

Biocidal Effects of the Electrochemically Activated Water

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Abstract

This article outlines the results on the antimicrobial action of electrochemically activated water solutions (anolyte/catholyte), produced in the anode and cathode chamber of the electrolytic cell. Under laboratory conditions the cell culture and suspensions of classical swine fever (CSF) virus were treated with the anolyte. After 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; viral growth during the infection of a cell monolayer with a cell culture virus was affected in the greatest degree by the anolyte in 1:1 dilution and less in other dilutions; whereas the viral growth at the infection of a cell suspension with cell culture virus was affected by the anolyte in dilution 1:1 in the greatest degree, and less by other dilutions; viral growth at the infection with a virus in suspension of the cell monolayer was affected by the anolyte in all dilutions. Unexpectedly, the stronger biocidal effect of the catholyte was observed when a strain of *E. coli DH5* was treated by the anolyte and

catholyte, respectively. In order to provide additional data about the antiviral activity of the electrochemically activated water and the distribution of H₂O molecules according to the energies of hydrogen bonds, the non-equilibrium energy spectrum (NES) and differential non-equilibrium energy spectrum (DNES) of the anolyte and catholyte were measured.

Keywords: anolyte, catholyte, *E. coli DH5*, CSF virus, NES, DNES

1. Introduction

The phenomenon of electrochemical activation of water (EAW) is a set of electrochemical and electrical processes that 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. In 1985 EAW was officially recognized as a new class of physical and chemical phenomena. As a result of the 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, accompanied by electrochemical processes and characterized by the abnormal activity levels of electrons, the redox potential, and other physico-chemical parameters (pH, E_h, ORP).

The main stage of electrochemical treatment of water is the electrolysis of water or aqueous solutions with low mineralization as aqueous solutions of 0,5–1,0 % sodium chloride (NaCl), which occurs in the electrolysis cell, consisting of a cathode and an anode separated by a special semipermeable membrane (diaphragm) which separates water to alkaline fraction – the catholyte and acidic fraction – the anolyte (Figure 1). When the electric current is passing through the water, the flow of electrons from the cathode as well as the removal of electrons from the water at the anode is accompanied by series of redox reactions on the surface of the electrodes. As a result, new elements are being formed, the system of intermolecular interactions, as well as the composition of water and the water structure are changed.

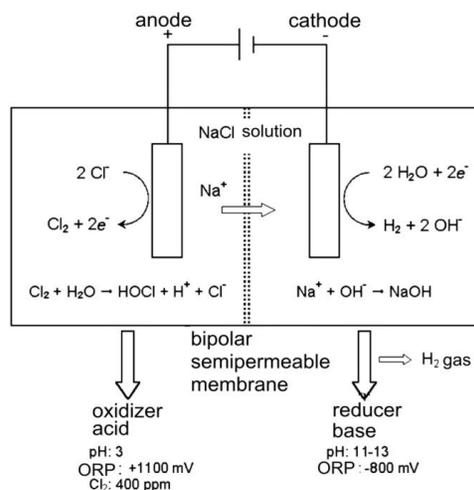


Figure 1. The diaphragm electrolysis method for the preparation of acid (anolyte) and alkali (catholyte)

solutions via the electrochemical activation of sodium chloride

The products of electrode reactions are the neutralized aqueous admixtures, gaseous hydrogen and oxygen generated during the electrolytic destruction of H₂O molecules, metal cations (Al³⁺, Fe²⁺, Fe³⁺) in the case of metal anodes made of aluminum and steel, and molecular chlorine. The gaseous hydrogen is generated at the cathode while the oxygen is produced at the anode. Water also contains a certain amount of hydronium ions (H₃O⁺) depolarizing at the cathode with formation of the atomic hydrogen:



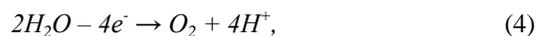
In an alkaline environment a disruption of H₂O molecules, accompanied by formation of atomic hydrogen and hydroxide ion (OH⁻) occurs:



