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Microwave Radiation Effects on the Process of *Escherichia coli* Cultivation

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Modern biotechnological industries have been attempting to improve the efficiency of bacterial strain cultivation. Millimeter wave electromagnetic radiation can have a varied influence on *E. coli* cultivation processes. The results of the study revealed that when a microwave radiation of low intensity is applied to positively adjust the conditions for the accumulation of bacterial culture biomass, a significant role is played not only by radiation parameters, but also by concomitant biological factors, which influence the reproducibility of the cultivation process and help obtain a useful biotechnological effect. The authors suggest a model that can be used to study the molecular mechanisms underlying the changes in the buildup of *E. coli* biomass under the influence of electromagnetic radiation.

Keywords: **Pharmaceutical biotechnology, NMR-relaxometry, epitaxy, biopolymers, microwave, living objects**

Introduction

Microwave radiation is divided into three ranges: decimeter, centimeter (SNF) and millimeter (EHF). The paper uses EHF-wave (10÷1 mm) of low intensity (energy flux density less than 10 mW/cm^2) corresponding to the frequency band 30 ÷ 300 GHz and photon energy 0.124 ÷ 1.24 μeV. This range is the boundary between radio waves and optical radiation and until recently was used insufficiently in comparison with SNF range. The specificity of EHF-band radio waves is their absorption by water, water-containing systems including living organisms, as well as atmospheric oxygen. The advantages of the EHF band are greater information capacity, higher resolution and smaller instrument dimensions. Therefore, during the past few years, this type of radiation has been used in mapping, high-speed radio relay communication, radio astronomy, meteorological radars, airport scanners, medicine, in road cameras, in special radars, for air traffic control at short distances, in telecommunications, in weapons (radars for fire control systems and anti-aircraft artillery systems, non-lethal weapons "Active Denial System"). Due to the increasing spread of EHF radiation, studies of the effects of low-intensity microwave radiation on living organisms are becoming increasingly relevant.

Strains of *Escherichia coli* are the most common microorganisms among those used in biotechnological and biopharmaceutical industries. There are a lot of works focused on the optimization of their cultivation process. In [1], we discussed the primary stages in the mechanism of low intensity electromagnetic radiation influence on the strain of *E. coli K12 TG1 with clone luxCDABE genes P. luminescens ZM* 1 (test system "Ecolum-8" for determining the acute chemical toxicity of drinking, surface fresh, ground, waste and treated wastewater, precipitation in laboratory and field conditions), whose cells are able to express luciferase. How-

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Table 1. Main components of the outer membrane.

Component	Number of molecules per cell ($\times 10^5$)	Amount of fatty acid residue per cell ($\times 10^5$)	Area occupied (μm^2)
Lipopolysacharides	34.6	242	4.9
Porines + OmpA	2	-	1.8
Lipoproteins	7	21	0.5
Phospholipids	87	174	4.1

ever, it is still difficult to explain such phenomena as change in the current of H^+ and K^+ ions through a cell membrane [2, 3], luciferase fluorescence and change of the culture growth characteristics. These are likely to be based on a fundamental mechanism which has not been researched yet.

While cells of the culture function, the unused protons are exported to the cell surface [4–6], where they are distributed and bound with the components of protecting lipopolysaccharide (LPS) and basic amino acids of the external cell wall of gram-negative bacteria [7]. LPS constitute the major component of the outer membrane and occupy a superior area of 4.9 μ^2 vs 4.1 μ^2 of phospholipids (Table 1) [8, 9]. LPS consist of a hydrophilic polysaccharide residue which is covalently attached to a hydrophobic lipidic residue. Hydrophilic polysaccharide chains of LPS are equipped by negatively charged functional groups and attract electron densities of the surrounding water molecules, which results in the formation of hydration shells.

It has been found [1] that the structural dynamic condition of the system "medium-culture" is related to its biological characteristics. It has been shown [3, 10] that on hydrophilic surfaces there can form layers of bound water, whose thickness can change under the influence of electromagnetic radiation of low intensity [11–14].

