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Studying the Virucidal and Biocidal Effects of Electrochemically Activited Anolyte and Catholyte Types of Water on Classical Swine Fever Virus (CSF) and Bactrerium *E. coli DH5*

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Abstract

This article outlines the results on the antimicrobial action of electrochemically activated water solutions (anolyte/catholyte). The two types of water solutions are produced in the anode and cathode chamber of the electrolitic cell, respectively. Under laboratory conditions a strain of *E. coli DH5*, as well as the cell culture and organ suspensions of classical swine fever virus (CSF) were treated with the anolyte and the catholyte. By inoculating them with cell cultures the viral presence (the presence of viral antigen) was measured using the immunoperoxidase technique. It was found that anolyte did not affect the growth of the cell culture PK-15; the viral growth in the infection of a cell monolayer with a cell culture CSF virus was affected in the greatest degree by the anolyte in 1:1 dilution and less by other dilutions; whereas the viral growth at the infection of a cell suspension with the cell culture of the CSF virus was affected by the anolyte in dilution 1:1 in the greatest degree, and less by other dilutions; whereas the viral growth at the infection with the CSF in suspension of the cell monolayer was affected by the anolyte in all applied

dilutions. Unexpectedly, the stronger biocidal effect of the catholyte was observed when a strain of *E. coli DH5* was treated by anolyte and catholyte, respectively. In order to provide additional data about the antiviral activity of the catholyte and the anolyte, and the distribution of H_2O molecules according to the energies of hydrogen bonds, the non-equilibrium energy spectrum (NES) and diferential non-equilibrium energy spectrum (DNES) of anolyte and catholyte were measured.

Keywords: anolyte, catholyte, cell culture, CSF virus, disinfection, NES, DNES

1. Introduction

The phenomenon of electrochemical activation of water (EAW) is a set of electrochemical and electrical processes occur in water in the electric double layer (EDL) type of electrodes (anode and cathode) with non-equilibrium electric charge transfer through EDL by electrons under the intensive dispersion in water the gaseous products of electrochemical reactions (Bahir et al., 1983). In 1985 EAW was officially recognized as a new class of physical and chemical phenomena.

As a result of treatment of water by a constant electric current at electric potentials equal to or greater than the decomposition potential of water (1.25 V), water goes into a metastable state, characterized by the abnormal activity levels of electrons, redox potential, and other physico-chemical parameters (pH, E_h, ORP) (Kirpichnikov et al., 1986). The passage of direct electric current through the water volume is accompanied by electrochemical processes, which occur as a result of redox reactions.

The main stage of electrochemical treatment of water is the electrolysis of water, which occurs in the electrolysis cell, consisting of the cathode and the anode separated by a special semipermeable membrane (diaphragm), which separates water to the alkaline fraction – the catholyte and the acidic fraction – the anolyte (Figure 1). When the passing over the electric current through water, the flow of electrons from cathode as well as the removal of electrons from water at the anode, is accompanied by series of redox reactions on the surface of the cathode and the anode. As the result, new compounds are formed, the system of intermolecular interactions, as well as the composition of water and the water structure are changed (Mosin, 2012; Dykstra, 1999).

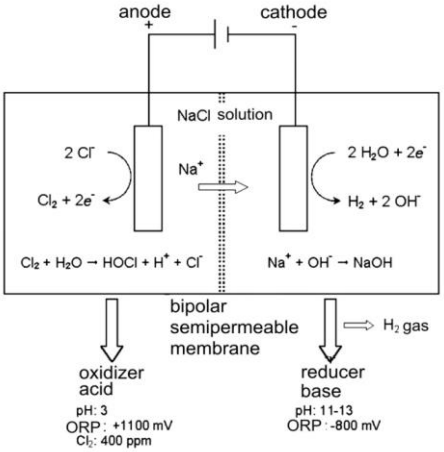

Figure 1. Laboratory setting of diaphragm electrolysis method for the preparation of acid (anolyte) and

alkali (catholyte) solutions in the electrochemical activation of NaCl

The products of electrode reactions are the neutralized aqueous admixtures, gaseous hydrogen and oxygen generated under the electrolytic destruction of H_2O molecules, the metal cations (Al^{3+}, Fe^{2+}, Fe^{3+}) in the case of metal anodes of aluminum and steel, and the molecular chlorine. Wherein at the cathode is generated the gaseous hydrogen, and at the anode – oxygen. Water also containes a certain amount of hydronium ions (H_3O^+) depolarizing at the cathode with formation of the atomic hydrogen:

$$H_3O^+ + e^- \rightarrow H + H_2O, \quad (1)$$

In an alkaline environment there occurs the disruption of H_2O molecules, accompanied by the formation of the atomic hydrogen and hydroxide ion (OH^-):

$$H_2O + e^- \rightarrow H + OH^-, \quad (2)$$

The reactive hydrogen atoms are adsorbed on the surfaces of the cathode, and after recombination formed the molecular hydrogen H_2, released in the gaseous form:

$$H + H \rightarrow H_2, \quad (3)$$

At the same time at the anode is released the atomic oxygen. In an acidic environment, this process is accompanied by the destruction of H_2O molecules:

$$2H_2O - 4e^- \rightarrow O_2 + 4H^+, \quad (4)$$

In an alkaline environment, the source of oxygen source is OH^- ions, moving under the electrophoresis from the cathode to the anode:

$$4OH^- \rightarrow O_2 + 2H_2O + 4e^-, \quad (5)$$

The normal redox potentials of these reactions compiles +1.23 V and +0.403 V, respectively, but the process takes place in certain conditions of overload.