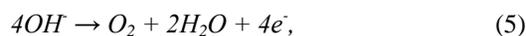
The reactive hydrogen atoms are adsorbed on the surface of the cathode, and molecular hydrogen H₂, released in the gaseous form after recombination are formed:



At the same time atomic oxygen is released at the anode. In an acidic environment, this process is accompanied by the destruction of H₂O molecules:



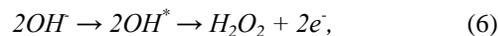
In an alkaline environment the OH⁻ ions moving under the electrophoresis from the cathode to the anode are a source of oxygen:



The normal redox potentials of these reactions compile +1,23 V and +0,403 V, respectively, but the process takes place in certain conditions of electric overload.

The cathodes are made of metals that require high electrical voltage (lead, cadmium) allowing for the generation of reactive free radicals as Cl[•], O[•], OH[•], HO₂[•], which react chemically with other radicals and ions.

In bulk oxidative processes products of electrolysis of water – oxygen (O₂), hydrogen peroxide (H₂O₂) and hydrochloric acid (HClO) play a special role. During the electrolysis, an extremely reactive compound H₂O₂ is formed. Its formation occurs due to the hydroxyl radicals (OH[•]) which are products of the discharge of hydroxyl ions (OH⁻) at the anode:



where OH[•] is the hydroxyl radical.

The chlorine-anion is transformed to Cl₂:



Gaseous Cl₂ forms highly active oxidants: Cl₂O; ClO₂; ClO₂⁻; HClO; Cl[•]; HO₂[•]. The parameters of pH, the redox potential, ORP and the electrical conductivity of the anolyte/catholyte depend on different factors including the ratio of water volumes in the two electric chambers, the material of electrodes, NaCl concentration, the temperature, electric voltage and processing time.

The electrolysis cell can be regarded as a generator of the above mentioned products, some of them, entering into a chemical interaction with each other and water impurities in the interelectrode space, provide additional chemical treatment of water (electrophoresis, electroflotation, electrocoagulation). 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 volume processes. Generally, they are initiated with the increase in both: the temperature of water during the electrolysis process and the pH value.

As a result the water treatment at the cathode (catholyte) becomes alkaline: its ORP decreases, the surface tension is reduced, the amount of dissolved oxygen is decreased, the concentration of hydrogen, hydroxyl ions (OH^-), increases, the conductivity of water decreases, the structure of hydration shells of ions changes. By external characteristics the catholyte appears as a soft, light liquid with an alkaline taste of $\text{pH} = 10\text{--}11$ and $\text{ORP} = -200\text{...}-800$ mV, sometimes with white sediment.

On physical and chemical parameters the catholyte has the significantly enhanced electron-donating properties, and getting into the physiological fluids of an organism can enhance the electron-background for a few tens of millivolts. The catholyte reportedly has antioxidant, immunostimulating, detoxifying properties, normalizing ORP, metabolic processes (increases the ATP synthesis, modification of enzyme activity), stimulates the regeneration of tissues, increases the DNA synthesis and stimulates the growth and division of cells by increasing the mass transfer of ions and molecules across the cell membrane, improves trophic processes in tissues and blood circulation. It was also reported that catholyte with the redox potential at $-700\text{...}-100$ mV favors the development of anaerobes, whereas the anolyte with the redox potential at $+200\text{...}+750$ mV supports the growth of aerobes. 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, there is bacteriostatic effect only.

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

C – alkaline catholyte ($\text{pH} > 9,0$; $\text{ORP} = 700\text{--}820$ mV), the active components – NaOH , O_2 , HO_2^- , HO_2^* , OH^- , OH^* , HO_2^- , O_2 ;

CN – neutral catholyte ($\text{pH} = 9,0$; $\text{ORP} = 300\text{--}500$ mV), the active components – O_2 , HO_2^- , HO_2^* , H_2O_2 , H^+ , OH^- .