Similar processes can occur in a culture medium located near the surface of a cell [1]. Electromagnetic radiation is likely to have a marked influence on the medium-culture system only under certain conditions. We suppose that the prolongation of the log phase and the increase of biomass buildup can occur due to hydration shell thickening on the membrane surfaces when microwave radiation is applied to the culture passing into the stationary growth phase. This effect may possibly be caused by the fact that when the pH factor is changed cell products, limiting growth of the culture, would encounter difficulties while passing through hydration shell barriers. This can lead both to the distortion of the signaling between the culture cells and to the change in the proton concentration gradient level, which contributes to the lower effect of limiting factors on the culture growth as well as has a direct impact on the enzyme-catalyst cell systems.

The purpose of the given research is either to confirm or to discredit the above suggestion and to establish the applicable value of the proposed approach with used industrial strain *E. coli* LEGM-18 VKPM V-6240.

Materials and Methods

The subject of the study was the industrial strain *E. coli* LEGM-18 VKPM V-6240 (patent RU 2065875). The strain was obtained it the Institute of Ecology and Genetics of Microorganisms of the Ural Branch of the Russian Academy of Sciences and deposited in the all-Russian collection of industrial microorganisms of the State Research Institute of Genetics and Selection of Industrial Microorganisms ("Genetika"). To culture the strain, the following media were used: resuspendable "Harvest broth for microorganism cultivation, dried fish meal hydrolysate" (FHM-broth, certified pharmacopeial description 42-3378-97) and meat-peptone broth. Cultures of strains were cultivated at 37℃.

Experimental apparatus for samples irradiation

The scheme of experimental device is shown in Fig. 1 [1]. Microwave generator 1 produce sinusoidal oscillations at the frequency of 37.04 GHz with the capacity of 20 mW. Ferrite insulator 2 is used to protect the generator from reflected waves. The microwave transformer 3 coordinates the wave resistances of the antenna-waveguide path and the free space. The emitting antenna with dielectric lens 5 forms weakly divergent beam of microwave radiation. Microwave beam with power flow of 0.4 mW/cm^2 irradiates the test tube with bacterial culture 5. To prevent the formation of standing waves, the tubes were placed in the far radiation zone of antenna 4. To protect personnel from radiation absorber 6 was mounted.

To determine the microwave power flow, special study was done. The level of microwave power at the output of the generator was measured with power meter. The

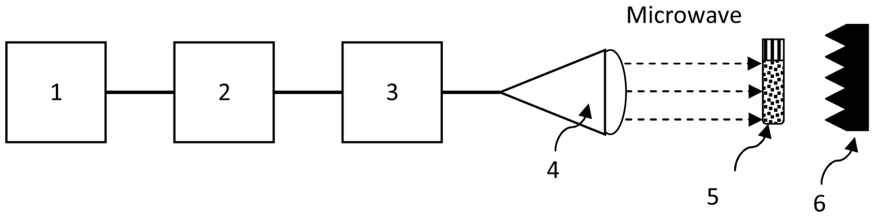

Fig. 1. Scheme of experimental device. Microwave generator 1, ferrite insulator 2, microwave transformer 3, antenna 4, the test tube with bacterial culture 5, absorber 6.

power losses in the microwave path were estimated by the method of comparison with the losses in calibrated attenuator. Then measuring antenna was placed in the plane of the test tube. By moving the antenna in horizontal and vertical directions and measuring the microwave power the radiation pattern of emitting antenna was obtained. This allowed us to calculate the power flow of microwave radiation. For current control, an opening was made in the absorber in which receiving antenna with detector was placed. The signal at the detector output confirmed the presence of microwave radiation.

NMR-relaxometry. NMR-relaxometry was performed with the use of the relaxometer "mq10 NMRAnalyzer" (Bruker, USA). It consists of: RF generator at frequency of 10 MHz, pulse programmer for magnetic system and the measuring sensor of the protons. The temperature of magnet stabilization is 40 degrees. Therefore, the samples were thermostated for 30 min at this temperature.

