EFFECT OF ARTESIAN WATER WITH A GAS NANOBUBBLES (Ar, O_2, N_2, CO_2, AIR) ON BIOCHEMICAL PROCESSES IN SPRING WHEAT SEEDS

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Abstract

The influence of artesian water with nanobubbles of different gases (Ar, O_2, N_2, CO_2, air) on the intensity of metabolic processes in seeds of spring wheat. The effect of pre-soaking the seeds and spraying of seedlings on yield of wheat was investigated in a field experiment. It was found that the degree of swelling of seeds in the source of artesian water and water with nano-bubbles was the same, but the kinetics of germination varied considerably. Metabolic processes in the seeds are accelerated in the presence of gas nanobubbles in water. The observed effect was similar to a temperature increase of several degrees. The most intensive metabolic processes occur in seeds in water with Ar and CO_2 nanobubbles. Soaking of the wheat seed by water with gas nanobubbles have contributed to a statistically significant increase in wheat yields. The greatest influence on this indicator was provided by the water with argon nanobubbles. Argon has no effect on metabolic processes in seeds and plants. The effect of water with argon nanobubbles can be explained only by the presence of the electrical double layer, which has redox properties and affects metabolic processes at different stages of wheat growth.

Key words: artesian water, gas nanobubbles, weat, seeds.

1. Introduction

In recent decades, many papers have presented case studies on the effects of micro and nanobubbles on the physiological activity of living organisms, such as promoting the growth of plants (Park et al., 2009; Liu et al., 2013), fish (Ebina et al., 2013; Ohnari et al., 2001; Ohnary et al., 2003) and cell cultures(Weber et al., 2005; Matsuno et al., 2014). Therefore, whether nanobubbles can remain for extended periods of time has still been a point to be discussed. For example, the lifetime of colloidal size air bubbles in water is very short. There is a calculation result indicating that the lifetime of air bubbles with radii between 10 and 100 nm ranged from about 1 to 100 μs (Ljunggren et. al, 1997). Furthermore, there is a report that stable nanobubbles could exist only in liquid under highly tensile stress or large negative pressure according to simulation results, and that nanobubbles observed under atmospheric pressure are those in the shrinking process or foreign substances that are mixed in (Matsumoto et al., 2008). On the other hand, results of an experiment confirmed the physiological activity promoting effect and sterilizing effect of water containing nanobubbles (Takahashi, 2006). Adding to this, the present authors confirmed experimentally and with good precision the existence of nanobubbles in water by the particle size distribution, zeta potential and proton spin–lattice relaxation time measurements (Ushikubo et al., 2010). Direct observation of nanobubbles were also aimed by (Uchida et al., 2011) and they observed oxygen nanobubbles that formed in pure water and compared the size distributions of nanobubbles obtained by a transmission electron microscope

Although some reports have attempted to describe the reason for which physiological activity is promoted, most have simply represented case studies without elaborating the mechanisms.

Previously (Butolin et al., 2016), the results of our researches of influence of water nanoparticle on blood biochemical parameters of laboratory rats with experimental diabetes have shown that water with nanoparticle exhibits properties similar properties to drugs-antioxidants. The reason for this effect is the presence of double electric layer on the surface nanomaterial.

In this work, we investigated the effect of gas nanobubbles on the redox processes in wheat seeds at different stages of growth.

2. Experimental

The object of the study were seeds of spring wheat variety Candle (germination rate - 98%, purity - 99.8%, mass of 1000 seeds was 33.4 g), treated by water with different gases nanobubbles. The variety is high yielding, early maturing. Variety milturum. Zoned in the Volga-Vyatka region in Russia.

The artesian water, purified from mechanical impurities by filtration and saturated with gases nanobubbles was used for seed treatment and germination of wheat. Element analysis of the treated water was carried out according to standard procedures. Saturation of water with gases (argon, nitrogen, oxygen, air, carbon dioxide) was carried out by barbotage. Analysis of the gas phase was carried out in the Prokhorov General Physics Institute (Moscow). The sizes of the gas nanobubbles were determined on the basis of data of lase modulation interferentional phase microscopy. Based on measurements of electrorinetic characteristics appreciated by their charges.

For the analysis of swelling of sample wheat seeds weighing 20 g at a temperature of $20^{o}\pm0.5^{o}C$ were placed in beakers of 200 ml and was poured with 30 ml of water. The swelling of the seeds carried in the source of artesian water and artesian water with gas (nitrogen, oxygen, air, argon, carbon dioxide) nanobubbles. After a certain period of time, the grains got, dried with filter paper and weighed. We calculated the degree of swelling according to standard methods by the formula: $\alpha = (m-m_0)/ m_0$ (where α is the degree of swelling of the seeds; m_0 – initial mass of the seed sample, g; m – weight of seed after swelling, g).