As a result of the anode (anolyte) treatment the water obtains acidic reaction, the ORP increases slightly, the surface tension is slightly reduced, the conductivity increases, the amount of the dissolved oxygen and chlorine in the water also increases, whereas the amount of hydrogen decreases. The anolyte is a brownish, acidic water with a characteristic odor and taste, and $\text{pH} = 4\text{--}5$ and $\text{ORP} = +500\text{...}+1100$ mV. The specific anolyte toxicity when being administered in the stomach and applying to the skin refers to the 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 being heated to 50°C the bactericidal activity of the anolyte is increased by 30–100 %.

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

A – acidic anolyte ($\text{pH} < 5,0$; $\text{ORP} = +800\text{...}+1200$ mV), the active components – HClO , Cl_2 , HCl , HO_2^* ;

AN – neutral anolyte ($\text{pH} = 6,0$; $\text{ORP} = +600\text{...}+900$ mV), the active components – HClO , O_3 , HO^- , HO_2^* ;

ANK – neutral anolyte (pH = 7,7; ORP = +250...+800 mV), the active components – HClO, ClO⁻, HO₂⁻, H₂O₂, O₂, Cl⁻, HO*;

ANKD – neutral anolyte (pH = 7,3; ORP = +700...+1100 mV), the active components – HClO, HClO₂, ClO⁻, ClO₂^{*}, HO₂^{*}, H₂O₂, O₂, O₃, Cl⁻, HO⁻, O*.

The anolyte has antibacterial, antiviral, antifungal, anti-allergic, anti-inflammatory, antiedematous and antipruritic effect, may be cytotoxic and may have antimetabolite action without harming the human tissue cells. 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, as staphylococcal Enterotoxin-A. One of them is the classical swine fever (CSF), prevalent in different regions of the world and 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 °C, and over 6 weeks at 4 °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 °C for 10 min, it is able to withstand at least 30 min at t = 68 °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 J.A. Sands and U.S. Springthorpe, 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. It is thought that to achieve the effective electrochemical disinfection it is necessary to irreversibly damage the RNA.

Investigations conducted by other authors were carried out with *E. coli*, using as a disinfectant 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, a full destruction of proteins, DNA and RNA was occurred. Supposedly the anolyte enters the cells provoking structural and functional damages on the cell's membrane and cell's wall. Similar research was performed by S.V. Kumar et al. 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 logCFU/ml) or fully destroyed (8,0 logCFU/ml) after 2 to 10 min inactivation by the anolyte in the temperature range from 4 °C to 23 °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 yet not been completely evaluated. Therefore, the purpose of this research was to study the virucidal effect: 1) of the anolyte in different dilutions on classical swine fever virus in cell culture and organ suspensions; 2) of the anolyte/catholyte on a strain of *E. coli DH5a*, and 3) to determine how the virocidal effect relates to local maximums in NES-spectra of the anolyte and catholyte¹.

2. Materials and methods

The studies of antiviral activity of the anolyte were performed at the National Reference Laboratory of 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. 0,3 % solution of chemically pure sodium chloride (NaCl) in distilled water was used. The anolyte had pH = 3,2 and ORP = +1070 mV. The interaction of the anolyte with the virus suspension was carried out at a temperature of 22 °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 the standard cell culture test virus 2,3 (Bulgaria) with a cell titre 107,25 TCID₅₀/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} TCID₅₀ 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 the infection of a cell monolayer, the mixture was removed after the end of the exposure period of 1 h. Upon the infection of a cell suspension, the mixture was not removed.

To establish the virucidal activity of the anolyte on the CSF virus in the suspension, a different dilution was used: the inoculum was mixed directly with the concentrated anolyte in anolyte-inoculum ratios 1:1; 3:1; 7:1 and 15:1 respectively. Since it is known that the growth of the 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 cm³ of tissue, which was homogenized in a mortar with 9 ml of the cell culture medium containing antibiotics, in order to obtain 10 % of organ suspension. Sterile sand was added to improve the homogenization. The samples were kept at room temperature for 1 h, after that they were centrifuged for 15 min at 2500 g. The supernatant was used to infect the cells. In case of cytotoxic effect, the parallel dilutions of the homogenates were prepared in proportions 1:10 and 1:100. From the suspensions into multi well

¹ Such a dependence was established between the local maximum (-0,1387 eV; 8,95 µm) in the NES-spectrum of the catholyte that suppresses the development of tumor cells (Ignatov & Mosin, 2014).