Turbidimetry was performed with the help of the optical device "Densi-La-Meter II" (Erba Rus, Russia). All the measurements were carried out at external temperature of 23 ± 2℃. For converting units McF in the concentration of cells/ ml the formula 1 McF = 3×10^8 cells/ml was used. Deviations from the value of calibration were in the range: 0.3 ÷ 3.0 McF ± 0.1 McF; 3.0 ÷ 10.0 McF ± 0.3 McF. Before each measurement, the cell suspension was thoroughly mixed in circular motion without bubble formation, after which about ½ min the sample was settled to release residual air bubbles which could distort the result.

pH-metry to determine integral acidity of the bacterial suspension was performed with the use of the ionometer "pX-150" (Antex, Belarus). The device has built-in temperature sensor. The temperature of samples was measured during microwave processing. Temperature deviations greater than the error value of device ± 1.0℃ was not detected.

Statistical analysis

All experiments were carried out at least of three times. During statistical processing, the arithmetic mean, standard deviation and confidence intervals were determined. The significance of differences was determined using student's *t*-test. Differences were considered significant at $p < 0.05$. The results were analyzed using MS office Excel 2016. The results in the tables are presented as an arithmetic mean and its error (M ± m).

Results and Discussion

Growth characteristics of the culture

To determine the transition of the culture from its log-phase into the stationary one, some curves were plotted representing the buildup of biomass (Fig. 2(A)) and specific growth rates (Fig. 2(B)). They were used to determine the major phases of culture *E. coli* LEGM-18 growth. It was found that the specific growth rate between 7 and 13 h of cultivation reduces when the initial concentration is 2.4×10^8 and 4.8×10^8 c/ml; if the concentration is 1.2×10^9 c/ml, specific growth rate increase is hardly seen. Thus, it can be stated that at the concentration of $1.2 \times 10^9 \div 1.4 \times 10^9$ c/ml the culture starts its transition from the "log-phase" into the stationary one. It is supposed that when the culture is being irradiated during its transition, the log-phase could be prolonged, which would affect the total biomass buildup.

NMR-relaxometry

NMR relaxometry method is based on the effect of constant magnetic field and radiofrequency pulse on hydro-

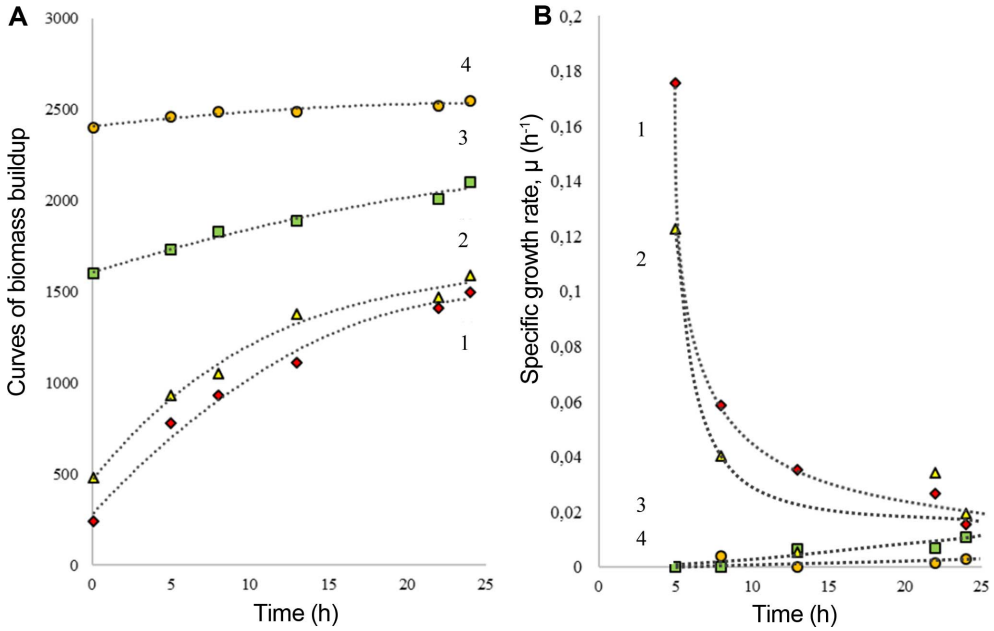

Fig. 2. (A) Curves of biomass buildup, (B) Specific growth rate of *E. coli* LEGM-18 depending on the initial cell concentration: 1) - 2.4×10^8 c/ml, 2) - 4.8×10^8 c/ml, 3) - 1.2×10^9 c/ml, 4) - 2.4×10^9 c/ml. The medium is FHM-broth. Specific growth rate is determined as a derivative of the microbial growth rate curve.