Analysis of the environment of swelling and germination were carried out by IR and UV spectroscopy. IR spectra were obtained on an IR Fourier-spectrometer FSM 1202 (Russia). UV spectra were obtained by UV- spectrometer Lambda 650 (Perkin Elmer).

The methodology of investigation of seeds, soaked and sprouted in water with different gas nanobubbles was following. Wheat seeds were kept in water with gas nanobubbles and kneaded in a mortar. Next received IR spectra. The spectrum of water which soaked the seeds was subtracted from each spectrum.

The germination of seeds in laboratory conditions was carried out directly in water and in air after soaking.

3. Results and discussion

Sparging gases of different nature have led to immaterial changes in the mineralization of the artesian water (table 1).

In the result of the bubbling is the supersaturation of water by gases and the formation of nanobubbles. So, by the method of polarography oxygen contents in the treated water exceeds the saturation limit by almost 3 times.

TABLE 1: The elemental composition of original and treated artesian water (mg/l)

Element	Nature of gas of nanobibbles					
	initial	Ar	Air	O_2	N_2	CO_2
K^+	4.2	4.2	2.9	4.2	3.0	3.5
Ca^{2+}	63.0	65.0	60.0	60.0	61.0	63.0
Mg^{2+}	71.0	71.0	70.0	70.0	70.0	70.0
NO_3^-	107.0	107.0	103.0	103.0	104.0	100.0
Cl^-	39.5	39.0	38.0	39.0	39.5	39.0
SO_4^{2-}	45.0	36.0	12.5	19.0	35.0	41.0

Fig. 1 shows the UV-spectra which were obtained relative to the initial artesian water. The increase in intensity in the region of 200 nm was observed. Comparison with results of pH measurement showed that the change in the intensity of the spectrum at 0.1 rel.ed. corresponds to a change in pH by 0.2-0.4 units.

It was found that the zeta potentials are in the range of 17-20 mV, 35-45 mV, mV 29-35, 20-27mV for nanobubbles of air, oxygen, nitrogen and carbon dioxide, respectively (Weihong Jia et al., 2013; Fernanda Yumi Ushikubo et al., 2010). The data in Fig. 1 show that with increase of zeta potentials increases the intensity of the UV spectra. This also increases the pH value: change in magnitude of the zeta potential example-but at 10-15 mV leads to a change in intensity in the region of 200 nm to 0.01 rel.ed.

The results of the study of the gas phase are as follows (Butolin et al., 2016):

- nanobubbles sizes of different gases which were generated under identical processing conditions vary slightly in the order of 200 - 300 nm;

- in average treatment times (10 – 30s) in the atmosphere of argon and oxygen, there is a slight decrease in the nanobubbles size compared to small and large treatment times;

- nanobubbles have a double electric layer; the values of the zeta potentials are of the order of 10-40 mV;

-gas nanobubbles increase water pH by 0.2-0.4 in a series of air< Ar <CO_2 <O_2< N_2 .

Effect of gas nanobubbles dissolved in the water, on the process of germination of seeds of spring wheat was studied under laboratory conditions.

3.1. The kinetics of swelling of seeds of spring wheat in the laboratory

The process of seed germination is a whole group of interconnected biochemical processes that begin even before there are signs of visible growth. For germination you must have certain conditions: free water, oxygen, and positive air temperatures, the main of which is water. Water enters the seeds in the gradient of water potential which is a sum of three potentials: osmotic, atmospheric and matrix. According to modern concepts, the flow of water in seeds can be divided into three stages. The first stage is mainly due to the matrix potential or the hydration force of which is subject to the availability of spare nutrient the seed of a large number of hydrophilic groups such as -OH, -COOH, $-NH_2$, which are formed around the hydration shell. In the second stage, the swelling of the seed, along with a matrix potential, the increasing role begins to play the osmotic potential. Its appearance is due to intense processes of hydrolysis of complex nutrients (fats, proteins, and polysaccharides) in the endosperm to simple (amino acids, amines, amides, monosaccharides, etc.). In the third stage, which occurs during the period of seeds germination when cells are stretched and appear vacuoles, the main force that causes the flow of water in seeds are the osmotic force. For intensity of the processes in the second and third stage, the decisive role played by enzymes in the embryo and endosperm, and produced *de novo*.