The cathodes made of metals that require high electrical voltage (lead, cadmium), allow to generate the reactive free radicals as Cl*, O*, OH*, HO_2*, which react chemically with other radicals and ions.

In bulk oxidative processes a special role plays products of electrolysis of water – oxygen (O_2), hydrogen peroxide (H_2O) and hydrochlorine acid (HClO). During the electrolysis, an extremely reactive compound is formed – H_2O_2, the formation of which occurs due to the hydroxyl radicals (OH*), which are the products of the discharge of hydroxyl ions (OH^-) at the anode:

$$2OH^- \rightarrow 2OH^* \rightarrow H_2O_2 + 2e^-, \quad (6)$$

where OH^* – the hydroxyl radical.

The chlorine-anion is transformed to Cl_2:

$$2Cl^- \rightarrow Cl_2 + 2e^-, \quad (7)$$

Gaseous Cl_2 forms highly active oxidants: Cl_2O; ClO_2; ClO^-; HClO; Cl^*; HO_2^*. As a result the surface tension will increase toghether with the electrical conductivity (Hsu, 2005; Bahir, 1992). The parameters of pH, redox potential, ORP and the conductivity of the anolyte/catholyte depend on different factors including the ratio of water volumes in the two chambers, the material of electrodes, NaCl concentration, the temperature, the electric voltage and processing time (Bahir, 1999).

The electrolysis cell can be regarded as a generator of the above products, some of which, entering into the chemical interaction with each other and water impurities in the interelectrode space, provide additional chemical treatment of water (electroflotation, electrocoagulation) (Bahir et al., 2001). These

secondary processes do not occur on the electrode surface, but in the bulk water. Therefore, in contrast to the electrode processes they are indicated as the volume processes. They generally are initiated by increasing the temperature of water during the electrolysis process and increasing the pH value.

As a result of the cathode (catholyte) treatment water becomes alkaline: its ORP decreases, the surface tension is reduced, decreasing the amount of dissolved oxygen in water, increases the concentration of hydrogen, hydroxyl ions (OH^-), decreases the conductivity of water, changes the structure of hydration shells of ions (Toropkov et al., 2001). By external characteristics the catholyte – is soft, light, with an alkaline taste liquid, sometimes with white sediment; its pH = 10–11, ORP = -200…-800 mV.

On physical and chemical parameters the catholyte has the significantly enhanced electron-donating properties, and getting into the physiological fluids can enhance the electron-background for a few tens of millivolts (Leonov et al., 1999). The catholyte has antioxidant, immunostimulating, detoxifying properties, normalizing ORP, metabolic processes (increases the ATP synthesis, modification of enzyme activity), stimulates the regeneration of tissues, DNA synthesis increases and stimulates the growth and division of cells by increasing the mass transfer of ions and molecules across the cell membrane, improves the trophic processes in tissues and blood circulation. It was also reported that catholyte with the ORP at -700…-100 mV favorizes the development of anaerobs, while anolyte with the ORP at +200…+750 mV supports the growth of aerobs (Kirkpatrick, 2009). The antibacterial effect of the catholyte is differentiated: the bactericidal effect is appeared relative to *Enterobacteriaceae*, resistant to it are enterococci and the group of streptococci B, and against Gram-negative microorganisms – only the bacteriostatic effect (Leonov et al., 1999).

The electrochemically activated solutions of catholyte, depending on the strength of the transmitted electric current may be of several types:

C – the alkaline catholyte (pH > 9.0, ORP = -700…-820 mV), the active components – NaOH, O_2, HO_2^-, HO_2*, OH^-, OH*, HO_2^-, O_2;

CN – the neutral catholyte (pH = 9.0, ORP = -300…-500 mV), the active components – O_2, HO_2^-, HO_2*, H_2O_2, H^+, OH^-;

As a result of the anode (anolyte) treatment water becomes acid reaction, the ORP increases slightly, the surface tension is slightly reduced, the conductivity increases, the amount of the dissolved oxygen and chlorine in water also increases, whereas the amount of hydrogen decreases (Toropkov et al., 1999). The anolyte is a brownish, acid, with a characteristic odor and taste the liquid with a pH = 4–5 and ORP = +500…+1100 mV. Specify anolyte toxicity when administered in the stomach and applying to the skin refers to class 4 of harmful substances according to the Russian Standard GOST 12.1.007-76 with the minimal toxicity within this class. When being inhaled the anolyte with oxidants content of 0.02 % and total mineralization 0.25–0.35 % does not irritate the respiratory system and mucous membranes of the eyes. When introduced into the organism, the anolyte has no immunotoxic action and increased chromosomal aberrations in the bone marrow cells and other tissues, and it has no cytogenetic activity. When heated to 50 ^{0}C the bactericidal activity of anolyte is reportedly increased by 30–100 % (Prilutsky et al., 1997).