gen nuclei. The intensity and duration of the reaction (relaxation time) depends only on the properties of sample and does not depend on it volume and shape. This is fast non-destructive method analysis of the internal structure of sample. NMR spectroscopy is an effective method of studying the dynamics of various molecular processes, such as proton exchange and diffusion.

In the given paper, the same as it has been done earlier in [1], we suppose that the main effect that microwave radiation of millimeter band has on biological properties of living objects is the change in the structural dynamic condition of the system "bound water – free water", i.e. it influences the process of transition from the free state into the adsorbed phase; the process being accompanied by the charge transfer. To confirm this hypothesis, NMR-relaxation parameters of the culture potentially industrial strain *E. coli* LEGM-18 were studied (T_1, which is the time of spin-grid relaxation, and T_2, which is the time of spin-spin relaxation, and the amplitudes of the signals A_1 and A_2). Time T_1 carries the information on the processes proceeding in the layers near the surface, while T_2 – the information on the mobility of water molecules over the whole sample volume. Paper [1] describes the parameters of NMR-relaxation of the sterile growth medium FHM-broth under the influence of electromagnetic radiation. NMR-relaxometry allows for analyzing the change of the condition of the "medium-cell" system through determining the relation of the amplitudes of particular components of the echo signal envelope when determining relaxation time.

NMR-relaxometry data analysis allows us to state that there are two phases in the system: the first one relates to short relaxation times T_{11} and the second phase – to long ones T_{12}. "Long" times of spin-grid relaxation T_{12} are common for more mobile nuclei ^{1}H of free water molecules, whereas for less mobile nuclei ^{1}H of biopolymers and bound water these are T_{11}, being by two orders of magnitude shorter.

Relaxation signal amplitudes A_{11} and A_{12} are proportional to the number of protons of water being in both bound and free phases. P_{11} and P_{12} represent populations of energy levels corresponding to the number of protons.

When the relation of P_{11} and P_{12} changes, it indicates the phase transition of a proportion of protons from the state of free water into the state of bound (adsorbed) water. The concentration of nuclei ^{1}H in this phase is about 4%, which can be confirmed by the presence of

Table 2. NMR parameters of *E. coli* LEGM-18 in FMH-broth medium.

