

Journal of Medicine, Physiology and Biophysics
ISSN 2422-8427 An International Peer-reviewed Journal
Vol. 13 2015

Preparation of Electrochemically Activated Water Solutions (Catholyte/Anolyte) and Studying Their Physical-Chemical Properties

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Abstract

The electrochemical treatment of water by the electric current is a promising modern approach in the water disinfection technique, resulting in obtaining the electrochemically activated water solutions (catholyte/anolyte) carrying new physical-chemical properties stipulated by changing of the electrochemical characteristics of water as ORP, E_h , pH. The electrochemically activated water solutions – the catholyte and anolyte can be used in medicine for treatment of various bacterial and viral diseases and in disinfection of water. The process of electrochemical water treatment includes several electrochemical processes associated with the transfer in a constant electric field the electrons, ions and other charged particles (electrolysis, electrophoresis, electroflotation, electrocoagulation), the main of which is the electrolysis of water. This article deals with the review of basic physical-chemical processes underlying the electrolysis of water and preparation of electrochemically activated water solutions (catholyte/anolyte) and studying their physical-chemical properties. The virucidal action of the anolyte and catholyte was studied on cell culture and suspensions of classical swine fever (CSF) virus.

Key words: electrochemical treatment of water, anolyte, catholyte, electrolysis, classical swine fever (CSF) virus.

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 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 physical-chemical parameters (pH, E_h , ORP) (Kirpichnikov *et al.*, 1986).

During the EAW occur four main processes:

- 1) Electrolytical decomposition of water by electrolysis on account of redox reactions on the electrodes due to the external electric field;
- 2) Electrophoresis – the movement in the electric field of a positively charged particles and ions toward the cathode and negatively charged particles and ions toward the anode;
- 3) Electroflotation – the gas formation and flocculation of aggregates consisting of fine-dispersed gas bubbles (H_2 at the cathode and O_2 at the anode) and suspended solids in water;
- 4) Electrocoagulation – the formation of colloidal aggregates of particles of deposited disperse phase through a process of anode dissolution of the metal and the formation of metal cations Al^{3+} , Fe^{2+} , Fe^{3+} under the influence of electric field.

The electrochemical processes, which occur at the passage of the direct electric current through the water volume, are accompanied as a result of redox reactions leading to coagulation of colloids, flocculation of suspended solids and subsequent flotation. The advantages of electrochemical water treatment is that it allows to correct the pH value and redox potential E_h , on which depends the possibility of occurrence of various chemical processes in water; increases the enzymatic activity of activated sludge in aeration tanks; reduces the resistivity and improves coagulation and sedimentation of organic sediments from water.

The purpose of this research was the investigation of the process of electrolysis of water, as well as the properties of the electrochemically activated water solutions – the anolyte and catholyte.

2. Material and Methods

The experiments were conducted with the diaphragm electrolysis apparatus “Wasserionisierer Hybrid PWI 2100”, equipped with four titanium electrodes coated with platinum. The voltage of the electric power supply – 220 V, the frequency of the electric current – 50 Hz, the power of the electric current 0.2–0.7 A; the time of electro processing – 30–40 min; the volume of the electroactivated water solutions: anolyte – 0.3 l; catholyte – 0.9 l; power consumption – 70 Watts.

The electrolysis cell was formed by two electrodes – a positively charged anode and a negatively charged cathode connected to different poles to a DC source. Interelectrode space was filled with water, which is an electrolyte capable of conducting the electrical current, or with 0.3 % solution of chemically

pure NaCl in distilled H₂O.

The anolyte had pH = 3,2 and ORP = +1070 mV; the active components – HClO, Cl₂, HCl, HO₂*;

The catholyte had pH = 9,0 and ORP = -300...-500 mV); the active components – O₂, HO₂⁻, HO₂*⁻, H₂O₂, H⁺, OH⁻.

The studies of the antiviral activity of the anolyte and catholyte were performed with the classic swine fever (CSF) virus and a strain of *E. coli* DH5α 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) as described in paper (Ignatov *et al.*, 2014).

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 of the anolyte, 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 the inocula in proportion 1:1 (100 µl of the CSF virus suspension and 100 µl of the appropriate anolyte concentration). Upon the infection of a cell monolayer, the mixture was removed after the end of the exposure period lasted for 1 h. Upon the infection of a cell suspension, the mixture, otherwise, 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 (24-well) plates were added 200 µl of the inoculums with coverage of 50–80 %. Cell cultures were incubated at t = +37 °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.