The electrochemically activated solutions of the anolyte are divided into four main types:

A – the acidic anolyte (pH < 5.0, ORP = +800…+1200 mV), the active components – HClO, Cl_2, HCl, HO_2*;

AN – the neutral anolyte (pH = 6.0, ORP = +600…+900 mV), the active components – HClO, O_3, HO^-, HO_2*;

ANK – the neutral anolyte (pH = 7.7, ORP = +250…+800 mV), the active components – HClO, ClO^-, HO_2^-, H_2O_2, O_2, Cl^-, HO*;

ANKD – the neutral anolyte (pH = 7.3, ORP = +700…+1100 mV), the active components – HClO, $HClO_2$, ClO^-, ClO_2*, HO_2*, H_2O_2, O_2, O_3, Cl^-, HO^-, O*.

The anolyte has antibacterial, antiviral, antifungal, anti-allergic, anti-inflammatory, antiedematous and antipruritic effect, and may exert the cytotoxic and antimetabolite action without harming the human tissue cells (Babtsova et al., 1999). The biocide elements in the anolyte are not toxic to somatic cells, as represented by oxidants, such as those ones produced by the cells of higher organisms.

Studies on the virucidal effect of the anolyte are rare and insufficient, basically on the possibilities of applying the anolyte in the implementation of effective control of viral diseases in humans and animals and especially on particularly dangerous viral infections. One of them is the classical swine fever (CSF), prevalent in different regions of the world, thus inflicting heavy economic losses. It is caused by enveloped viruses belonging to the genus *Pestivirus* of the family *Flaviviridae*. The resistance and inactivation of the virus of CSF virus is a subject of extensive research. Although it is less resistant to external stresses other than non-enveloped viruses, it retains its virulence for a long period of time: in frozen meat and organs – from a few months up to one year; in salted meat – up to three years; in dried body fluids and excreta – from 7 to 20 days. In rotting organs it dies for a few days and in urine and faeces – for approx. 1–2 days. In liquid fertilizer it can withstand 2 weeks at +20 ^{0}C, and over 6 weeks at +4 ^{0}C. Its thermal resistance may vary depending on the strain type, but the inactivation is dependent mostly on the medium containing the virus. Although the CSF virus loses its infectivity in cell cultures at +60 ^{0}C for 10 min, it is able to withstand at least 30 min at t = +68 ^{0}C in defibrinated blood. It is relatively stable at pH = 5–10, and the dynamic of the inactivating process below pH = 5 depends on the temperature.

According to Sands et al., (1979) and Springthorpe et al., (1990), the effective disinfection of viruses whose infectivity is associated with the elements of the casing is achieved by disinfectants dissolving fats, surfactants, disinfectants or fatty acids, organic solvents (ether and chloroform), detergents, proteases, and common disinfectants. It is believed that 2 % solution of sodium hydroxide is most suitable for the disinfection of spaces contaminated with them. According to Wittmann (1967), to achieve effective disinfection it is necessary to irreversibly damage the nucleic acid.

Investigations conducted by other authors (Zinkevich et al., 2000) were carried out with *E. coli*, using as a desinfectant the anolyte with ORP equal or greater than +1100 mV and pH = 5.5, obtained via electrolysis of diluted NaCl solution on planktonic cells of a strain of *E. coli JM109*. It was demonstrated that within 5 min of influence all cells were inflated and burst. Also, it was occurred a full destruction of proteins, DNA and RNA. Supposedly the anolyte enters the cells provoking structural and functional damages on the cell‘s membrane and the cell‘s wall.

Similar research was performed by S.V. Kumar et al. (1999). They evaluated the inactivation efficacy of anolyte of pH = 2.7 and ORP = + 1100 mV on *Escherihia coli O157:H7*, *Salmonela enteritidis* and *Lusteria monocytogenes*. As it was demonstrated on five strains of *E. coli E06* (milk), E08 (meat), E10 (meat), E16 (meat) and E22 (calf feces), all patogens were significantly reduced (7.0 log CFU/ml) or fully

destroied (8.0 log CFU/ml) after 2 to 10 min inactivation by the anolyte in the temperature range from +4 ^{0}C to +23 ^{0}C. Supposedly, the low pH value of the anolyte makes sensitive the outer cell‘s membrane, thus facilitating HClO to enter the cell and further destroy it.

However, it should be noted that the pharmacological studies of electro-activated solutions of water and their virucidal effects and toxicity have not yet been completely studied. The purpose of this research was to study the virucidal effect: 1) of the anolyte in different dilutions on CSF virus in cell culture and organ suspensions; 2) of the anolyte/catholyte on a strain of *E. coli DH5a*, and 3) to observe how the virocidal effect relates to local maximums in NES-spectra of the anolyte and the catholyte[1].