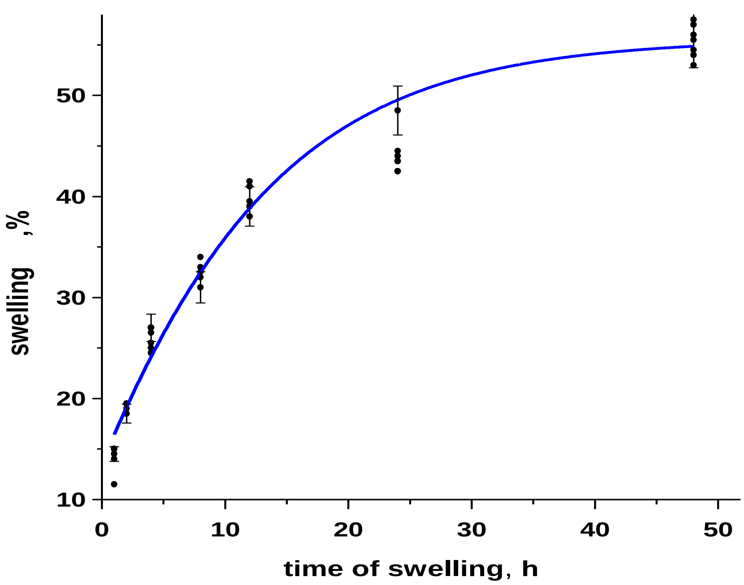

Fig. 2. The kinetics of swelling of seeds of spring wheat

Fig. 2 shows the dependence of the degree of swelling from the time the seeds in water. According to the obtained results the degree of swelling of seeds in the source of artesian water and water with nanobubbles was the same, but the kinetics of germination varied considerably. The kinetics of seed germination depending on the duration of soaking at temperatures 20ºC and

25ºC shown in table 3 and table 4. These data indicate that germination in the air is not pre-soaked seeds was lower than that of seeds, pre-soaked. The increase in temperature and pre-soaking in water with gas nanoparticle resulted in increased seed germination in the air, and this increase is almost independent from the nature of the gas. Gas nanobubbles acted on seed germination about as well as an increase in temperature.

TABLE 2: The kinetics of swelling of seeds of spring wheat at 20º ±0.5ºC

The duration of soaking the seeds in water, h	The duration of seed germination in the air, h	The nature of gas nanobubbles in water					
		Initial water	O_2	N_2	air	CO_2	Ar
0	8.5	0	0	0	0	0	0
	10	0	0	0	0	0	0
	12	1.0±0.2	3.0±0.3	2.0±0.2	2.0±0.1	3.0±0.2	3.0±0.2
	18	12.3±1.2	23.4±1.2	20.0±2.5	20.2±1.3	26.0±1.5	20.0±1.5
1	8.5	0	0	0	0	0	0
	10	1.0±0.2	3.5±0.2	4.4±1.0	3.0±0.3	7.0±1.0	3.0±0.5
	12	2.0±0.1	5.3±0.7	4.2±0.2	4.2±0.2	8.0±1.0	4.2±0.2
	18	30.3±1.0	50.4±5.5	65.4±5.0	75.0±5.0	80.6±4.2	66.6±1.6
2	8.5	1.0±0.3	1.0±0.3	1.0±0.3	2.0±0.5	0	0
	10	7.0±1.5	1±0.5	3.0±1.0	6.0±1.5	5.0±1.0	5.0±1.5
	12	10.5±2.5	14.5±2.5	14.0±1.5	14.0±2.0	12.0±2.0	10.0±2.0
	18	83.5±3.5	85.0±2.0	85.0±3.0	88.5±1.5	89.0±4.0	87.0±3.0
4	8.5	1.0±0.5	4.0±1.0	3.0±1.0	2.0±0.5	4.0±0.5	1.0±0.2
	10	9.0±2.0	10.5±1.5	6.0±1.5	17.0±2.0	12.0±2.5	1.0±0.2
	12	36.5±1.5	37.5±2.5	23.0±2.5	52.0±1.3	35.4±1.2	32.4±1.4
	18	72.3±1.3	80.3±4.5	89.5±6.3	91.5±4.0	86.5±2.5	95.0±3.0
12	0	1.0±0.3	4.0±1.0	4.4±1.1	5.5±0.5	1.0±0.2	2.0±0.2
	2	2.0±1.0	7.0±1.0	5.0±1.5	6.5±1.5	3.0±0.5	4.0±0.5
	3	3.5±1.0	9.0±1.5	5.5±1.5	6.0±1.0	4.0±0.5	6.4±1.4
	5	4.5±1.0	13.5±1.5	7.0±0.5	9.0±2.0	6.0±1.5	8.0±2.0
	7	15.0±3.5	22.0±1.5	26.3±1.1	22.3±1.1	10.3±1.5	20.0±1.0
	12	90.0±9.0	89.5±7.5	93.5±3.5	85.0±5.0	87.5±4.5	88.5±7.5
16	0	2.0±0.3	4.4±1.1	11.5±1.0	6.0±1.0	8.5±1.5	6.2±1.2
	8.5	80.0±8.0	84.0±3.0	85.0±5.0	90.0±6.0	86.0±8.0	94.0±6.0
24	0	6.0±	14.5±2.5	19.0±2.0	10.3±2.1	10.3±1.0	8.5±1.5
	8.5	73.5±6.0	81.0±7.5	85.5±5.5	83.0±9.0	86.0±9.0	87.5±9.5