The cell cultures were incubated for 72–96 h at t = +37 °C in a CO₂ incubator. The procedure with preparation of the positive and negative control samples was similar. The positive control sample was a reference strain of the CSF virus. The immunoperoxidase technique with using a horseradish peroxidase was used for the enzymatic detection of antigen-antibody complexes in cell cultures. The fixation of the plates was carried out thermally for ~3 h at t = +80 °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 % H₂O₂ 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) 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 Viral RNA Mini Kit, Qiagen Hilden (Germany). The initial volume of the biological material was 140 μ l, and the elution volume – 60 μ l.

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

Bacterial strain used in the experiment was *E. coli DH5 α* . 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 1.0 % bactotryptone; 0.5 % yeast extract; 1.0 % NaCl at $t = +37^{\circ}\text{C}$. After overnight cultivation of bacteria 100 μ l samples of culture liquids were taken, centrifuged for 1 min at 10000 g and the pellet of bacterial cells was resuspended in 100 μ l 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 $t = +7^{\circ}\text{C}$ 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 %.

3. Results and Discussion

3.1. Electrolysis of water

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 % NaCl (Morita *et al.*, 2000), which occurs in the electrolysis cell, consisting of the cathode and the 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 passing of 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 anode (Petrushanko *et al.*, 2001). As the result, new elements are formed, the system of intermolecular interactions, as well as the composition of water and the water structure are changed (Dykstra, 1999; Mosin, 2012).

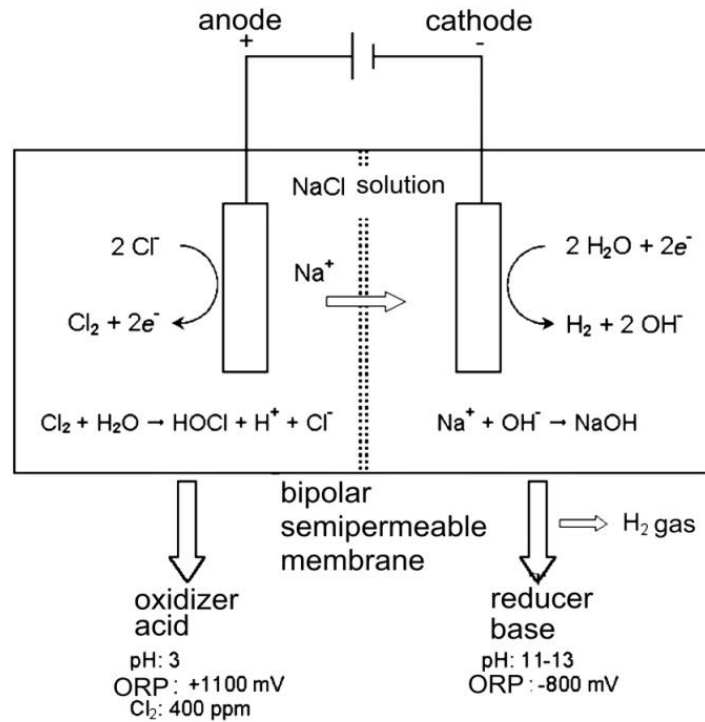


Figure 1. The diaphragm electrolysis method for the preparation of acid (anolyte) and alkali (catholyte) solutions through the electrochemical activation of NaCl

The typical apparatus for electrochemical treatment of water comprises water preparation unit (1), the electrolyzer (2), the processing unit after the electrochemical treatment of water (3) (Figure 1).

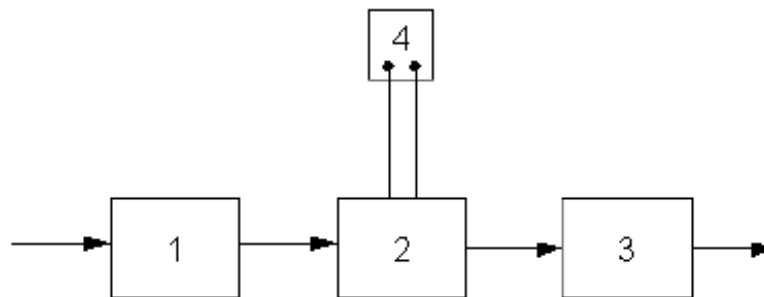


Figure 2. The apparatus for electrochemical water treatment: 1 – water treatment unit; 2 – electrolyzer; 3 – the block of post-treatment; 4 – rectifier of electric current.

The main element of the apparatus is electrolyzer consisting of one or more electrolysis cells (Figure 2). The electrolysis cell is formed by two electrodes – a positively charged anode and a negatively

charged cathode connected to different poles to a DC source (Stoner, 1982). Interelectrode space is filled with water, which is an electrolyte capable of conducting electrical current. As a result it is transferred electric charges through the water – electrophoresis, i.e. migration of the polar particle charge carriers - ions for the electrode having an opposite sign. Wherein the negatively charged anions are moved toward the anode, whereas the positively charged cations are moved toward the cathode. At electrodes the charged ions lose charge and become depolarized, turning into the decay products. In addition to these charged ions, in the electrophoresis participate the polar particles with different particle sizes, including solid particles (emulsified particles, gas bubbles, etc.), but the main role in the transfer of electrochemical charges play the ions possessed by the greatest mobility.