2. Material and Methods

The studies of antiviral activity of the anolyte were performed at the National Reference Laboratory "Classical and African Swine Fever" section "Exotic and Especially Dangerous Infections" of the National Diagnostic and Research Veterinary Medical Institute(Sofia, Bulgaria). Experiments were conducted with the anolyte obtained by the electrolysis apparatus "Wasserionisierer Hybrid PWI 2100", equipped with four titanium electrodes coated with platinum. The disinfectant had pH = 3.2 and ORP = 1070 mV. 0.3 % solution of chemically pure NaCl in distilled water was used. The interaction of the anolyte with the CSF virus suspension was carried out at a temperature of +22 ^{0}C.

A cell culture of porcine origin sensitive to the CSF virus was used: a continuous cell line was PK-15. Contamination of cell cultures was carried out with cell culture test virus 2.3 Bulgaria with titre 107.25 $TCID_{50}$/ml and organ suspension of internal organs (spleen, kidney, lymph node) of wild boar originating from the last outbreak of CSF in Bulgaria in 2009. The titer of the established virus in the suspension was $10^{4,75}$ $TCID_{50}$ ml.

To establish the virucidal activity, the inocula prepared for contamination of cell culture (cell culture virus) were treated with the following dilutions of the anolyte in sterile distilled water: 1:1 (50 %), 1:2 (33.33 %), 1:3 (25 %), 1:4 (20 %). These dilutions were mixed with inocula in proportion 1:1 (100 µl of virus suspension and 100 µl of the appropriate anolyte concentration). The time of action was conformed to the period, which is methodologically necessary to “capture” any virus present on the cell culture. Upon infection of a cell monolayer, the mixture was removed after the end of the exposure period of 1 h. Upon infection of a cell suspension, the mixture was not removed.

To establish the virucidal activity of the anolyte on the CSF virus in organ suspension, a different formula was used: the inoculum was mixed directly with concentrated anolyte in anolyte-inoculum ratios respectively 1:1; 3:1; 7:1 and 15:1. Since it is known that the growth of the CSF virus does not cause a cytopathic effect, therefore, for demonstration of its presence, immunoperoxidase plates dyeing were used. The cells were fixed and the viral antigen was detected after binding to a specific antibody labeled with peroxidase. The organs exude 1 cm^3 of tissue, which is homogenized in a mortar with 9 ml of cell cultures medium containing antibiotics, in order to obtain 10 % organ suspension. Sterile sand was added to

[1] Such a dependence was established between the local maximum (-0.1387 eV; 8.95 µm) in the NES-spectrum of the catholyte solution that suppresses the development of mice tumor cells (Ignatov & Mosin, 2014).

improve the homogenization. The samples were left at room temperature for 1 h. They were centrifuged for 15 min at 2500 g. The supernatant was used to infect the cells. In case of cytotoxic effect, parallel dilutions of the homogenates were prepared in proportions 1:10 and 1:100. From the suspensions into multiwell (24-well) plates were added 200 μl of the inoculum to cells with coverage of 50–80 %. Cell cultures were incubated at t = +37 ^{0}C for 1 h in order to "capture" an eventual virus if presented, they were rinsed once with PBS and fresh media were added. Alternatively, the plate was filled directly (cell suspension), since the preliminary studies had found that anolyte did not induce a cytotoxic effect.

Cell cultures were incubated for 72–96 h at t = +37 ^{0}C in a CO_2 incubator. The procedure with the positive and negative control samples was similar. The positive control sample was a reference strain of the CSF virus. The immunoperoxidase technique was used. The fixation of the plates was carried out thermally for 3 h at t = +80 ^{0}C in a desiccator. In the processing was used a primary monoclonal antibody C 16 diluted 1:50, and secondary antibody RAMPO diluted 1:50. For immunoperoxidase staining was used 3 % H_2O_2 and AEC (dimethylformamide and 3-amino-9-ethylcarbazole) in acetate buffer. The antibody-antigen complex was visualized by the reaction of the peroxidase with the substrate.

A polymerase chain reaction (PCR) to amplify the segments of the RNA was carried out in real time scale. The cell culture and organ suspensions were examined for the presence of the CSF viral genome by the PCR in real time (real-time RT-PCR, one step, TagMan), one-step according to Protocol of the Reference Laboratory for CSF of EU. For RNA extraction was used the test QIAamp Vital RNA Mini Kit, Qiagen Hilden (Germany). The initial volume of the biological material was 140 μl, and the elution volume – 60 μl.

For amplification of PCR was used the test Qiagen OneStep RT-PCR Kit in a total volume of 25 μl, and template volume of 5 μl. In the PCR were used primers A 11 and A14, and probe TaqMan Probe–FAM–Tamra.

PCR studies were carried out with a thermo cycler machine "Applied Biosystems 7300 Real Time PCR System" with the temperature control for reverse transcription at t = +50 ^{0}C – 30:00 min, inactivation of reverse transcriptase and activation of Taq at t = +95 ^{0}C – 15:00 min, denaturation at t = 95 ^{0}C – 00:10 min, extension at t = +60 ^{0}C – 00:30 min for 40 cycles.

The second study on the antimicrobial activity of anolyte/catholyte was performed at the Institute of Molecular Biology at the Bulgarian Academy of Sciences (BAS). The two solutions were prepared with using the Activator-I, developed at the Institute of Information and Communication Technologies at BAS. For this, drinking water without aditional quantity of NaCl was used. This led to pH = 3.0 and ORP = +480 for the anolyte, and pH = 9.8 and ORP = -180 mV for the catholyte.