(24-well) plates were added 200 μl of the inoculum to cells with coverage of 50–80 %. Cell cultures were incubated at $t = 37\text{ }^{\circ}\text{C}$ for 1 h in order to “capture” an eventual virus if presented, then 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 the anolyte did not induce a cytotoxic effect.

Cell cultures were incubated for 72–96 h at $t = 37\text{ }^{\circ}\text{C}$ 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 virus of CSF. The immunoperoxidase technique was used. The fixation of the plates was carried out thermally for 3 h at $t = 80\text{ }^{\circ}\text{C}$ in a desiccator. In the processing was used a primary monoclonal antibody C 16 diluted in proportion 1:50, and secondary antibody RAMPO diluted in proportion 1:50. For the 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 peroxidase reaction with the substrate.

A polymerase chain reaction (PCR) was carried out in real time scale. The cell culture and organ suspensions were examined for the presence of CSF viral genome by the polymerase chain reaction 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 Viral RNA Mini Kit, Qiagen Hilden (Germany). The initial volume of the biological material was 140 μl , and the volume of elution – 60 μl .

For amplification of PCR the test Qiagen OneStep RT-PCR Kit in a total volume of 25 μl , and template volume of 5 μl was used. In the reaction were included 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 $t = 50\text{ }^{\circ}\text{C} - 30:00$ min, inactivation of reverse transcriptase and activation of Taq at $t = 95\text{ }^{\circ}\text{C} - 15:00$ min, denaturation at $t = 95\text{ }^{\circ}\text{C} - 00:10$ min, extension at $t = 60\text{ }^{\circ}\text{C} - 00:30$ min for 40 cycles.

The second study on the antimicrobial activity of anolyte/catholyte was performed at the Institute of Molecular Biology of the Bulgarian Academy of Sciences (BAS). The two solutions were prepared using the Activator-I, developed at the Institute of Information and Communication Technologies at BAS. For this, drinking water without additional quantity of NaCl was used. This led to $\text{pH} = 3,0$ and $\text{ORP} = +480$ mV for the anolyte, and $\text{pH} = 9,8$ and $\text{ORP} = -180$ mV for the catholyte.

Bacterial strain used in these experiments was *E. coli* DH5a with genotype: *fhuA2 lac(del)U169 phoA glnV44 Φ 80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*.

The assay Colony Forming Units (CFU) was used to assess cellular viability. The conditions for the bacterial cultures growth. The bacterial cells were cultivated on the LB-medium ($\text{pH} = 7,5$) with 1 % bactotryptone; 0,5 % yeast extract; 1,0 % NaCl at $t = 37\text{ }^{\circ}\text{C}$. 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. Bacterial samples re-suspended in non-activated water were used as control samples. Different dilutions of cells were spread on LB-agar Petri plates. After the overnight incubation at $t = 37\text{ }^{\circ}\text{C}$ the appeared bacterial colonies were counted. The viable cells were calculated as a percentage from the CFU. The CFU obtained from culture liquids treated with non-electrochemically activated water were accepted as 100 %.

The NES method was used for the estimation of energy of hydrogen bonds of anolyte, catholyte and

deionized water in order to make a supposition about the spectrum peculiarities. The device measures the angle of evaporation of water drops from 72° to 0° . The average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between individual H_2O molecules in water's samples was used as the main estimation criterion. The spectrum of water was measured in the range of energy of hydrogen bonds 0,08–0,387 eV or $\lambda = 8,9-13,8 \mu m$ with using a specially designed computer program.

3. Results and discussions

3.1. Research into the effects of electro-activated aqueous sodium chloride (anolyte) on the classical swine fever virus

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



Figure. 2. The established presence of viral antigen in cell cultures (left) and a negative control sample (right).