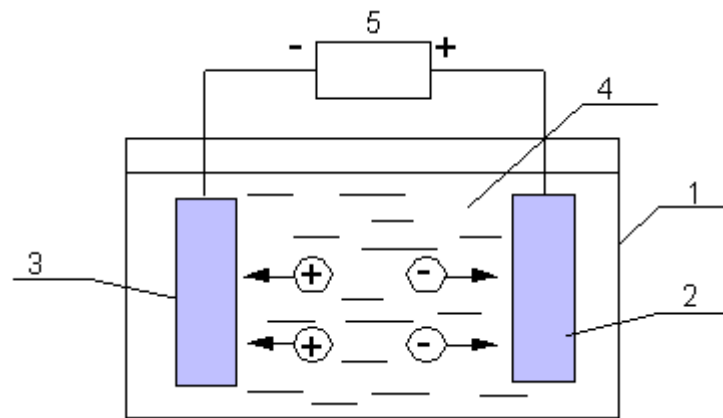


Figure 3. Scheme of the electrolysis cell: 1 – the case; 2 – anode; 3 – cathode; 4 – interelectrode space; 5 – DC power source.

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



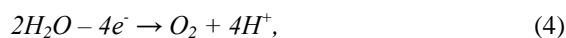
In an alkaline environment there occurs the disruption of H_2O molecules, accompanied by formation of the atomic hydrogen and hydroxide ion (OH^-):



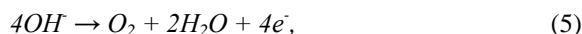
The reactive hydrogen atoms are adsorbed on the surfaces of the cathode, and after recombination are formed the molecular hydrogen H_2 , released in the gaseous form:



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:



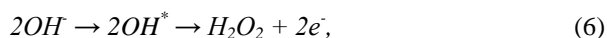
In an alkaline environment, the source of oxygen source is OH^- ions, moving under the electrophoresis from the cathode to the anode:



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), 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_2) and hydrochlorine acid ($HClO$). During the electrolysis, an extremely reactive compound 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:



where OH^* – the hydroxyl radical.

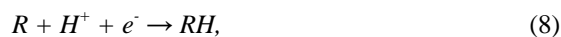
The chlorine-anion is transformed to Cl_2 :



Gaseous Cl_2 forms highly active oxidants: Cl_2O ; ClO_2 ; ClO^- ; $HClO$; Cl^* ; HO_2^* . 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 (Bahir, 1992).

The electrolysis cell can be regarded as a generator of the above mentioned products, some of them, entering into the chemical interaction with each other and water impurities in the interelectrode space, providing additional chemical treatment of water (electrophoresis, electroflotation, electrocoagulation) (Hsu, 2005). 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 with increasing the temperature of water during the electrolysis process and with increasing the pH value.

There are distinguished the cathodic and anodic oxidation. When the cathodic oxidation the organic molecules absorbed on cathodes, accepting free electrons and reduced. The reduction process usually takes place in one step:



where R – the organic compound; RH – the hydrated form of a compound.

In other cases, the cathodic reduction takes place in two stages: at the first stage (9) the organic molecule is converted into an anion, in the second (10) – the hydrated anion interacts with the proton of H_2O water:



The cathodes made of materials that require high electrical voltage (lead, cadmium), allow for large amounts of electricity to generate the reactive free radicals – particles having on the outer orbits of atoms or molecules free unpaired electrons (Cl^* , O^* , OH^* , HO_2^*). The latter circumstance makes the free radicals the reactivity, i.e. to react chemically with other radicals and ions.

At the anodic oxidation the organic molecules, adsorbed on the anode, give up electrons to simultaneous or prior hydration:



The anodic oxidation of organic compounds often results in the formation of free radicals, which further transformations is defined by their reactivity. Anodic oxidation processes are multistage and proceed with the formation of intermediate products. Anodic oxidation lowers the chemical resistance of organic compounds and facilitates their subsequent destruction in volume processes.

The rate of the anodic oxidation depends on the temperature and the pH value. Often in the process of oxidation of organic compounds are formed intermediates, which differ from the original compounds by the resistance to further transformations and indicators of toxicity.