The bacterial strain used in these experiments was *E. coli* DH5α with genotype: *fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*. The Colony Forming Units (CFU) technique was used to assess the cellular viability. The bacterial cells were cultivated on the LB-medium (pH = 7.5) with 1 % bactotryptone; 0.5 % yeast extract; 1.0 % NaCl at t = +37 ^{0}C (Maniatis *et al*., 1982). After overnight cultivation of bacteria 100 μl samples of culture liquids were taken, centrifuged for 1 min at 10000 g and the pellet of bacterial cells was resuspended in 100 μl of the anolyte or the catholyte. As control samples were used the bacterial samples, re-suspended in non-electroactivated water. Different dilutions of cells were spread on LB-agar Petri plates. After the overnight incubation at t = +7 ^{0}C

the appeared bacterial colonies were counted. The viable cells were calculated as a percentage from the CFU. The CFU obtained from culture liguids treated with non-electrochemically activated water were accepted as 100 %.

NES and DNES methods were used for the estimation of energy of hydrogen bonds of anolyte, catholyte and deionized water in order to make a supposition about the spectrum characteristics. The device measures the angle of evaporation of water drops from 72^{0} to 0^{0}. As the main estimation criterion was used the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water's samples. The spectrum of water was measured in the range of energy of hydrogen bonds 0.08–0.387 eV or λ = 8.9–13.8 μm with using a specially designed computer program.

3. Results and discussions

3.1. Research into the effects of electrochemical NaCl solution (anolyte) on the CSF virus

As shown in Figure 2 the cytoplasm of the cells infected by the CSF virus (positive reaction) was stained in dark reddish brown color, whereas in the uninfected cells it was colorless. That indicates on the presence of the viral antigen in the samples.

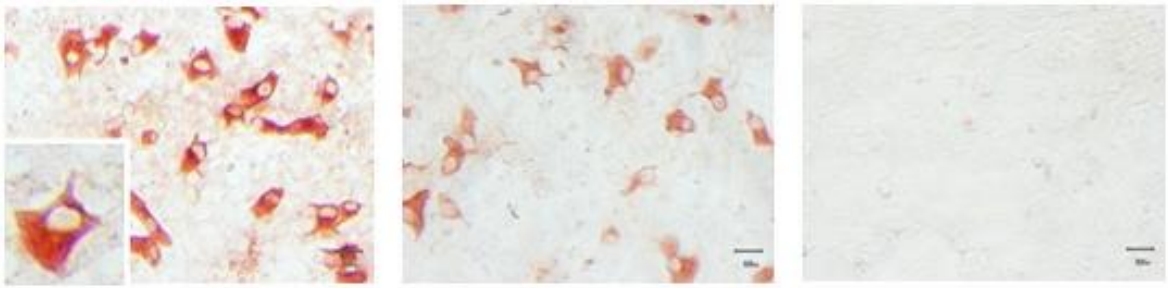

Figure. 2. The established presence of the viral antigen (left) and a negative control (right).

Table 1 summarizes the results of different experiments. Upon treatment of the viral inoculum of the CSF with the anolyte in a 1:1 dilution, there was no viral growth in the four infected wells of the plate, upon 1:2 dilution there was no growth in two of the wells, the other two were reported as positive. Upon treatment with the anolyte at dilutions 1:3 and 1:4, the result was identical: no growth in one of the contaminated wells of the plate, and poor growth – in the other three. The results obtained by infection of a cell monolayer and cell suspension with CSF virus were identical.

Table 1: Virucidal action of the anolyte on the cell culture suspension of the CSF virus upon infecting cell monolayer PK-15

Contamination of CC with:	Dilutions of anolyte (100 μl)	Total volume of the inoculum (μl)	Concentration of anolyte in %	Number of wells	Result: positive/negative
Virus 200 μl	–	200		4	4/0
Virus 100 μl	1:1	200	25	4	0/4

Virus 100 µl	1:2	200	16.51	4	2/2
Virus 100 µl	1:3	200	12.5	4	3/1
Virus 100 µl	1:4	200	10	4	3/1

Table 2 summarizes the results of studies aimed at the evaluation of the virucidal effect of the anolyte on organ suspension containing CSF virus upon infecting a cell monolayer PK-15. Upon treatment of the viral inoculum (organ suspension) of the CSF with the anolyte in all dilutions, there is no viral growth in the four infected wells of the plate.

