vivo*. To create it, special devices are used – anaerostats, from which air oxygen is removed or replaced with another inert gas. Oxygen-free conditions can also be created by boiling the medium or using chemicals that actively absorb oxygen from the space where the dishes and test tubes with cultures are placed.

Thus, when developing methods for the influence of low-intensity, specially organized EMFs on plant seeds and microorganisms, for the purposeful modification of their functional properties, it is relevant to study the role of individual gases, in particular oxygen, as a separate factor determining the viability of biological objects, as well as a link in the model of perception and transmission of electromagnetic energy.

Purpose of the work: to study the role of oxygen in the process of modifying the functional indicators of the state of wheat seeds of soft varieties and strains of lactobacilli by irradiating them with low-intensity EMF on the resonant absorption lines of oxygen, hydrogen and ozone, with additional enrichment of water with oxygen during its irradiation and subsequent soaking of seeds in it, as well as by creating conditions for cultivating bacteria in an environment with normal and reduced oxygen content.

Materials and methods

The studied objects were soft wheat seeds, as well as standard probiotic strains of lactobacilli: *L. rhamnosus*, *L. acidophilus* and a strain of *L. plantarum* extracted from the intestines of bees.

A measuring stand was prepared to carry out the experimental work.

Generators G4-141 ($f = 37,5\text{--}53,57$ GHz) and G4-142 ($f = 53,57\text{--}78,33$ GHz) were used as EMF sources in narrow frequency bands of the EHF range, the radiation power of which did not exceed 5 mW. The waveguide outputs of the generators were loaded with horn antennas with apertures in $6,0 \times 5,0$ cm² and $8,5 \times 6,5$ cm². Irradiation



was carried out in the near zone of the antenna, at a distance of 5–7 cm from the opened horn. The power flux density was 0,1 mW/cm² with uneven irradiation at the location of the objects no more than 3dB.

The electromagnetic effect on wheat seeds was carried out indirectly, by soaking dry seeds in water pre-irradiated with EMF and aerating it with oxygen. To carry out the electromagnetic effect, frequencies in the EHF range were selected corresponding to the resonant absorption lines of atmospheric gases: 61,0 GHz for oxygen, 58,0 GHz for hydrogen, 42,2 GHz for ozone. The effect time was 5, 10, 30 and 60 minutes.

To enrich water with oxygen, a device was made, consisting of a container for carrying out chemical reactions and a system of tubes that ensure the delivery and uniform distribution of gas in water. Oxygen was produced in a laboratory manner as a result of the reaction of a solution of hydrogen peroxide with potassium permanganate. Aeration of water with oxygen was carried out during its irradiation with EMF for 15 minutes.

Seed germination was carried out in Petri dishes of 50 pieces each, which were placed in a specially made thermostat and kept at a temperature of 23 ± 1 °C.

The main indicators of the biological activity of plant seeds were assessed – germination energy (E_g), average length of roots (L_{rmid}) and sprouts (L_{smid}) for 72 hours of observations carried out in accordance with state standards [12, 13].

Irradiation of lactobacilli strains was carried out in the frequency ranges of 42,2 and 61,0 GHz for 3 hours. Cultivation of bacteria was carried out in aerobic (at normal oxygen content *in vitro* conditions ~20 %) and microaerophilic conditions (at low oxygen content, simulating *in vivo* conditions).

Microaerophilic conditions for the cultivation of bacteria were created in microanaerostats using gas-generating packages Generator GENboxmicroaer (bioMerieux, France) or a gas mixture manufactured in a factory and consisting of O_2 – 5 %, CO_2 – 10 % and N_2 – 85 %.

The following features were assessed:

1) The quantity of glucose consumed by strains of *L. rhamnosus*, *L. acidophilus* and *L. plantarum* from the nutrient medium using the glucose oxidase method. This



indicator determines the potential ability of cells to actively develop. It is used to judge the metabolic activity of microorganisms, as well as the activation or inhibition of catabolic processes [14].