The source of active chlorine and its oxygen-containing compounds are chlorides generated in the electrolyser, and NaCl, which is added into the electrochemically treated water before the electrolysis. As a result of the anodic oxidation of Cl⁻ anions is generated the gaseous Cl₂. Depending on the pH value Cl₂ is either hydrolyzed to form hypochlorous acid (HOCl), or forms hypochlorite ions (ClO⁻). The equilibrium of the reaction depends on the pH value; at pH = 4–5 all chlorine is present in the form of HClO, and at pH = 7 – half in the form of ClO⁻ ion and half – in the form of HClO (Figure 4).

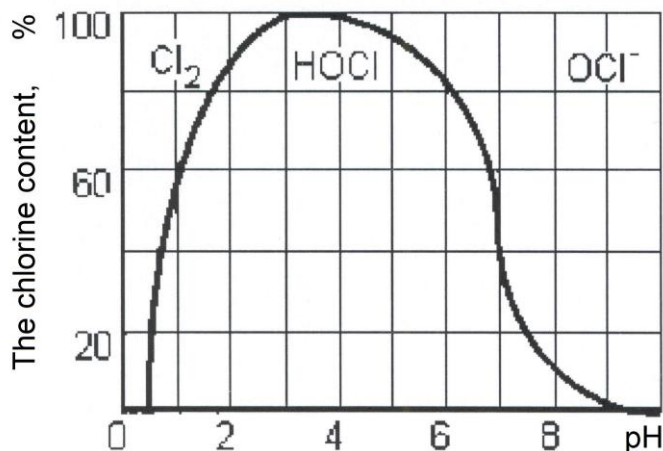


Figure 4. Content (%) of various forms of chlorine (Cl₂, HOCl, OCl⁻) in the electrochemically activated water depending on the pH value

The mechanism of interaction between the hypochlorite ions (ClO⁻) with the oxidizing agent described by the following equation:



where A – the oxidizing substances; C – the oxidation product.

The electrochemical oxidation of organic compounds by hypochlorite ions (ClO⁻) is accompanied by an increase in the redox potential E_h, indicating the predominance of oxidative processes (Bahir, 2001). The E_h value growth depends on the ratio of active chlorine concentration in the interelectrode space to the

content of organic impurities in water. As the purification and reducing the amount of impurities, the ratio increases, which leads to an increase of E_h , but after some time the rate stabilizes.

The amount of substance reacted at the electrodes by passing a constant electric current through the Faraday's law, is directly proportional to the current strength and the time of the electrochemical treatment:

$$G = A I_{cur} t, \quad (13)$$

where A – the electrochemical equivalent of an element (g/A·h); I_{cur} – the amperage (A); t – the processing time (h).

The electrochemical equivalent of an element is defined by the formula:

$$A = \frac{M}{26,8 z}, \quad (14)$$

where M – the atomic mass of the element (g); z – the valence.

The values of the electrochemical equivalents of some elements are shown in Table. 1.

Table 1: Electrochemical equivalents of some elements

Element	The electrochemical equivalent of an element, g/A·h
H ₂	0.0376
O ₂	0.289
Fe (II)	1.042
Fe (III)	0.695
Al (III)	0.336
Cr (III)	0.647
Cr (VI)	0.324
Cu (II)	1.186
Zn (II)	1.22
Cl ₂	1.324
Ca (II)	0.748

The actual amount of a substance, generated during the electrolysis is less than the theoretical, calculated from the formula (13) as part of the electric power is expended on heating the electrodes and water. Therefore, at calculations take into account the current efficiency $\eta < 1$, the value of which is determined experimentally.

The electrode processes are accompanied by an exchange of charged particles and ions between the electrodes and the electrolyte – water. For this the equilibrium must be established to provide an electric

potential minimum value, which depends on the sort of the redox reaction and the water temperature at +25 °C (Table 2).

Table 2: The electrode potentials of some elements

Electrode reaction	Electric voltage [V]	Electrode reaction	Electric voltage [V]
$\text{Al} \rightarrow \text{Al}^{3+} + 3\text{e}^-$	-1.66	$\text{Cu} \rightarrow \text{Cu}^{2+} + \text{e}^-$	+0.345
$\text{Zn} \rightarrow \text{Zn}^{2+} + 2\text{e}^-$	-0.763	$4\text{OH}^- \rightarrow 2\text{H}_2\text{O} + \text{O}_2 + 4\text{e}^-$	+0.401
$\text{Fe} \rightarrow \text{Fe}^{2+} + 2\text{e}^-$	-0.44	$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$	+1.23
$\text{Cd} \rightarrow \text{Cd}^{2+} + 2\text{e}^-$	-0.403	$2\text{Cl}^- \rightarrow \text{Cl}_2 + 2\text{e}^-$	+1.36
$\text{H}_2 \rightarrow 2\text{H}^+ + 2\text{e}^-$	0.0001	$\text{Cl}^- + \text{H}_2\text{O} \rightarrow \text{HClO} + \text{H}^+ + 2\text{e}^-$	+1.49

The electrical voltage generated in the electrode cell, should be sufficient to cause oxidation-reduction reactions at the electrodes. The voltage depends on the ionic composition of water, the presence of impurities in water, such as detergents, the electric current density (its power per unit area of the electrode), the electrode material, and others. Other things being equal the task of selecting the electrode material is to undergo the oxidation-reduction reactions at the electrodes, the voltage required to be minimized since it reduces the cost of electricity.