Table 1 summarizes the results of different experiments of the virucidal action of the anolyte on the cell culture suspension of the CSF virus upon infecting cell monolayer PK-15. As is shown in Table 1, upon treatment of the viral inoculum 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 were identical.

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 with the virus. According to the data, upon treatment of the viral inoculum (organ suspension) with the anolyte in all dilutions, there is no viral growth in the four infected wells of the plate.

Table 1: Virucidal action of the anolyte on cell culture suspensions 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:
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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: The virucidal action of the anolyte on organ suspensions 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

Evidently, the anolyte has a destructive influence on the envelope of the CSF virus, where the main antigens (proteins) are localized. Studies of the viral inocula used in the tests by means of polymerase chain reaction (PCR) in real time demonstrate the presence of a genome (RNA) in them, 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 under the treatment of the viral inocula. The longer the exposure of processing with the anolyte, the sooner the presence of the RNA virus in the PCR was detected. According to one of our co-authors (Stoil Karadzhev), this may serve as an indirect indication that anolyte destroys the virus envelope, which, in its turn, facilitates the extraction of RNA and its more rapid reading by the fluorescent signal. However, there is still no sufficient convincing evidence on the impact of different concentrations of the anolyte on viral particles. Experiments carried out by Russian and German researchers were mainly with the concentrated anolyte. The maximum virucidal effect detected in those experiments confirmed a strong virucidal action of the electrochemically activated aqueous solution of NaCl. The difference in the results evidently is due to the use of lower concentrations of NaCl in our experiments. We attributed essential significance to the fact that we determined the concentration limit (25 %) of the well demonstrated by the virucidal activity. In this aspect the further studies on reducing the time of action, and the conducting of experiments in the presence of biofilms which protect viruses would be promising.

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

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 catholyte and anolyte. After the treatment of bacterial cells the colonies appearing on the plates with 2 % agar were obtained, produced by survived cells and further counted by the

CFU method. Therefore, the number of colonies was presented on figure 3 as a percentage of viable cells. It can be seen from the figure 3 that bacterial cells treated with the catholyte hardly survived the treatment with only approximately 15 % of the cells being survived. This clearly shows that the electrochemically activated water produced from the cathode possesses a strong bacteriocidal activity.

Notably, the anolyte also showed slight antibacterial effect. Thus, approximately, 73 % of the bacterial cells survived the electrochemical treatment with the anolyte. In summary, it is clear that the both types of the electrochemically activated water solutions (catholyte/anolyte) possess antibacterial effect, however it is obvious that the catholyte has a stronger bacteriocide effect than the anolyte.

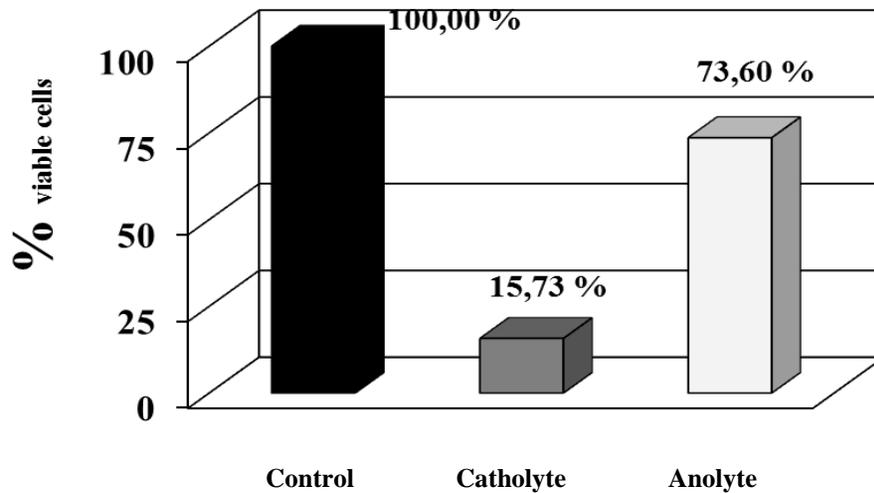


Figure 3. Percentage of viable cells of *E. coli DH5a* after the electrochemical treatment with the catholyte and anolyte relative to the non-electrochemically activated water.