2) Fractional composition of *L. plantarum* exometabolites, which was determined by gel filtration chromatography (exclusive, gel permeation or sieve chromatography) [15]. This method is based on the separation of substance molecules by size due to their different penetrating abilities into the pores of the carrier.

Exometabolites are metabolic products released by microorganisms into the environment. They play an important role in inter- and intrapopulation communications. Exometabolites include high and low molecular weight peptides, the molecules of which are built from two or more amino acid residues. Antimicrobial peptides that can kill microbial cells are distinguished from the total number – these are bacteriocins or plantaricins (due to *L. plantarum*). Plantaricins are cationic thermostable peptides with a molecular weight of less than 10 kDa (most often in the range of 2–6 kDa) [16, 17].

The results obtained were processed in accordance with the rules of variation statistics [18] using standard programs.

Research results

In the first experiment the changes in the functional parameters of soft wheat seeds, which were soaked in water pre-irradiated with EMF and additionally saturated with oxygen, were studied. The measurement results are presented in table. 1.

As a result of soaking wheat seeds in water pre-enriched with oxygen without EMF irradiation, stimulation of all considered functional indicators was observed. At the same time, a significant increasing 1,2 times ($p < 0,05$) was found only when measuring the average root length. These data are taken as control ones for assessing the indirect water effect of EMF on seeds when it is additionally enriched with oxygen («oxygen control») (Fig. 1, 2).



Table1 - The influence of low-intensity EMFs on the functional parameters of soft wheat seeds, carried out indirectly through irradiated water enriched with oxygen (* – $p < 0,05$; ** – $p < 0,001$)

Exposure mode			Functional indices		
O_2	EMF				
$T, \text{ min}$	$f, \text{ GHz}$	$T, \text{ min}$	$E_g, \%$	$L_{r \text{ mid}}, \text{ mm}$	$L_{s \text{ mid}}, \text{ mm}$
Control			90,0	19,9	15,6
15	oxygen control		92,1	24,0*	16,3
	61,0	5	90,4	28,4*	18,2*
		10	94,0	31,9**	19,3**
		30	93,3	21,7	15,8
		60	92,8	24,9	18,0
	58,0	5	91,3	26,5	16,8
		10	91,7	25,1	16,0
		30	91,7	24,0	16,6
		60	91,0	20,7*	15,0
	Control			91,4	18,4
15	oxygen control		92,1	24,0*	16,3
	42,2	5	90,7	13,7**	13,3*
		10	92,0	14,6**	14,3
		30	90,7	17,8**	15,7
		60	92,0	15,6**	14,5

Author's development

Irradiation of wheat seeds in the range of 61,0 GHz through water enriched with oxygen, led to additional stimulation relative to the «oxygen control» of the $L_{r \text{ mid}}$ and $L_{s \text{ mid}}$ indicators by an average of 1,2 times with short-term effect of 5 and 10 minutes. At the same time, a 10-minute effect turned out to be more effective ($p < 0,001$). Increasing the effect time of EMF to 30 and 60 minutes did not give significant changes. At 30 minutes of signal exposure a tendency was observed towards inhibition of the average length of roots and sprouts (see Fig. 2).

When wheat seeds were soaked in water irradiated in the frequency range of 58,0 GHz, there was a tendency for E_g decreasing relatively to the «oxygen control» under all effect modes. A reliable result was obtained with signal exposure for 1 hour – inhibition of the average root length by 1,2 times ($p < 0,05$).