Some redox reactions are competing – they occur simultaneously and mutually inhibit each others. Their flow can be regulated by changing the electric voltage in the electrolytic cell. Thus, the normal potential of the reaction of formation of molecular oxygen is +0.401 V or +1.23 V; when the voltage increases to +1.36 V (the normal potential of the reaction of formation of molecular chlorine) at the anode will be allocated only oxygen, and at the further increase in capacity – both oxygen and chlorine, and the evolution of chlorine will occur with insufficient intensity. At the voltage +5 V the oxygen evolution will be almost ceased, and the electrolytic cell will only generate chlorine.

3.2. Calculation the basic parameters of the electrolysis of water

The basic parameters of the electrolysis include the amperage and the density of the electric current, the voltage in the electrode cell, as well as the velocity and the residence time of water in the interelectrode space.

The amperage I_{cur} – a value determined depending on the desired capacity of the generated product, as determined by the formula:

$$I_{cur} = \frac{G}{At\eta} \quad (15)$$

This formula is obtained by transformation the formula (13), based on current utilization factor η .

The density of the electric current – the amperage per unit area of the electrode (A/m^2), for example, for the anode is determined by the formula:

$$i_{an} = \frac{I_{cur}}{F_{an}} \quad (16)$$

where F_{an} – the anode area (m^2).

The density of the electric current has a decisive effect on the electrolysis: with the increasing of the density of the electric current the electrode processes are intensified and electrode surface area is reduced, but also increases the voltage in the electrolytic cell and as a consequence of the process – the energy consumption. The increased increase in the density of electric current in its turn intensifies the release of electrolysis gases, leading to the wildness and dispersion of insoluble products of electroprocessing of water. With increasing the density of electric current is also enhanced the passivation of electrode consisting in blocking the incoming electrons by the surface deposits of the anode and cathode, which increases the electrical resistance of the electrode cells and inhibits the redox reactions occurring at the electrodes.

Anodes are passivated by the formation on their surfaces the thin oxide films due to adsorption of oxygen at the anodes and other components which, in turn, adsorb the particles of other impurities. At the cathode are formed mainly the calcareous deposits, especially in the case of treating water with high hardness. For these reasons, the density of electrical current during the electrolysis of water should be administered the minimal at the conditions necessary for sustainable flow of the redox reactions during the process.

The voltage in the electrode cell is determined by the formula:

$$V_{\bar{x}} = \frac{1}{\chi_R} i_{an} \cdot \Delta \cdot K_z \quad (17)$$

where i_{an} – the density of electric current (A/m^2); Δ – the distance between electrodes (width of the interelectrode channel) (m); χ_R – the electrical conductivity of water, $(Ohm \cdot m)^{-1}$; K_z – the coefficient of the gas filling of the interelectrode space, $K_z = 1,05-1,2$.

Formula (17) does not take into account the electrical resistance of the electrode due to their low values, but while the passivation these resistances are significant. The width of the electrode channel is accepted minimal (3–20 mm) under the terms of clogging by impurities.

The electrical conductivity of water χ_R depends on a number of factors, among which the most important are the temperature, pH, ionic composition and concentration of ions (Figure 5). With increasing the temperature χ_R increases and the voltage on the contrary decreases (Figure 6). The minimum value of the electrical conductivity corresponds to a value of pH=7. In addition, the electrolysis process takes place under the rise of temperature and pH. If pH>7, then we can expect a decrease in the electrical conductivity of water χ_R , whereas at pH<7, the electrical conductivity of water χ_R , on the contrary, increases (Figure 5).

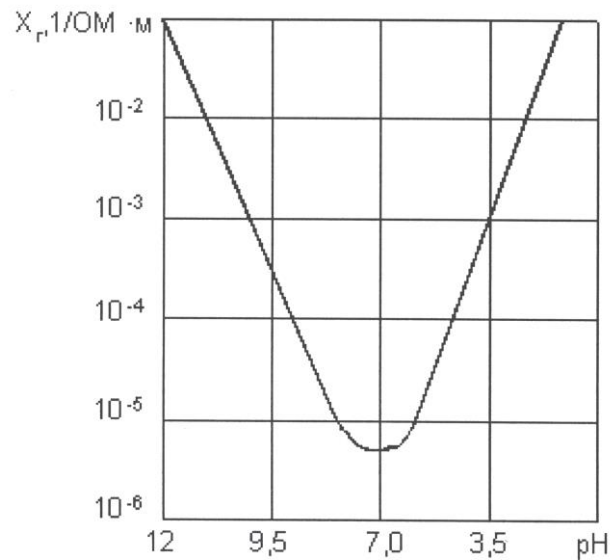


Figure 5. The dependence of the electrical conductivity on the pH of water at $t = +5\text{ }^{\circ}\text{C}$