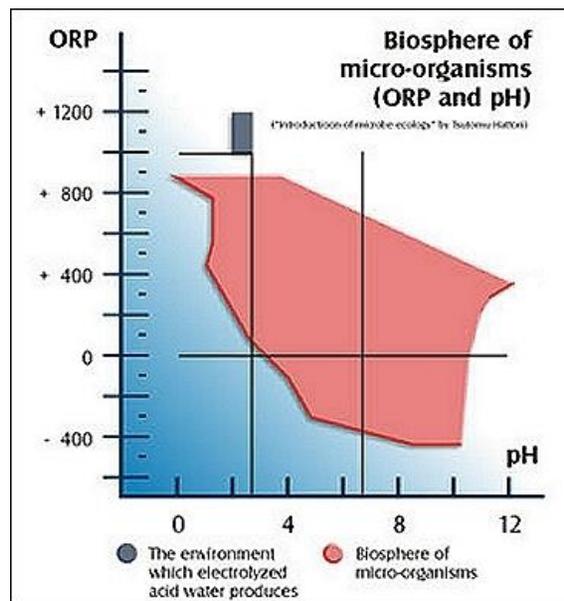


Figure 4. The dependence between the acidity and basicity (pH) of electrochemically activated solution of NaCl and the oxidation-reduction potential (ORP) on the biosphere of microorganisms.

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 the microorganisms will hardly survive. The disinfecting effect is strengthened by the residual chlorine destructing fat acids and protein in the cell membrane.

3.3. NES and DNES methods in spectral analysis of the anolyte and catholyte

Other method for obtaining useful information about the structural changes in water and the average energy of hydrogen bonds is the measuring of the energy spectrum of the water state. It was established experimentally that at evaporation of water droplet the contact angle θ decreases discretely to zero, whereas the diameter of the droplet changes insignificantly. By measuring this angle within a regular time intervals a functional dependence $f(\theta)$ can be determined, which is designated as “the spectrum of the water state”. For practical purposes by registering the spectrum of water state 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 [29]. 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:

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

where $b = 14,33 \text{ eV}^{-1}$; $\theta = \arccos(1 - bE)$

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 $\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water})$ – is designated as the “differential non-equilibrium energy spectrum of water” (DNES) [30].

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 characteristic NES-spectrum of deionized water made from 25 independence measurements performed in a period of one year.

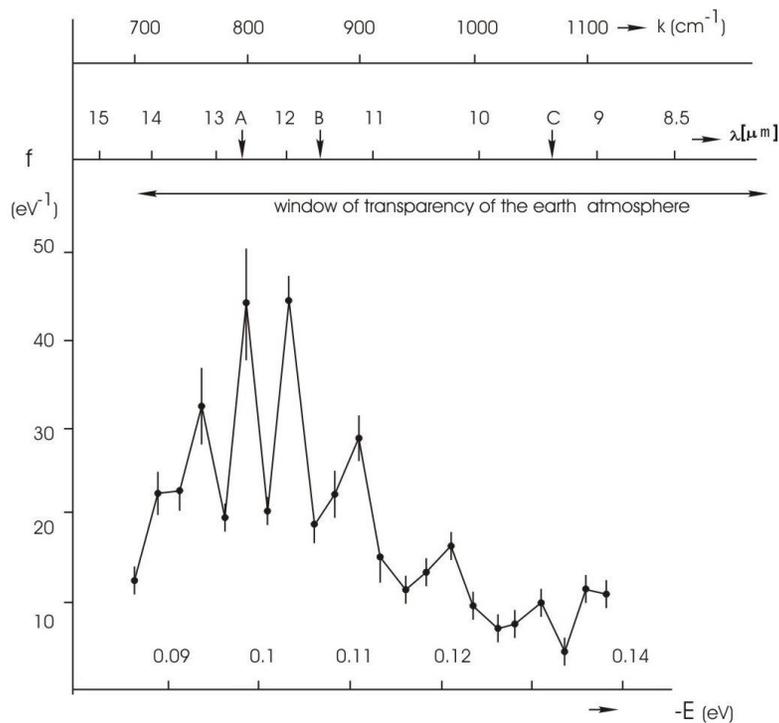


Figure 5. NES-spectrum of deionized water (chemical purity – 99,99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm). The horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV). The vertical axis – energy distribution function – f (eV⁻¹). 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 H₂O was calculated