Fig. 3 shows the IR spectra of the environment of swelling. The main direction of biochemical processes occurring during germination, is intensely flowing hydrolysis of macromolecular compounds under the influence of the corresponding enzymes, the activity of which is greatly increased from the first hours of the germination process. As the germination of the grain decreases the total content of protein nitrogen and increases the total amount of water-soluble substances (amino acids and carbohydrates). Undergoes the greatest conversion of starch - the main carbohydrate reserve of the grain. Approximately 20% of the whole amount hydrolyzed: 8-9% is spent on breathing, 3-4% on the construction of the stem and roots and 8-10% remains in the form of sugar. Proteins also undergo considerable changes. Total nitrogen content during the whole period of germination remains virtually the same, the

TABLE 3: The kinetics of swelling of seeds of spring wheat at 25º±0.5ºC
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The duration of soaking the seeds in water, min	The duration of seed germination in the air, h	The nature of gas nanobubbles in water					
		Initial water	O_2	N_2	air	CO_2	Ar
0	11	0	0	0	0	0	0
	13	0	0	0	0	0	0
	20	20.5±3.2	36.5±2.2	32.3±3.3	32.3±4.3	36.5±4.5	32.5±2.5
	26	28.0±2.5	48.0±5.0	46.0±6.0	44.5±2.3	46.5±3.3	44.5±5.3
	35	65.5±4.3	72.5±8.2	70.0±8.5	70.0±3.5	71.0±4.3	70.0±3.5
10 min	11	0	1.0±0.3	4.0±0.5	3.0±0.3	2.0±0.3	1.0±0.2
	13	8.0±2.0	14.0±1.3	16.0±1.3	11.0±2.5	12.0±2.0	10.0±2.5
	20	56.0±1.3	78.0±2.3	79.5±3.5	74.0±3.0	71.5±1.5	72.5±2.5
	26	87.0±4.3	93.0±3.0	96.0±5.3	93.3±6.1	90.0±4.5	92.0±0.5
	35	97.0±5.5	96.0±4.0	97.0±4.0	96.3±3.3	96.3±5.1	95.5±2.5
30 min	11	3.0±1.0	3.3±1.1	4.3±1.1	2.5±0.5	6.5±1.5	5.5±1.5
	13	21.3±2.1	17.3±2.1	29.0±1.5	23.0±2.0	32.3±2.1	21.3±0.5
	20	82.0±3.0	87.0±4.3	90.0±5.5	88.0±5.3	88.0±3.3	82.0±2.5
	26	93.0±2.0	96.0±1.5	97.0±2.0	95.0±2.0	94.0±1.0	92.0±3.0
	35	96.0±1.5	98.0±1.0	97.0±2.0	96.0±2.0	95.0±2.5	98.0±2.0

60 min	11	4.0±1.0	4.3±1.0	3.3±1.1	3.3±1.2	4.5±1.5	3.5±1.0
	13	16.0±2.0	43.0±1.5	25.0±1.5	32.3±1.0	30.3±1.0	24.5±1.5
	20	70.0±2.0	87.0±1.5	83.0±1.0	91.0±2.0	86.0±2.3	82.0±1.5
	26	87.0±1.0	93.0±2.5	94.0±2.5	94.0±2.0	95.0±1.0	91.0±1.0
	35	94.0±1.0	93.0±2.0	95.0±2.0	95.0±2.3	96.0±1.5	91.0±1.0

content of amine nitrogen increases dramatically at 6 to 8 days, and then the growth rate slowed down. Source of seed proteins are hydrolyzed to approximately 55%, of which about 23% is concentrated in the germ in the form of qualitatively different proteins.