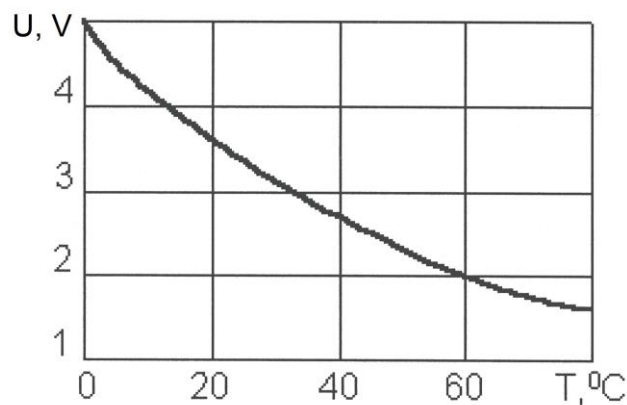


Figure 6. Dependence of the electrical voltage on the electrodes on the water temperature.

The electrical conductivity of natural water of medium mineralization makes up $0,001\text{--}0,005\text{ (Ohm}\cdot\text{m)}^{-1}$, municipal waste water – $10.0\text{--}0.01\text{ (Ohm}\cdot\text{m)}^{-1}$ (Tanaka *et al.*, 1996). In the electrolysis the electrical conductivity should be between $0.1\text{--}1.0\text{ (Ohm}\cdot\text{m)}^{-1}$ (Aider, 2012). If the initial water has the insufficient electrical conductivity the salinity should be increased (Figure 7). Typically this is performed using NaCl, which doses are determined experimentally and often comprise $500\text{--}1500\text{ mg/l}$ ($8\text{--}25\text{ mEq/l}$) (Kloss, 1988). NaCl is not only suitable for the conditions of use and safety (storage, preparation of solution, etc.), but in the presence of NaCl the passivation of the electrodes is slowed down. Being dissociated in water, NaCl saturates water with Cl^- anions and Na^+ cations. Cl^- anions are small and penetrating through the passivation deposition to the surface of the anode thus destroying these deposits. In the presence of other anions, particularly sulphate ions (SO_4^{2-}), the depassivation influence of Cl^- anions decreases. The stable

operation of the electrolysis cell is possible if the Cl^- anions are at least 30 % of the total anions in water. Na^+ cations are moved as a result of the electrophoresis to the cathode, at which are generated hydroxide ions OH^- , and interacting with the latter, form sodium hydroxide (NaOH) which dissolves the carbonate deposits on the cathode.

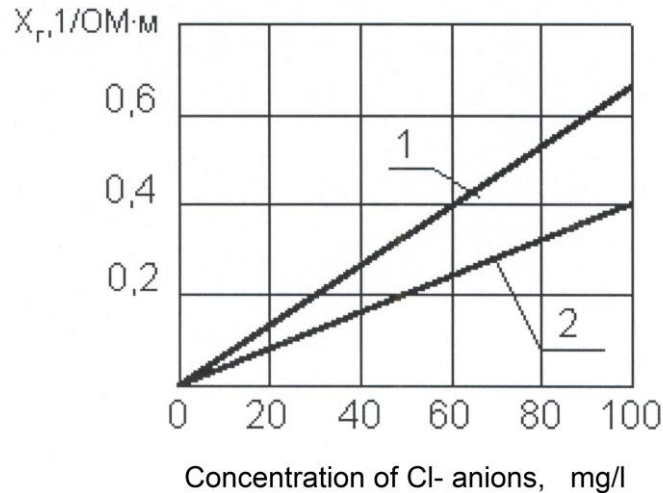


Figure 7. The dependence of electric conductivity on the concentration of Cl^- anions: 1 – at $t = +25\text{ }^\circ\text{C}$; 2 – at $t = +5\text{ }^\circ\text{C}$

Power consumption (W) of the electrolysis cell is determined by the relation:

$$N_{\text{nomp}} = \eta_{\text{э}} I_{\text{cur}} V_{\text{э}} \quad (18)$$

where $\eta_{\text{э}}$ – the efficiency of the electrolysis cell; $\eta_{\text{э}} = 0,7-0,8$; I_{cur} – the amperage (A); $V_{\text{э}}$ – the voltage (V).