NMR parameters, relaxation time T (ms)						
Initial concentration	T_{11}	% relative to control	T_{12}	% relative to control	T_2	% relative to control
0	34 ± 8	0	3210 ± 32	0	1565 ± 11	0
2.4×10^8	36 ± 8	5.9 ($p = 0.75$)	3380 ± 41	5.3 ($p < 0.001$)	1594 ± 12	1.9 ($p < 0.05$)
4.8×10^8	38 ± 7	11.8 ($p = 0.55$)	3760 ± 46	17.1 ($p < 0.001$)	1690 ± 14	8.0 ($p < 0.001$)
1.2×10^9	32 ± 5	-5.9 ($p = 0.73$)	3700 ± 44	15.3 ($p < 0.001$)	1648 ± 11	5.3 ($p < 0.001$)
2.4×10^9	28 ± 4	-17.6 ($p = 0.3$)	3610 ± 42	12.5 ($p < 0.001$)	1621 ± 12	3.6 ($p < 0.001$)
NMR parameters, amplitude A (%)						
Initial concentration	A_{11}	% relative to control	A_{12}	% relative to control	A_2	% relative to control
0	-5.1 ± 0.4	0	-161.6 ± 5.7	0	41.5 ± 1.4	0
2.4×10^8	-4.7 ± 0.3	-7.8 ($p = 0.24$)	-123 ± 3.1	-24 ($p < 0.001$)	39.59 ± 1.1	-4.6 ($p = 0.14$)
4.8×10^8	-4.7 ± 0.4	-7.8 ($p = 0.29$)	-134.3 ± 4.4	-17 ($p = 0.003$)	35.88 ± 0.5	-13 ($p < 0.005$)
1.2×10^9	-5.3 ± 0.4	3.9 ($p = 0.57$)	-130.4 ± 4.8	19.3($p = 0.002$)	39.3 ± 0.9	-5.3 ($p = 0.09$)
2.4×10^9	-5.6 ± 0.4	9.8 ($p = 0.2$)	-147.4 ± 5.1	-8.8 ($p = 0.03$)	41.58 ± 1.3	0.2 ($p = 0.95$)
NMR parameters, population P (%)						
Initial concentration	P_{11}	% relative to control	P_{12}	% relative to control	P_2	% relative to control
0	3.16 ± 0.09	0	96.84 ± 0.09	0	99.8 ± 0.2	0
2.4×10^8	3.82 ± 0.1	21 ($p = 0.005$)	96.18 ± 0.1	-0.7 ($p = 0.005$)	95.2 ± 0.2	-4.6 ($p < 0.001$)
4.8×10^8	3.5 ± 0.12	11 ($p = 0.06$)	96.5 ± 0.12	-0.4 ($p = 0.06$)	86.29 ± 3.8	-14.2 ($p = 0.01$)
1.2×10^9	4.06 ± 0.11	29 ($p = 0.002$)	95.94 ± 0.11	-0.9 ($p = 0.002$)	94.52 ± 0.5	-6.1 ($p < 0.001$)
2.4×10^9	3.8 ± 0.05	20 ($p = 0.005$)	96.2 ± 0.05	-0.7 ($p = 0.005$)	100	0.2 ($p = 0.23$)

The minus sign indicates decline compared to control
Population P (%) amplitude rated value = $A/A_{max} \times 100\%$, $P_{11} + P_{12} = 100\%$
Initial concentration $\ll 0 \gg$ – sterile growth medium

1.8% pancreatic hydrolysate of fish meal flour, culture cells, metabolites, culture medium residue from previous cultivation and bound water in the system.

First, the technique described in [1] was applied to study NMR-relaxation parameters of the culture that was not irradiated. The results of the experiment are presented in Table 2.

It has been found that initially when concentration of cells rise, spin-spin and spin-grid relaxation times increase too but if concentration reaches the value of 1.2×10^9 c/ml they decrease. For concentration 1.2×10^9 c/ml, the value of population P_{11} is statistically larger than at other concentrations, which indicates a bigger concentration of nuclei ^{1}H forming a less mobile fraction.

Study of EMR effects on the bacterial culture

At the next stage, growth characteristics of *E. coli* LEGM-18 were studied when the culture was exposed to EMR of different duration. The research results are presented in Table 3. Analysis of the obtained data revealed difference in the growth characteristics of *E. coli* LEGM-18 and *E. coli K12 TG1 with clone luxCDABE genes P. luminescens ZM* 1 on FHM-broth medium [1].

During the comparative study of biological characteristics of *E. coli* LEGM-18 on FHM-broth medium, it has been revealed that its biomass buildup increases when the parameters are as follows: initial concentration is 1.2×10^9 c/ml, duration of exposure is 20 min (maximum buildup); initial concentration is 2.4×10^9 c/ml with the exposition time of 60 min.

It is shown that the positive effect of microwave irradiation on bacterial culture occurs when its concentration reaches $1.2 \times 10^9 \div 2.4 \times 10^9$ cells/ml. In this case bacterial culture passes from the log phase to the stationary one (Fig. 2B). In the process of culture growth, the total surface area of bacterial cells increases. As it was noted above hydrophilic areas of LPS and phospholipids occupy the majority of their surface and have negative

Table 3. Biomass buildup when exposed to EMR of low intensity at frequency of 37.04 GHz depending on the initial cell concentration of *E. coli* LEGM-18 on the FHM-broth medium.