for the catholyte and anolyte by NES- and DNES-methods. We studied the distribution of local maximums in catholyte and anolyte solutions. The local maximum for catholyte in the NES-spectrum was detected at -0,1285 eV, for 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 anolyte (+1,8±0,0011 eV). These results suggest the restructuring of $\Delta E_{H...O}$ values among individual H₂O molecules with a statistically reliable increase of local extremums DNES-spectra of the catholyte and 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 a decrease in the NES-spectrum compared with the control sample of cells from healthy mice. There was a decrease of the local extremum at -0,1387 eV, or 8,95 μm in DNES-spectra. Notably, was that the local extremums at 8,95 μm was with a negative value. It should be noted that for the catholyte the local extremums in the DNES-spectrum was detected with the positive value at +133,3 eV⁻¹.

For the catholyte the biggest local maximum in the DNES-spectrum was detected at -0,1312 eV, or 9,45 μm. The aluminum hydroxide Al(OH)₃ is a part of medical drugs for the influenza treatment. The local maximum in this case was measured at -0,1326 eV, or at 9,35 μm.

The evaluation of the possible number of hydrogen bonds as percent of H₂O molecules with different values of distribution of energies is presented in Table 4. These distributions are basically connected with the restructuring of H₂O molecules with the same energies. This serves as a base for evaluating the mathematical model explaining the behavior of the anolyte and catholyte regarding the distribution of H₂O molecules to the energies of hydrogen bonds.

Table 3: Local extremums of catholyte and anolyte solutions in NES- and DNES-spectra

-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)
0,0937	0	0	0	0	0	0,1187	0	66,7	66,7	-66,7
0,0962	0	0	0	0	0	0,1212	66,7	0	0	66,7
0,0987	0	0	0	0	0	0,1237	0	0	0	0
0,1012	66,7	66,7	33,3	33,4	33,4	0,1262	0	0	66,7	-66,7
0,1037	0	0	33,3	-33,3	-33,3	0,1287	0	0	66,7	-66,7
0,1062	0	0	0	0	0	0,1312	33,3	100	33,3	0
0,1087	0	0	0	0	0	0,1337	33,3	33,3	33,3	0
0,1112	0	0	0	0	0	0,1362	0	0	0	0
0,1137	0	66,7	66,7	-66,7	0	0,1387	200	66,7	66,7	133,3
0,1162	0	0	0	0	0	–	–	–	–	–

Table 4: Energy distribution of catholyte and anolyte solutions in electrochemical activation of sodium

chloride

-E(eV) x-axis	Catholyte y-axis, % (-E _{value})/ (-E _{total value})	Anolyte y-axis, % (-E _{value})/ (-E _{total value})	-E(eV) x-axis, % (-E _{value})/ (-E _{total value})	Catholyte y-axis, % (-E _{value})/ (-E _{total value})	Anolyte y-axis, % (-E _{value})/ (-E _{total 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	-	-	-

5. Conclusion

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 catholyte and anolyte, respectively. Unexpectedly, the catholyte with ORP \approx -180 mV and pH = 9,8 demonstrated better biocidal effect than the anolyte with ORP \approx +500 and pH = 3,9. We tried to relate the antimicrobial and antiviral action of electrochemically activated water with the characteristics of the energy 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 could be 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 suppresses 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 μ m; 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 μ m; at 9,35 μ m

- occurred the effect of inflammation from virus of influenza;
7. The mathematical model of the catholyte and anolyte regarding the distribution of H₂O molecules to the energies of hydrogen bonds was evaluated.

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