Table 2: The virucidal action of the anolyte on organ suspension containing CSF virus upon infecting cell monolayer PK-15

Contamination of CC with:	**Dilutions of anolyte (100 µl)**	**Total volume of the inoculum (µl)**	**Concentration of anolyte in %**	**Number of wells**	**Result: positive/negative**
Virus 200 µl	–	200		4	4/0
Virus 100 µl	1:1	200	50	4	0/4
Virus 50 µl	3:1	200	75	4	0/4
Virus 25 µl	7:1	200	87	4	0/4
Virus 12,5 µl	15:1	200	94	4	0/4

Judging from these data, the anolyte has a destructive influence on the envelope of the CSF virus, wherein the main cell antigens (proteins) are localized. Studies of the viral inocula used in the tests with using the PCR reaction in real time demonstrate the presence of a viral genome (RNA) in them, remarkably, also after the treatment with the anolyte. Some shortening of the time is proved (the decreased number of amplification cycles), required for the formation of a fluorescent signal, respectively, a positive reaction for genome, closely correlated with the exposure of the treatment with the viral inocula. The longer the exposure of the processing time with the anolyte, the sooner the presence of the viral RNA in the PCR reaction is detrected. According to one of the co-authors (S. Karadzhov), this is an indirect indication that the anolyte destroys the virus envelope, which, in its turn, facilitates the extraction of nucleic acid and its more rapid reading by the fluorescent signal. There is still no sufficient convincing evidence on the impact of different concentrations of the anolyte on viral CSF particles. The similar experiments carried out by Russian and German researchers were dealt mainly with the concentrated anolyte (Zinkevich et al., 2000). The full virucidal effect confirms our opinion for a strong virucidal action of electrochemically activated aqueous solution of NaCl. The differences in results obtained by us are due to the use of lower concentrations of active substances in our experiments. We attribute essential significance to the fact that we determined the concentration limit (25 %) of a well demonstrated by a virucidal activity. Further studies to reduce the time of action, and the conducting of experiments in the presence of biofilm that protects viruses would be promising.

3.2. Research into the effects of anolyte and catholyte on a strain of *E. coli DH5a*

In order to assess the effect, if any, of the electrochemically activated water solutions on bacterial cells, we treated cultures of *E. coli DH5a* by the catholyte. After the treatment of bacterial cells the colony appearing on agar plates were accepted as resulted from survived cells and counted CFU. Therefore, the number of colonies was presented on Figure 3 as percentage of viable cells. It can be seen from the figure that bacterial cells treated with the catholyte hardly survive the treatment. Only approximately 15 % of the cells survived. This clearly shows that the electrochemically activated water from the cathode possesses a strong bacteriocidal activity. Notably, the anolyte has also shown slight antibacterial effect. Thus, approximately, 73 % of the bacterial cells survived the treatment with it. In summary, both types of activated water possess antibacterial effect, however it is obvious that the catholyte has a stronger bacteriocide effect than the anolyte.

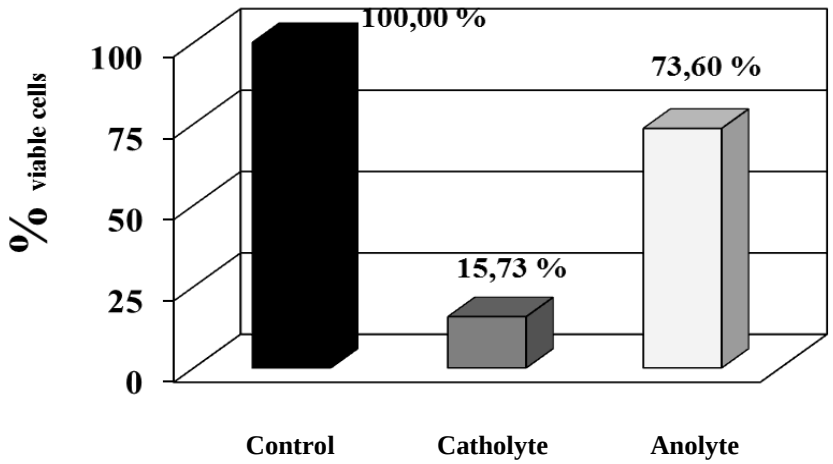

Figure 3. Percentage of viable cells of *E. coli DH5a* after the treatment with catholyte and anolyte relative to the non-electroactivated water.

Figure 4 shows the dependence between the acidity and basicity (pH) of electrochemically activated solution of NaCl and the oxidation-reduction potential (ORP). The pH value within the interval from 3 to 10 units and the ORP within the interval from -400 mV to +900 mV characterize the area of the biosphere of microorganisms. Outside these ranges of pH and ORP the microorganisms will hardly survive. The disinfecting effect in this case is strengthened by the residual chlorine in electrochemically activated solution of NaCl, destructing unsaturated fatty acids, phospholipids and protein in the cell membrane.

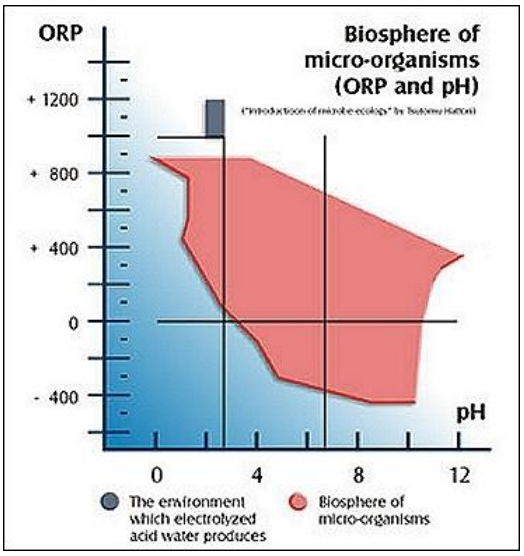

Figure 4. The dependence between acidity and basicity (pH) of NaCl solution and the ORP on the biosphere of micro-organisms.