In IR spectra we can to observe the changes (qualitative and quantitative) in medium of germination and in seed during germination of wheat seeds. Wave range 1000-1200 cm^{-1} corresponds to valence vibrations of C-O groups in the polysaccharide molecules. 1400-1700 cm^{-1} region of the IR spectrum. responsible for valent and deformation vibrations of the groups C=O and C-N included in the composition of amino acids, peptides and proteins. Fig. 4 shows the IR spectra of the seeds, seasoned in different environments of germination for 12, 24 and 48 h. Major qualitative changes occur in the qualitative and quantitative composition of the absorption bands caused by vibrations of protein structures. Moreover, differences in processes in various environments, most noticeable after 24 h of soaking.

Fig. 5 shows the IR spectra of different environments on successive stages of germination. The visible qualitative differences between samples are observed in the region 3000-3200 cm^{-1} corresponding to the stretching vibrations of N-H. The most noticeable difference of the absorption of the amino group in the source water and in water with dissolved gases visible to 24 hours of soaking. In addition, it is possible to make some estimates on the changes in the absorption region of proteins and polysaccharides.

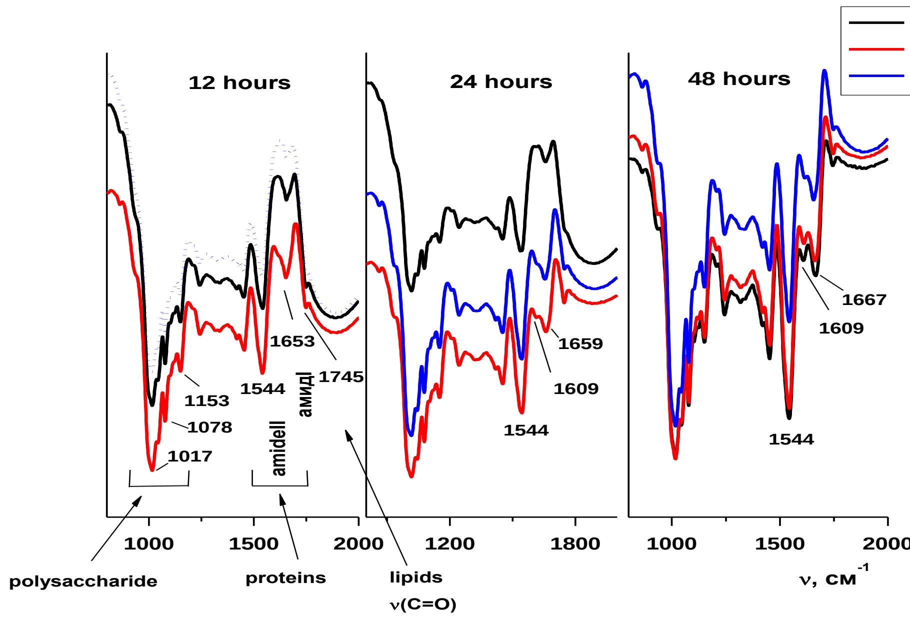

Fig. 4. IR spectra of wheat seeds after storage in water with gas nanobubbles.

Fig. 6 shows the values of the total area of the absorption bands of each of these structures in different environments after 12-48 h of germination. The character of dependencies allows you to note specific features of the processes of hydrolysis of macromolecular structures in water with nanoparticle nanoparticle Ar and CO_2.