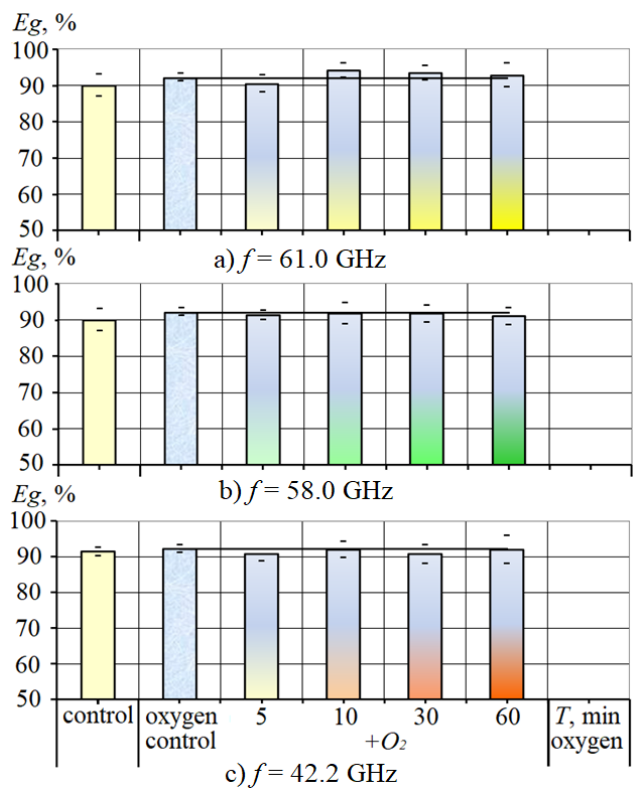


Figure 1 - Changes in germination energy during indirect EMF irradiation of soft wheat seeds through oxygen-enriched water

Author's development

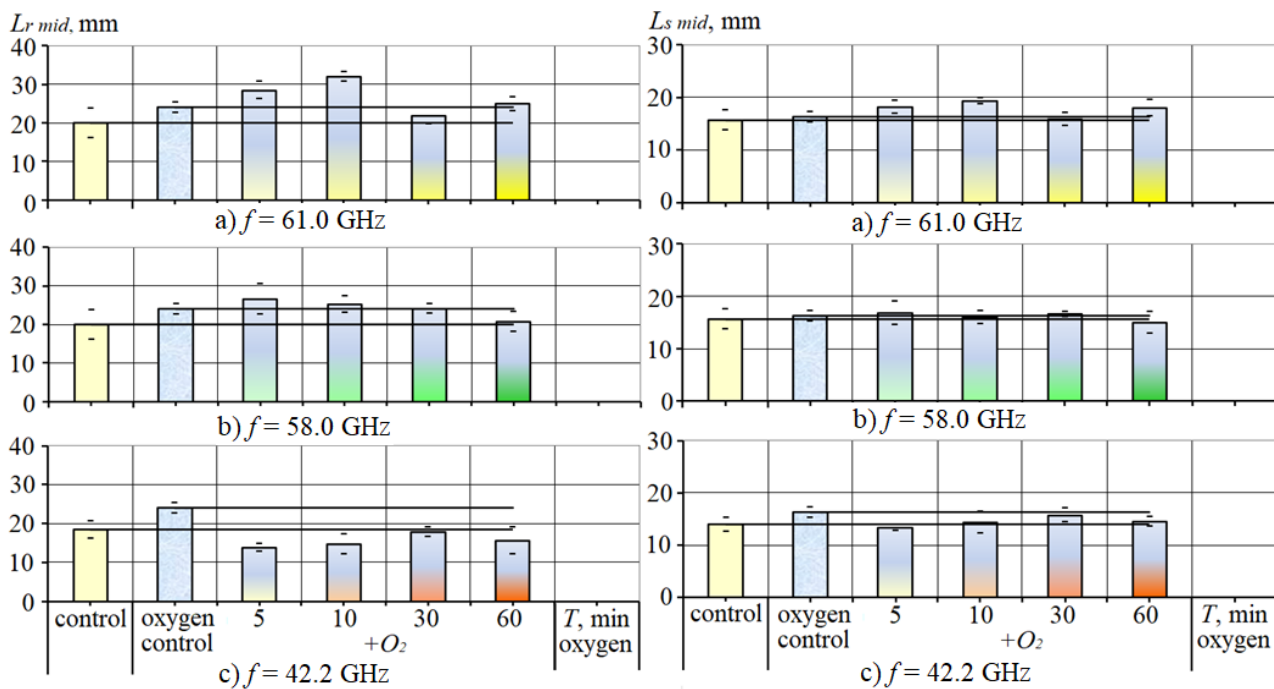


Figure 2 - Changing the average length of roots and sprouts during indirect EMF irradiation of soft wheat seeds through oxygen-enriched water