3.3. Research into biophysical effects of the anolyte and catholyte with NES and DNES spectral methods

Other method for obtaining the useful information about the structural changes in water and the average energy of hydrogen bonds among individual H_2O molecules in samples is the measuring of the energy spectrum of the water state (ESWS). It was established experimentally that at the evaporation of the water droplet the contact angle θ decreases discretely to zero, whereas the diameter of the droplet changes insignificantly (Antonov, 2005). By measuring this angle within a regular time intervals a functional dependence f(θ) can be determined, which is determined by the ESWS (Ignatov, 2005; Ignatov, 2012; Ignatov & Mosin, 2013). For practical purposes by registering the ESWS it is possible to obtain information about the averaged energy of hydrogen bonds in an aqueous sample. For this purpose the model of W. Luck was used, which consider water as an associated liquid, consisted of O–H…O–H groups (Luck *et al*., 1980). The major part of these groups is designated by the energy of hydrogen bonds (-E), while the others are free (E=0). The energy distribution function f(E) is measured in electron-volts (eV^{-1}) and may be varied under the influence of various external factors on water as temperature and pressure.
For calculation of the function f(E) experimental dependence between the water surface tension measured by the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = bf(\theta) / [1 - (1 + bE)^2]^{1/2}, \qquad (8)$$

where $b = 14.33\ eV^{-1}$; $\theta = arcos(1 - bE)$

The energy of hydrogen bonds (E) measured in electron-volts (eV) is designated by the spectrum of energy

distribution. This spectrum is characterized by non-equilibrium process of water droplets evaporation, thus the term "non-equilibrium energy spectrum of water" (NES) is applied.

The difference Δf(E) = f (samples of water) – f (control sample of water)

– is designated the "differential non-equilibrium energy spectrum of water" (DNES).

The DNES-spectrum measured in milielectron volts (0.001 eV) is a measure of changes in the structure of water as a result of external factors. Figure 5 shows the characteristical NES-spectrum of deionized water measured with using 25 independence measurements done in a period of one year.

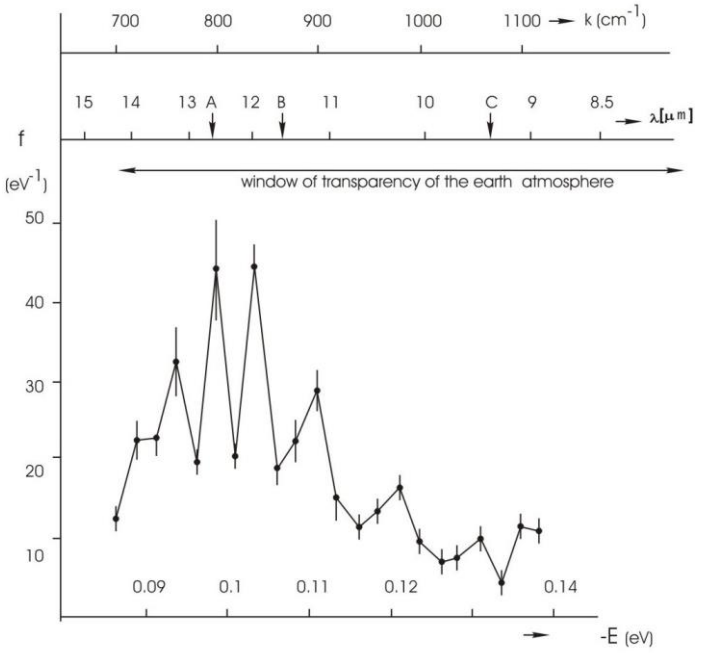

Figure 5. NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6.5–7.5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm). The horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV). The vertical axis – energy distribution function – f (eV^{-1}). k – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm).

The average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds among individual molecules H_2O was calculated for the catholyte and anolyte by NES- and DNES-methods. We studied the distribution of local maximums in the catholyte and anolyte solutions of the electrochemically activated water. The local maximum for the catholyte in the NES-spectrum was detected at -0.1285 eV, for the anolyte – at -0.1227 eV and for the control sample of deionized water – at -0.1245 eV. The calculations of $\Delta E_{H...O}$ for the catholyte with using the DNES method compiles (-0.004±0.0011 eV), and for the anolyte (+1.8±0.0011 eV). These results suggest the restructuring of $\Delta E_{H...O}$ values among individual H_2O molecules with a statistically reliable increase of local maximuns in DNES-spectra of the catholyte and the anolyte (Table 3).

For the catholyte the biggest local maximum was detected at -0.1387 eV, or at 8.95 μm. In 1992 A. Antonov performed experiments with the impact of different types of water on tumor mice cells. It was detected a decrease in the NES-spectrum compared with the control sample of cells from healthy mice. At

the same time there was a decrease of the local maximum at -0.1387 eV, or 8.95 μm. In the DNES-spectrum the local maximum at 8.95 μm was with the negative value. It should be noted that for the catholyte the local extremum in the DNES-spectrum was detected with the positive value at 133.3 eV^{-1}.