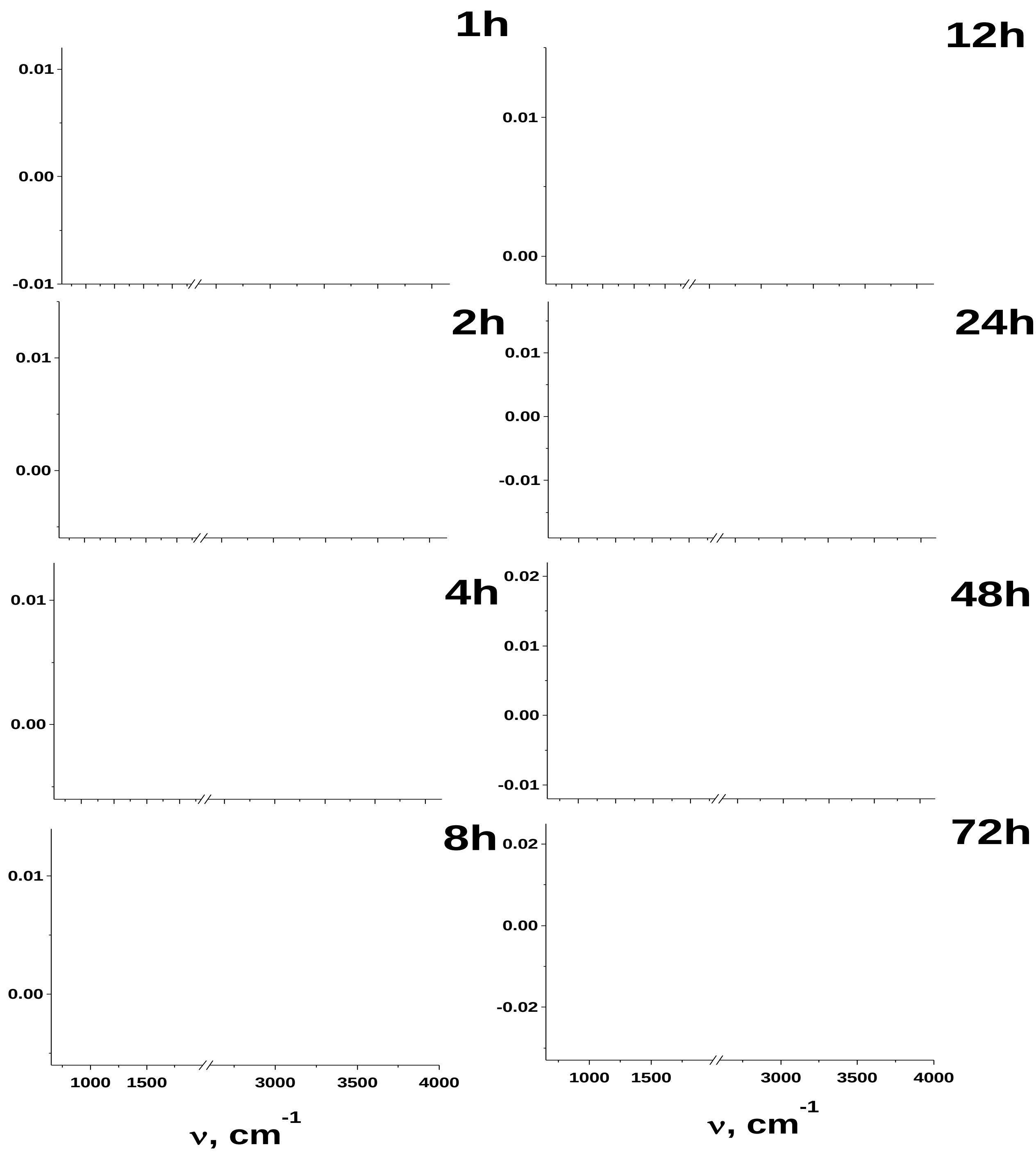

Fig. 6. IR spectra of the environment of swelling, depending on exposure of the seeds in water with gas nanobubbles

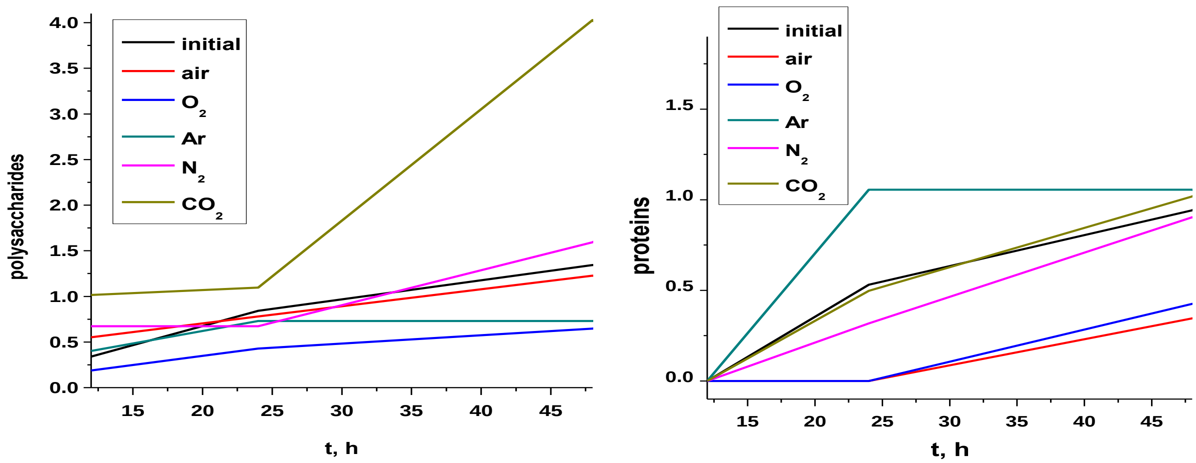

Fig. 7. The sum of the integral intensities of IR absorption bands corresponding to vibrations in the bonds of molecules of proteins and polysaccharides.