Author's development



Irradiation of water in the 42,2 GHz frequency band while enriching it with oxygen and subsequent soaking of seeds in it led to inhibition of all the studied functional parameters of the seeds, regardless of the time of signal effect. Thus, the average length of sprouts decreased on average by 1,6 times ($p < 0,001$). Irradiation for 5 minutes turned out to be the most effective with the average length of roots decreasing by 1,2 ($p < 0,05$), and sprouts by 1,8 times ($p < 0,001$) (see Fig. 1, 2).

Previously, indirect EMF irradiation of soft wheat seeds through water was carried out under similar effect modes, but without additional enrichment of water with oxygen [20].

Comparing the published data [19] with the results presented in Table 1, we can note the general positive role of oxygen on seed germination. When oxygen is activated in the frequency range of 61,0 GHz, additional stimulation of the state functionals of wheat seeds is observed with short-term irradiation for 5 and 10 minutes. Increasing the effect time is less effective.

When water enriched with oxygen is irradiated in the hydrogen absorption band of 58,0 GHz, a decreasing the efficiency of electromagnetic influence is observed. Tendencies of inhibition of the functional state of wheat seeds intensify with increasing signal exposure.

Irradiation of water enriched with oxygen at the ozone absorption line of 42,2 GHz leads to additional inhibition of the functional state of wheat seeds. The value of inhibition varies depending on the time of electromagnetic effect.

In the next experiment, we studied the influence of EMF in the frequency ranges of 42,2 and 61,0 GHz on the functional parameters of microorganisms, the cultivation of which was carried out under different conditions: at high (aerobic) and low (microaerophilic) oxygen content.

The studied microorganisms were strains of lactobacilli: *L. plantarum*, *L. rhamnosus*, *L. acidophilus*. The condition of the bacteria was assessed by changes in their enzymatic activity, namely, by the quantity of glucose consumed from the nutrient medium.

For most organisms glucose is a universal source of energy and also serves as an



indicator of the potential rate of development of subpopulations. Therefore, determining the quantity and rate of its utilization is very important in the selective search for strains producing biologically active substances and assessing their stability with subsequent use in biotechnology.

As a result of preliminary studies of the influence of the cultivation atmosphere on the quantity of glucose consumed by lactobacilli, it was defined that microaerophilic conditions contributed to its increase (Table 2).

Table 2 - The quantity of glucose consumed by lactobacilli strains under different cultivation conditions (* – $p < 0,05$)

Strains <i>Lactobacillus spp.</i>	Cultivation conditions	
	aerobic	microaerophilic
<i>L. plantarum</i>	13,2±0,16*	14,7±0,06*
<i>L. rhamnosus</i>	11,4±0,07*	12,6±0,07*
<i>L. acidophilus</i>	12,5±0,07*	14,5±0,06*

Author's development

In all studied strains of *Lactobacillus spp.* the quantity of glucose consumed increased on average by 12,6 % ($p < 0,05$), it may be associated with a changing the rate of metabolic processes under conditions of oxygen deficiency.

In the next part of the experiment lactobacilli *L. plantarum*, *L. rhamnosus* and *L. acidophilus* were subjected to electromagnetic irradiation in the frequency ranges of 42,2 and 61,0 GHz for 3 hours. The results of measuring the quantity of glucose consumed by lactobacilli cultivated under aerobic conditions are presented in table. 3.