Exposure time, min	0 (control)	20	40	60
Initial concentration 2.4×10^8 c/ml				
Opacity, McF	5 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	5.8 ± 0.2
Concentration, c/ml	1.5×10^9	1.41×10^9	1.35×10^9	1.74×10^9
% relative to control[b]	0	-6	-10	16
Initial concentration 4.8×10^8 c/ml				
Opacity, McF	5.3 ± 0,2	4.9 ± 0.2	4.5 ± 0.1	4.5 ± 0.1
Concentration, c/ml	1.59×10^9	1.47×10^9	1.35×10^9	1.35×10^9
% relative to control[b]	0	-8	-15 [a]	-15 [a]
Initial concentration 1.2×10^9 c/ml				
Opacity, McF	7 ± 0.2	9.3 ± 0.4	6.4 ± 0.3	6.3 ± 0.2
Concentration, c/ml	2.1×10^9	2.79×10^9	1.92×10^9	1.89×10^9
% relative to control[b]	0	32.9[a]	-9	-10
Initial concentration 2.4×10^9 c/ml				
Opacity, McF	8.5 ± 0.2	9.4 ± 0.3	8.2 ± 0.2	11 ± 0.4
Concentration, c/ml	2.55×10^9	2.82×10^9	2.46×10^9	3.3×10^9
% relative to control[b]	0	10.6	-4.5	29.4 [a]

[a] $p < 0.05$ relative to non-irradiated samples
[b] The minus sign indicates decline compared to control

charge. This determines the membrane potential of the cell. The surfaces are able to absorb water molecules on themselves due to their polarization and form multilayer hydrate shells that participate in the transport of substances through the cell membrane [14].

During the life of bacterial cultures, including *E. coli*, acidic compounds accumulate in the nutrient medium, which causes an increase in the concentration of H+ protons [4, 5]. As a result, changes the concentration gradient of protons ΔμH+ on the outside of the cell membrane [12–14]. Thus, in the process of bacterial culture growth, charge redistribution occurs both in the nutrient medium and in the near-surface space of cells. This causes changes in the concentration gradients of charged particles and membrane potential. Consequently, the transition of bacterial culture from log phase to stationary phase depends not only on the amount of nutrients in medium, but also on the concentration gradients of charged particles on both sides of membrane and membrane potential.

External influences, for example, by electromagnetic radiation, leading to changes in the natural processes of

Table 4. Biomass buildup when exposed to EMR of low intensity at frequency of 37.04 GHz depending on the initial cell concentration of *E. coli* LEGM-18 on the meat-peptone broth.

Exposure time, min	0 (control)	20	40	60
Initial concentration 2.4×10^8 c/ml				
E (pan 10 mm), D540 nm	0.84 ± 0.2	0.85 ± 0.3	0.89 ± 0.2	0.9 ± 0.2
% relative to control	0	1.8	3.9	0.9
Initial concentration 1.2×10^9 c/ml				
E (pan 1 mm), D540 nm	0.5 ± 0.1	0.91 ± 0.3	0.47 ± 0.1	0.45 ± 0.1
% relative to control[b]	0	82[a]	-6	-9

[a] $p < 0.05$ relative to non-irradiated samples
[b] The minus sign indicates decline compared to control

redistribution of charges can have an impact on the transport of charged particles through the membrane, and on the life of the cell as a whole.

Culture growth characteristics have also been studied on meat-peptone broth (Table 4). The research shows that the use of meat-peptone broth as a growth medium when cultivating strain *E. coli* LEGM-18 provides a larger biomass buildup compared to FHM-broth medium. Differentiation of growth characteristics could be due to the amount of nutritional ingredients, their adequacy and the amino acid profile that are consumed by the culture.