Fig. 4 shows UV spectra of spectra of different environments on sequential stages of seed germination. Differences in the kinetics of exchange processes in different environments of germination is most apparent after 12, 24 and 48 hours soaking.

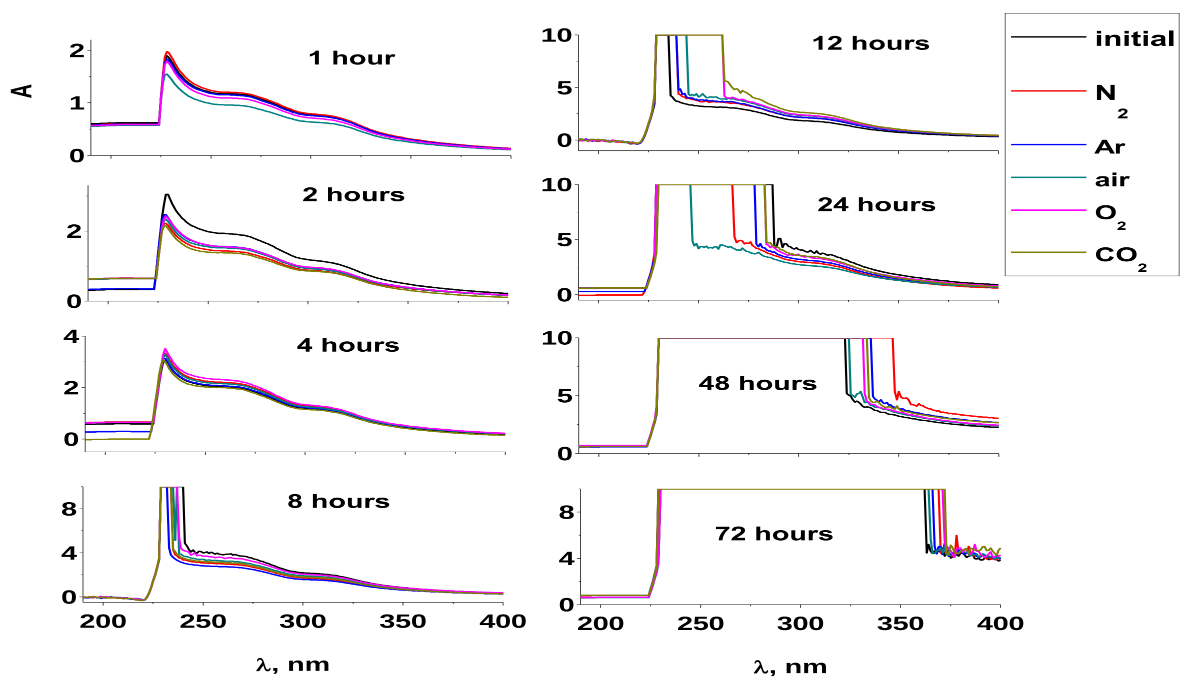

Fig. 8. UV spectra of the medium swelling on successive stages of artificial aging of seeds wheat in water with gas nanoparticle.

Analysis of the data provided in table 2, table 3 and figures 1-8, show that gas nanobubbles have influenced the course of physiological processes in germinating wheat seeds and on their rate.

According to the hypothesis of the release of seeds from dormancy as a result of oxidation, to activate the required acceptors of hydrogen [Cao et al., 1994; Tuan-Hua David Ho., 1980). As such acceptors can be negatively charged gas nanobubbles with a double electric layer. During germination of seeds in they generate reactive oxygen species that can cause oxidative tissue damage (Zenkov et al., 1993). Protection from this effect is due to the use of the antioxidant system consisting of low - and high-molecular compounds. The effects of the components of the antioxidant defense system mainly due to the suppression of free radicals, maintain the normal level of free radical processes and lipid peroxidation in the tissues (Kenia met al., 1993).

Previously (Butolin et al., 2016) based on studies of rats with experimental diabetes have shown that water with gas nanobubbles acts like drugs – antioxidants. It is possible that the same action nanobubbles have on wheat seeds. Therefore, the speed of germination of seeds in water with nanobubbles of gases of different nature is higher than in the source water.

Most often the increased number of germinated seeds was observed in water with nanoparticle CO2, which was accompanied by increased content of polysaccharides and a low content of proteins in an environment swelling with seed protein content was increased. The physiological role of carbon dioxide, in our opinion, is associated with the activation of the processes required to initiate stretching of cells: the accumulation of osmotically active substances and acidification of cell walls, leading to their loosening and allowing it to begin the "acidic growth". In the standard conditions of "acidic growth" starts due to the activation plasmalemmal N+ = ATF - phase (Obrucheva, Antipova, 1994). The interaction of carbon dioxide with water leads to the formation of carbonic acid and thereby to further acidification of the liquid phase surrounding the shell of the seed.