Table 3 - The influence of low-intensity EMFs on the quantity of glucose consumed by lactobacilli strains when they are cultivated under aerobic conditions (* – $p < 0,05$)

Strains <i>Lactobacillus spp.</i>	Cultivation conditions		
	Control	42,2 GHz	61,0 GHz
<i>L. plantarum</i>	13,9±0,16	13,0±0,05	14,2±0,06
<i>L. rhamnosus</i>	12,0±0,07	15,6±0,1*	16,2±0,09*
<i>L. acidophilus</i>	12,5±0,07	14,0±0,07*	14,5±0,08*

Author's development



The data obtained indicate increasing the quantity of glucose consumed in most strains after irradiation. Significant changes are observed in strains of *L. rhamnosus* and *L. acidophilus*. When effected to the 42,2 GHz range, the quantity of glucose consumed increased by 30 and 12 % ($p < 0,05$), respectively. When irradiated in the range of 61,0 GHz, increasing was 35 and 20 % ($p < 0,05$) (Fig. 3).

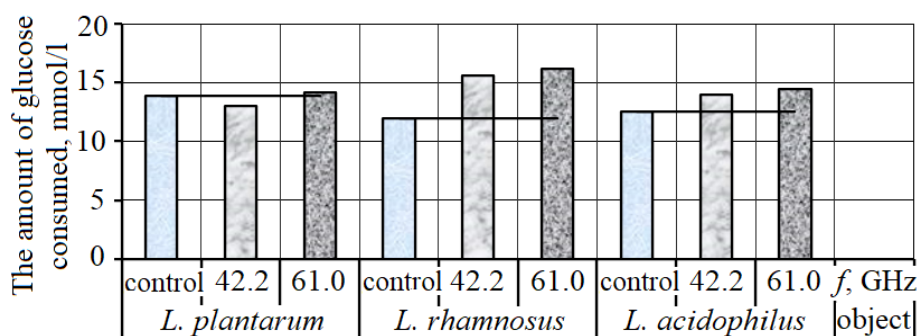


Figure 3 - Changes in the average quantity of glucose consumed by lactobacilli: *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, cultivated under aerobic conditions, after EMF effect

Author's development

Thus, one can see a frequency dependence of the effectiveness of electromagnetic influence on the functional state of lactobacilli strains cultivated under aerobic conditions. The greatest stimulation of consumed glucose was observed for irradiation in the 61,0 GHz frequency band in the *L. rhamnosus* strain.

The positive effect of lactobacilli on the human organism is due not only to their ability to colonize, but also to their high antagonistic abilities in the biocenosis with pathogens, and they, in turn, depend on the production of antimicrobial compounds of protein origin (plantaricins). The activity and level of their production is determined by the conditions in which lactobacilli are found.

A study of the fractional composition of *L. plantarum* exometabolites under different conditions of the gas composition of the cultivation atmosphere was carried out (Table 4).



Table 4 - Influence of cultivation conditions and low-intensity EMF on the share of peptide fractions (%) *L. plantarum*

Fractions	Molecular weight, Da ($M \pm m$)	Aerobic conditions			Microaerophilic conditions		
		control	f , GHz		Control	f , GHz	
			42,2	61,0		42,2	61,0
0	≥ 12000	5,2	7,7	2,9	4,7	7,5	5,2
Aa	7300 \pm 370	–	–	27,3	–	–	–
A	5580 \pm 87	51,3	51,5	2,6	58,8	59,4	59,5
B	3710 \pm 125	8,8	8,4	6,6	7,3	6,2	3,7
C	3020 \pm 29	10,9	9,2	–	7,9	8,4	12,3
D	2330 \pm 63	–	1,8	5,0	2,8	–	–
E	1780 \pm 36	3,4	2,7	2,9	2,7	2,6	3,4
F	1460 \pm 16	5,5	5,1	4,8	4,7	5,0	5,4
G	1220 \pm 25	4,2	3,2	14,2	3,2	3,1	3,2
H	870 \pm 20	6,5	4,9	14,3	4,4	4,3	4,0
I	550 \pm 4	4,2	5,5	9,4	3,5	3,5	3,3

Author's development

In the studied exometabolites of the *L. plantarum* strain the low molecular weight peptides were extracted – protein components of the nutrient medium (fractions I – E), as well as peptides with a relatively high molecular weight: 1195–7670 Da – presumably plantaricins (molecular weight 2–6 kDa, fractions D–A). Peptides with molecular weight ≥ 12 kDa were not selected.