Parameters of NMR-relaxation of the irradiated culture

At the next stage, NMR-relaxation parameters of the culture were studied when it was exposed to microwave radiation of low intensity for periods of different duration and possible mechanism of observed phenomena is proposed. The results are presented in Fig. 2. The data obtained indicate that the processes taking place when the duration of NMR increases and protons ^1H pass from one fraction into another are non-linear. We suppose that in terms of quantum electronics applying microwave radiation to a bacterial culture is so called "energy pumping". The energy absorbed gets water molecules to pass into a higher metastable excited state shifting electronic densities and causing phase transitions. The processes mentioned trigger the mechanisms of epitaxial growth of bound water phase and forced ^1H transfer outside it (Fig. 3A). In the case of gradual energy genera-

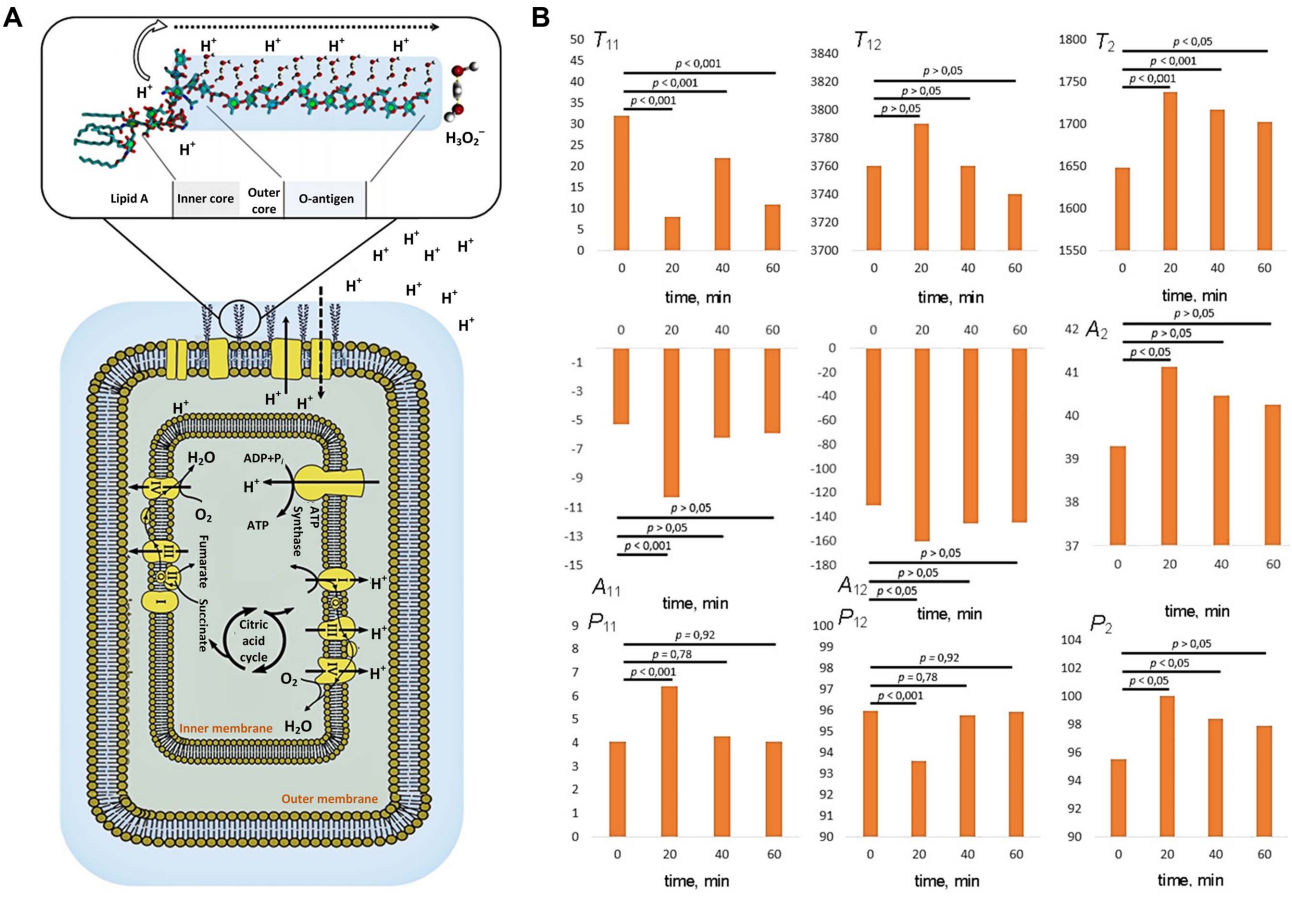

Fig. 3. (A) Shows transfer of protons inside and outside the cell. The reference callout on a larger scale on the right presents the structure of polysaccharides on the surface of the outer membrane as well as transfer of protons in hydrate shells from its surface to the near-surface space. (B) Parameters of NMR-relaxation of *E.coli* LEGM-18 depending on the duration of exposure at the frequency of 37.04 GHz. The medium is FHM-broth. The initial concentration is 1.2×10^9 c/ml. Markers with black filling are amplitude, markers with white filling are relaxation time.

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tion, the system can turn into a short-lived excited state, where there is a high probability of the initiation of the process of induced quantum transition into the initial state.

A simplified formula $H_3O_2^-$ [15–17] corresponds to the hydrate shell so the equations (a) $2H_2O \leftrightarrow H_3O_2^- + H^+$ and (b) $4H_2O \leftrightarrow H_3O_2^- + H_5O_2^+$ are correct. It is suggested that the reaction (a) can result in a significant change of ΔμH+ proton concentration gradient beyond hydrate shells [13]. Reaction (b) causes ions $H_3O_2^-$ and $H_5O_2^+$ to form; those playing a crucial role in charge transfer in water [18–25]. In [26] it is shown that the anionic complex $H_3O_2^-$ liberates protons easier than OH^- ions and is one of the basic elements determining abnormal mobility of protons in water and extremely high speeds of enzyme-catalyst reactions [27–30].