The length of stay of water in the interelectrode space of the electrolysis cell is limited by the time required to generate the required amount of electrolysis products, as well as by the duration of the corresponding bulk reactions, and is determined experimentally.

The velocity of movement of water in the interelectrode space is calculated with regard to the conditions of the electrolytic removal of electrolysis products and other impurities; moreover, the velocity of movement of water depends on the turbulent mixing of water, which affects the bulk reactions. As the residence time of water in the interelectrode space, the water velocity is determined on the basis of experimental data.

During the electrolysis of water are generated H_2 and O_2 . The mixture of these gases is explosive, and hydrogen-air mixture has explosive concentrations of hydrogen at 4.0 vol.%. Under the terms of the security electrolyzers are equipped with the exhaust ventilation systems that provide air dilution of the produced H_2 at concentration of less than 0.4 vol.%, i.e., 10 times lower than the threshold explosiveness.

The capacity of ventilation systems for continuous electrolyzers (m^3/h) is calculated by the formula:

In the case of batch electrolyzers the formula becomes the view:

$$Q_{air} \geq 0,1 I_{cur} \frac{W_{el} (273 + T)}{t \cdot 273} \quad (19)$$

where W_{el} – the useful capacity of the electrode chamber (m^3); t – duration of electrochemical treatment (h).

In cases when the outdoor electrolytic cell is used and the generated hydrogen flows directly into the room, the ventilation rate (h^{-1}) is calculated from the formula:

$$m_g = \frac{Q_{air}}{W} \quad (20)$$

where W – the room volume (m^3).

Electrolysis of water is always accompanied by evolution of heat and heating of water, which may affect the conditions for its further use or release of water into the system.

The consumption of heat during the electrolysis of water (kJ/h) equals:

$$Q_T = 3,62 I_{cur} (V_{\vartheta} + \Delta V) \quad (21)$$

where ΔV – voltage of decomposition of NaCl. If $i_{an} = 200 \text{ A/m}^2$, so $\Delta V = 2.1 \text{ V}$.

The temperature ($^{\circ}\text{C}$) of water in the electrolysis is determined from the formula:

$$\Delta T = \frac{Q_T \cdot 10^{-3}}{C q_w} \quad (22)$$

where C – volumetric heat capacity of water ($\text{kJ}/(l^{\circ}\text{C})$); q_w – the consumption of water (m^3/h).

3.3. The physical-chemical properties of the catholyte and the anolyte

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 (Shimada *et al.*, 2000). By external characteristics the catholyte – is a soft, light, with an alkaline taste liquid, sometimes with white sediment; its $\text{pH} = 10\text{--}11$, $\text{ORP} = -200\text{...}-800 \text{ mV}$.

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 (Toropkov *et al.*, 2001). 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 (Petrushanko & Lobyshev, 2004). It was also reported that catholyte with the redox potential at $-700\text{...}-100 \text{ mV}$ favors the development of anaerobes, whereas the anolyte with the redox potential at $+200\text{...}+750 \text{ mV}$

mV supports the growth of aerobs (Prilutsky & Bakhir, 1997). The antibacterial effect of the catholite 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 the catholite, 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, O₂, HO₂⁻, HO₂^{*}, OH⁻, OH^{*}, HO₂⁻, O₂;

CN – the neutral catholyte (pH = 9.0; ORP = -300...-500 mV), the active components – O₂, HO₂⁻, HO₂^{*}, H₂O₂, 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. 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 (Yahagi *et al.*, 2000; Inoue *et al.*, 1997). 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 % (Leonov *et al.*, 1999b).

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, Cl₂, HCl, HO₂^{*};

AN – the neutral anolyte (pH = 6.0; ORP = +600...+900 mV), the active components – HClO, O₃, HO⁻, HO₂^{*};

ANK – the neutral anolyte (pH = 7.7; ORP = +250...+800 mV), the active components – HClO, ClO⁻, HO₂⁻, H₂O₂, O₂, Cl⁻, HO^{*};

ANKD – the 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 antimetabolite action without harming the human tissue cells (Kirkpatrick, 2009). 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 (Suzuki *et al.*, 2002). 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 (Edwards, A. & Edwards, S, 2000). 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 the 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 (Sands *et al.*, 1979) and U.S. Springthorpe (Springthorpe & Sattar, 1990), the effective disinfection of viruses whose infectivity is associated with the elements of the virus casing is achieved by disinfectants dissolving poly-saturated fats, fatty acids, lipids, protein and proteases. It is thought that to achieve the effective electrochemical disinfection it is necessary to irreversibly damage the RNA (Kim *et al.*, 2000).