Under aerobic cultivation conditions 9 fractions of microbial peptides with a molecular weight from 546 to 5667 Da were extracted from exometabolites of the *L. plantarum* strain. Share of peptide fractions with molecular weight up to 2 kDa was 23,8 %, from 2 to 6 kDa – 71 %, ≥ 12 kDa – 5,2 % of the total quantity.

When cultivated under microaerophilic conditions, 10 fractions of microbial peptides were extracted. Fraction D appeared, which was absent under aerobic cultivation conditions. At the same time, the share of low molecular weight peptides with molecular weight up to 2 kDa significantly decreased by 1,3 times ($p < 0,05$) and amounted to 18,5 %, and the share of peptides with molecular weight 2–6 kDa increased to 76,8 %.

Thus, it was experimentally shown that microaerophilic cultivation conditions at



a reduced partial pressure of oxygen contribute to the stimulation of the production of peptides with molecular weight 2–6 kDa by the *L. plantarum* strain (presumably plantaricins – antimicrobial compounds of protein origin) and a decreasing the share of low molecular weight proteins in the nutrient medium, which indirectly indicates increasing the antagonistic activity of the strain and, accordingly, an improvement in its absorption of nutrients from the medium. These data were taken as reference ones for further assessment of the effectiveness of electromagnetic influence.

The results of studying the fractional composition of exometabolites of the *L. plantarum* strain after its irradiation with EMF under different cultivation conditions are presented in Table. 4.

After irradiation of the *L. plantarum* strain in the frequency band 42,2 GHz, 10 fractions of microbial peptides with a molecular weight from 546 to 5667 Da were extracted from exometabolites obtained under aerobic cultivation conditions. In this case, fraction D with share of 1,8 % missing in the control was extracted. When comparing the obtained data with the reference ones, the share of low-molecular and high-molecular fractions on average decreased slightly.

When irradiated in the range of 61,0 GHz under aerobic cultivation conditions, 10 fractions of microbial peptides with a molecular weight from 546 to 7670 Da were also extracted. The share of fractions with a molecular weight up to 2 kDa was 45,6 %, from 2 to 6 kDa – 24,2 %, ≥ 12 kDa – 2,9 %. At the same time, fraction Aa was extracted from exometabolites of *L. plantarum* – 27,3 % of the total quantity of microbial peptides, which was absent in other studied samples. Fraction C was not extracted, whereas in the control the share of these peptides was 10,9 %. When comparing these data with the reference ones, it is clear that the share of low molecular weight peptides with a molecular weight of up to 2 kDa significantly increased by 1,9 times ($p < 0,001$), and the share of putative plantaricins (2–6 kDa), on the contrary, decreased by 2,9 times ($p < 0,001$), and even taking into account the Aa fraction, the decreasing was 1,4 times (Fig. 4, a).