For the catholyte the biggest local maximum was at -0.1312 eV, or 9.45 μm. It should be noted that for the treatment of influenza part of medical drugs composes aluminum hydroxide $Al(OH)_3$. The local maximum in this case was detected at -0.1326 eV, or at 9.35 μm.

Table 3: Local maximums of catholyte and anolyte solutions in NES- and DNES-spectra (Ignatov & Mosin, 2014).

-E(eV) x-axis	**Catholyte**	**Anolyte** y-axis (eV^{-1})	**Control sample** y-axis (eV^{-1})	**DNES Catholyte**	**DNES Anolyte**	**-E(eV)** x-axis	**Catholyte** y-axis (eV^{-1})	**Anolyte** y-axis (eV^{-1})	**Control Sample** y-axis (eV^{-1})	**DNES Catholyte** y-axis (eV^{-1})	**DNES Anolyte** y-axis (eV^{-1})
0.0937	0	0	0	0	0	0.1187	0	66.7	66.7	-66.7	0
0.0962	0	0	0	0	0	0.1212	66.7	0	0	66.7	0
0.0987	0	0	0	0	0	0.1237	0	0	0	0	0
0.1012	66.7	66.7	33.3	33.4	33.4	0.1262	0	0	66.7	-66.7	-66.7
0.1037	0	0	33.3	-33.3	-33.3	0.1287	0	0	66.7	-66.7	-66.7
0.1062	0	0	0	0	0	0.1312	33.3	100	33.3	0	66.7
0.1087	0	0	0	0	0	0.1337	33.3	33.3	33.3	0	0
0.1112	0	0	0	0	0	0.1362	0	0	0	0	0
0.1137	0	66.7	66.7	-66.7	0	0.1387	200	66.7	66.7	133.3	0
0.1162	0	0	0	0	0	–	–	–	–	–	–

Table 4: Energy distribution of alkaline (catholyte) and acid (anolyte) solutions in electrochemical activation of sodium chloride.

-E(eV) x-axis	**Catholyte** y-axis (%((-Evalue)/ (-Etotal value)	**Anolyte** y-axis (%((-Evalue)/ (-Etotal value)	**-E(eV)** x-axis (%((-Evalue)/ (-Etotal value)	**Catholyte** y-axis (%((-Evalue)/ (-Etotal value)	**Anolyte** y-axis (%((-Evalue)/ (-Etotal value)
0.0937	0	0	0.1187	0	16.7
0.0962	0	0	0.1212	16.7	0
0.0987	0	0	0.1237	0	0
0.1012	16.7	16.7	0.1262	0	0
0.1037	0	0	0.1287	0	0
0.1062	0	0	0.1312	8.4	24.8
0.1087	0	0	0.1337	8.4	8.4

0.1112	0	0	0.1362	0	0
0.1137	0	16.7	0.1387	49.8	16.7
0.1162	0	0	–	–	–

The evaluation of the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies is presented in Table 4. These distributions are basically connected with the restructuring of H_2O molecules with the same energies. This serves as the mathematical model explaining the behavior of anolyte and catholyte regarding the distribution of H_2O molecules to the energies of hydrogen bonds (Ignatov, Mosin, 2012).

5. Conclusions

The experimental results prove the strong influence of different types of electrochemically activated water solutions (catholyte/anolyte) on various microbes and viruses. They are in accordance with the results obtained by other researchers, and demonstrate the strong biocidal effect of the anolyte toward the CSF virus. Also, the interesting results on the antibacterial effect were obtained when a strain of *E. coli DH5a* was treated with the catholyte and anolyte, respectively. Unexpectidely, the catholyte with ORP ≈ -180 mV and pH = 9.8 demonstrated the better biocidal effect than the anolyte with ORP ≈ +500 and pH = 3.9. We tried to relate the antimicrobial and antiviral action of electrochemically activated water with the characteristics of the NES-spectrum. There is an indication about such a connection but more thorough research is needed to prove it. For example, the inverse biocidal effect between the catholyte and anolyte in case of a strain of *E. coli DH5a* requires a clear explanation.

The results of the research are formulated as follows.

1. The anolyte did not affect the growth of the cell culture PK-15;
2. The anolyte administered at a concentration of 25 %, exerts a strong virucidal effect on a cell culture virus, and a weaker antiviral activity at concentrations of 16.51 %, 12.5 % and 10 %;
3. The anolyte exerted a strong virucidal effect at concentrations of 50 %, 75 %, 87 % and 94 % over the CSF virus in cell culture suspensions;
4. The catholyte supresses the growth of *E. coli* up to 85 % while the anolyte is at least three times less effective;
5. The local maximum in the DNES-spectrum of the catholyte was detected at 9.85 μm; there was a decrease of this local maximum in water with mice tumor cells;
6. The local maximum in the DNES-spectrum of the anolyte was detected at 9.45 μm; at 9.35 μm occurred the effect of inflammation from virus of influenza;
7. The mathematical model of the catholyte and anolyte regarding the distribution of H_2O molecules to the energies of hydrogen bonds was evaluated.

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