The analysis of biomass buildup data (Table 3) and NMR-relaxation parameters (Fig. 3B) allows us to draw a conclusion that there is a direct dependence between the culture growth, amount of bound water and proton concentration gradient. Under the 20 min exposure to electromagnetic radiation, the time of spin-spin relaxation T_2 increases and so does its amplitude A_2, which is connected with the increase of proton mobility inside the system, which indicates the increase of the system mobility owing to the formation of new weaker H-bonds between water molecules.

The change in population P_{11} (concentration ^1H) observed when studying NMR-relaxation describes the change in the number of protons participating in the formation of hydrate shells. Insignificant change in population P_{11} is in accord with the modern theory of phases, which states that when the volume occupied by the adsorbed water phase expands, the process of charge redistribution takes place, being accompanied by pushing the protons outside. The paper [1] provides an example showing how to calculate the number of water molecules participating in the phase transition $2H_2O \leftrightarrow H_3O_2^- + H^+$. The results are presented in Table 5.

Improvement of the efficiency of bacterial strain cultivation process is of great importance for industry. One of the techniques to optimize this process is the exposition to microwave radiation of low intensity. In the course of experiments, it has been established that reproducibility and obtaining of a useful biotechnological effect depend not only on frequency and radiation power flux but also on some other critical parameters, such as:

- stage of the culture growth at which exposition to EMR takes place;
- duration of the exposition;
- culture medium (percentage of nutrients, amino acid profile).

The study of the low intensity microwave radiation influence on the processes of culturing bacterial strains, including *E. coli*, requires a large body of data, so to understand the mechanisms and effects better a more profound study is necessary. Currently millimeter range intensively mastered. Its component base is expanding and cost of generators decreases. In future, this creates prerequisites for the commercial use of proposed technology to optimize the cultivation of microorganisms.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

Table 5. The increase of the amount of bound water relative to the control in the *E. coli* suspension in the FHM-broth medium at frequency 37.04 GHz.

Exposure time, min	0 (control)	20	40	60
E. coli LEGM-18, 1.2×10^9 c/ml				
P_{12} %[b]	95.94	93.57	95.74	95.93
P_{11} %[b]	4.06	6.43	4.26	4.07
% relative to bound water molecules control	0	116[a]	9	1

[a] $p < 0.05$ relative to non-irradiated samples

[b] population P (%) rated value of the amplitude = $A/A_{max} \times 100\%$, $P_{11} + P_{12} = 100\%$

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