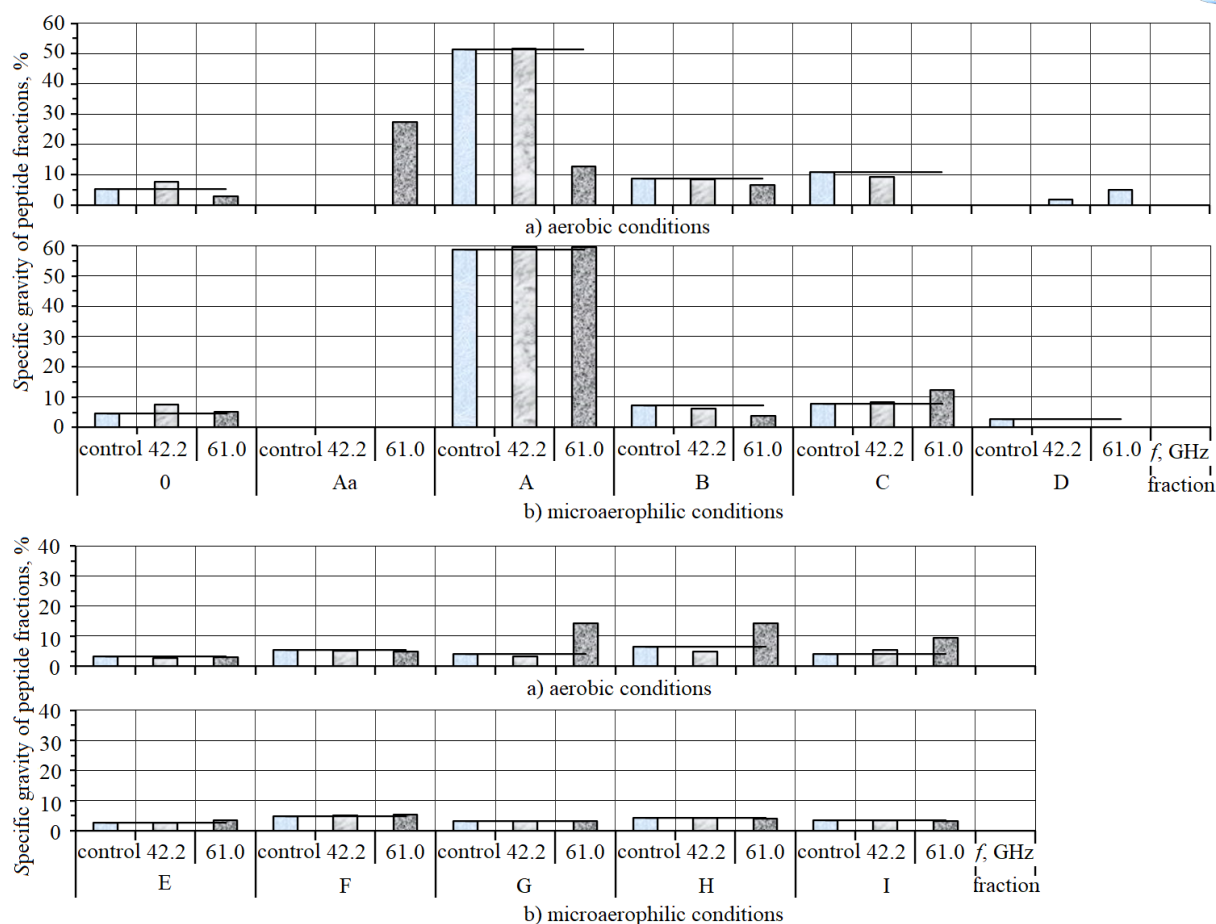


Figure 4 - Changes in the share of peptide fractions of *L. plantarum* cultivated under aerobic conditions after EMF effect

Author's development

Under microaerophilic cultivation conditions, 9 fractions of microbial peptides with a molecular weight from 546 to 5667 Da were extracted from exometabolites of *L. plantarum* obtained after its irradiation with EMF in the frequency ranges of 42,2 and 61,0 GHz. In this case, fraction D was absent, which in the reference was 2,8 % (Table 4). Effect in the range of 42,2 GHz did not significantly affect the changing the fractional composition of exometabolites of the *L. plantarum* strain.

Irradiation of *L. plantarum* in the range of 61,0 GHz when cultivated in microaerophilic conditions contributed to the stimulation of the formation of peptides with a molecular weight from 1 to 2 kDa by 1,1–1,2 times ($p < 0,05$), with a molecular weight of 2991–3049 Da – by 1,55 times ($p < 0,05$) and inhibition with a molecular weight of 3585–3835 Da by 1,97 times ($p < 0,05$) (Fig. 4, b).