Increased the number of germinated seeds, in some cases, was observed in seed treatment with water nanoparticle argon and nitrogen. In addition, Ar nanobubbles contributed to the increase in the content of water soluble nitrogen compounds in the environment swelling. The physiological effects of these gases nanomaterial apparently associated with the destruction or inhibition of the hormone responsible for the dormant period in seed - azizova acid.

Oxygen had a two-fold impact on the process of seed germination. As he was the main factor, which sharply increased breathing swollen seed, with prolonged soaking (more than 4 hours) it would cap the process of their germination. However, the presence of gas nanobubbles in water resulted in oxidative tissue damage and suppression of the synthesis of polysaccharides and proteins that lowered content of proteins and polysaccharides in the environment of swelling and, in some cases, reduced the number of germinated seeds.

3.2. Field experiment with wheat

The yield of agricultural crops cultivated in the experience is the most objective indicator of the effectiveness of the studied factors. The influence of activation of water with nanobubbles on wheat yield is shown in table 5. It was established that all kinds of effects on seeds and seedlings of wheat had

TABLE 5: Wheat yield depending on the type of treatment of seeds and seedlings.

Option experience		Productivity, g/m^2	Deviation, g/m^2	
			from absolute control	From control 1 or 2
The sowing of dry seeds (absolute control)		71.3±23.1	-	-
	Initial water (control 1)	81.7±15.4	10.4	-
	Water with O_2 nanobubbles	84.3±18.0	13.0	2.6

Soaking of seeds in the water	Water with air nanobubbles	91.5±26.9	20.2	9.8
	Water with CO_2 nanobubbles	95.0±29.3	23.7	1.3
	Water with Ar nanobubbles	91.0±16.4	19.7	-5.9
Spraying shoots water	Initial water (control 2)	75.4±11.6	4.1	-
	Water with Ar nanobubbles	84.2±32.3	2.9	-19.3
	Water with O_2 nanobubbles	74.6±12.7	3.3	-0.8
	Water with air nanobubbles	85.1±26.8	13.8	9.7
	Water with CO_2 nanobubbles	93.9±11.1	22.6	18.5
Least significant difference		-	9.9	9.9

an impact (positive or negative) on the growth and development of plants, which is reflected, ultimately, on their productivity. Table 5 show that even a simple soaking of seeds in the source of artesian water for 1 min contributed to statistically reliable hanging wheat yield of 10.4 g/m2 or 14.6%. Soaking seeds is quite well known agronomic technique, which allows to accelerate the process of seed germination and make it more stable. It is very widely used in vegetable production, although the period of soaking, tend to be more significant.

Activation of water by nanobubbles of various gases contributed to further increase productivity, statistically significant only in the variants with carbon dioxide and argon.

Artesian water spraying of wheat in the phase of germination at the dose of 0.25 liter per 1 m^2 stimulated the increase of grain yield by 4.1 g/m^2 (6%). In agronomic practice, such low dose of spraying water not used practically, but they are widely used for various chemical treatments of plant growth regulators, microfertilizers, protective means. Activation of water by nanobubbles of various gases contributed to the increase in grain yield of wheat: air nanobubbles (13.8 g/m^2 or 19%), carbon dioxide nanobubbles (22.6 g/m^2 or 32%) and the most significant was water with argon nanobubbles (32.2 g/ m^2 or 45%).

4. Conclusion

The degree of swelling of seeds in the source of artesian water and water with nanobubbles was the same, but the kinetics of germination varied considerably. Metabolic processes in the seeds in water are accelerated in the presence of nanobubbles of gases of different nature similar to a temperature increase of several degrees. The most intensive metabolic processes occur in water with Ar and CO_2 nanobubbles.

Soaking of the wheat seed in water gas nanobubbles have contributed to a statistically significant increase in wheat yields by 10.4 and 23.7 g/m^2 (15-33%). The greatest influence on this indicator was provided by the water with argon nanobubbles.

Spraying of wheat by water with gas nanobubbles statistically significantly increased the grain yield by 13.8-32,2 g/m^2 (19 - 45%). The greatest influence on this indicator was provided by the water with argon nanobubbles.

Oxygen, nitrogen and carbon dioxide can have an influence on metabolic processes in seeds and plants. Argon has no effect on metabolic processes in seeds and plants. The effect of water with argon nanobubbles can be explained only by the presence of the electrical double layer, which has redox properties and affects metabolic processes at different stages of wheat growth.

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