Thus, it has been obtained that the activation of oxygen by EMF in the EHF range has a diverse effect on the functional indicators of aerotolerant lactobacilli, for which microaerophilic conditions are a priority. In this case, stimulation of some functions is observed, for example, glucose uptake, and, consequently, increasing the colonization ability of the population, and inhibition of others, for example, antimicrobial abilities, occurs, which is further aggravated under aerobic cultivation conditions.

Conclusions.

1. The possibility of targeted modification of the functional parameters of soft wheat seeds and lactobacilli through their irradiation with low-intensity EMF in the EHF range on the resonant absorption lines of oxygen, hydrogen and ozone has been defined.

2. The role of oxygen in the life activity of biological objects of different classes is shown: wheat seeds and lactobacilli. The possibility of stimulating the germination process of soft wheat seeds by soaking them in water previously enriched with oxygen has been found. For lactobacilli, the priority of microaerophilic cultivation conditions is shown with a reduced partial pressure of oxygen and an increased content of carbon dioxide, which imitates the conditions for the presence of bacteria *in vivo* (inside the body). Thus, in strains of lactobacilli under microaerophilic conditions the following are observed:

- stimulation of enzymatic activity, namely increasing the quantity of glucose consumed, which indicates increasing growth rate of subpopulations;
- stimulation of the production of peptides, presumably plantaricins (with a molecular weight of 2–6 kDa) and a decreasing the share of low molecular weight proteins in the nutrient medium, which, accordingly, indicates increasing the antagonistic activity of probiotic strains in the biocenosis with pathogenic bacteria and an improving the absorption of nutrients from the environment.

3. The dependence of the effectiveness of low-intensity irradiation in the EHF range on frequency has been defined. The possibility of stimulating seed germination, when irradiated indirectly through oxygen-enriched water at the oxygen resonance



absorption line of 61,0 GHz with a short signal exposure of 5 and 10 minutes, has been shown. Increasing the effect time does not contribute to stimulation, it may be caused by the formation of active and reactive oxygen forms under the influence of EMF at a resonant frequency. Irradiation in frequency ranges not associated with oxygen resonance is less effective. Thus, when effected to hydrogen absorption lines at 58,0 GHz, tendencies toward suppression are observed. Irradiation at the ozone resonance frequency of 42,2 GHz leads to a significant suppression of the studied parameters. Thus, the relevance of water-dissipative and gas models of interaction of EMF with matter has been confirmed.

4. A non-monotonic dependence of the biological response on the time of signal effect, which is individual for each frequency range, is shown. The most significant modes of effect have been identified.

5. It has been obtained that when irradiated with low-intensity EMFs in the EHF range on the cultures of *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, a dispersion dependence on frequency is observed. The magnitude of the influence depends on the cultivation conditions. Thus, effect in the frequency range of 42,2 and 61,0 GHz contributed to increasing the quantity of glucose consumption by lactobacilli when cultivated under aerobic conditions. At the same time, the 61,0 GHz frequency range turned out to be more efficient.

6. When assessing the share of peptide fractions of exometabolites of the *L. plantarum* strain after irradiation in the frequency range of 61,0 GHz under aerobic cultivation conditions, inhibition of the production of high molecular weight peptide fractions (presumably plantarcins – antimicrobial compounds of protein origin) and increasing low molecular weight proteins of the nutrient medium were observed. Under microaerophilic cultivation conditions, changes after irradiation turned out to be insignificant. This confirms the unfavorable effect of oxygen on lactobacilli when they are *in vitro*. Effect of EMF in the frequency range of 42,2 GHz under aerobic and microaerophilic cultivation conditions also had a depressing result, which manifested itself to a lesser extent.

The results obtained open up the prospect of using electromagnetic technologies



in agriculture when preparing seeds for sowing and in medicine, in particular in the development of new generation drugs based on lactobacilli with increased colonization and antagonistic properties towards pathogens.