Investigations conducted by other authors (Zinkevich *et al.*, 2000) were carried out with *E. coli*, using as a disinfectant the anolyte with the ORP equal or greater than +1100 mV and pH = 5.5, obtained via electrolysis of diluted NaCl solution on 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 the destruction of proteins, DNA and RNA. Supposedly the anolyte enters the cells, provoking structural and functional damages on the cell's membrane.

Similar research was performed by S.V. Kumar (Kumar *et al.*, 1999) in order to evaluate the inactivation efficacy of the anolyte at pH = 2.7 and ORP = +1100 mV on *E. coli O157:H7*, *Salmonella enteritidis* and *Listeria monocytogenes*. As it was demonstrated on five strains of *E. coli E06* (milk), E08 (meat), E10 (meat), E16 (meat) and E22 (calf feces), all pathogens were significantly reduced (7.0 log CFU/ml) or fully destroyed (8.0 log CFU/ml) after 2 to 10 min inactivation by the anolyte within 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 into the cell and further destroy it. 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.

The virucidal action of the anolyte was studied by us on cell culture and suspensions of classical swine fever (CSF) (Atanasov *et al.*, 2014; Karadzhev *et al.*, 2014). 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 the CSF 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.

Table 3 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 3, 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 CSF virus a cell monolayer and cell suspension were identical.

Table 4 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 CSF viral inoculum (organ suspension) with the anolyte in all dilutions, there was no viral growth in the four infected wells of the plate.

Table 3: The 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 (%)	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 4: 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 (%)	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 antigens (proteins) are localized. Studies of the viral inocula used in the tests by means of polymerase chain reaction (PCR) in real time demonstrated the presence of a genome (RNA) in them, also after the treatment with the anolyte. Some shortening of the time was proved (the decreased number of amplification cycles), required for the formation of a fluorescent signal, respectively, a positive reaction for a 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 viral RNA in the PCR was detected. According to one of our co-authors (Stoil Karadzhov), this may serve as an indirect indication that the

anolyte destroys the CSF virus envelope, which, in its turn, facilitates the extraction of viral 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 CSF viral particles. The analogous experiments carried out by Russian and German researchers were dealt mainly with the concentrated anolyte (Zinkevich *et al.*, 2000). The maximum virucidal effect detected in those experiments confirmed a strong virucidal action of the electrochemically activated aqueous solution of NaCl on the CSF virus. 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 the virucidal action, and the conducting of experiments in the presence of biofilms which protect viruses would be promising.

In order to assess the effect, if any, of the electrochemically activated water solutions (catholyte/anolyte) on bacterial cells we treated the cultures of a strain of *E. coli DH5a* by the catholyte. After the treatment of bacterial cells the colonies appearing on the plates with 2 % agar were obtained, produced by survived cells, which were further counted by the CFU method. Therefore, the number of colonies was presented on Figure 8 as a percentage of viable cells. It can be seen from Figure 3 that bacterial cells of *E. coli DH5a* 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 on the strain of *E. coli DH5a*.

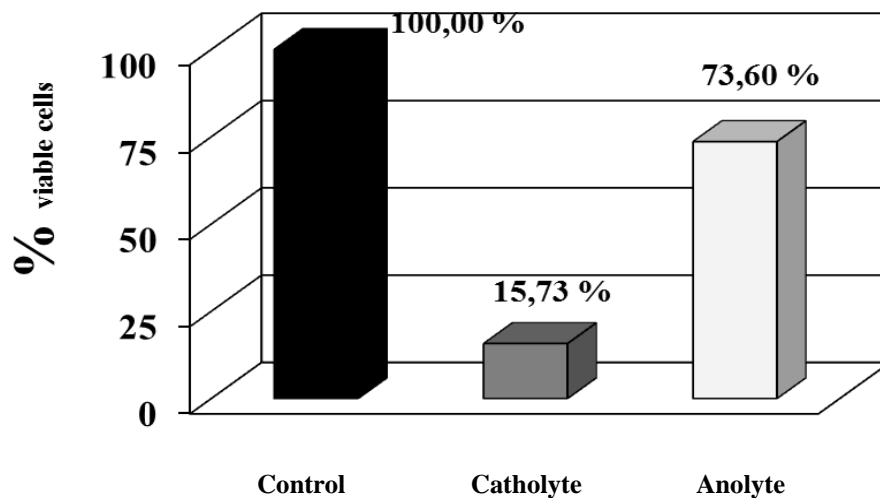


Figure 8. Percentage of viable cells of *E. coli DH5a* after the electrochemical treatment with the catholyte and anolyte relative to the non-electrochemically activated water.

Notably, the anolyte also showed slight antibacterial effect. Thus, approximately, 73 % of the bacterial cells of *E. coli DH5a* survived the electrochemical treatment with the anolyte. In summary, it is assumed that both types of the electrochemically activated water solutions (catholyte/anolyte) possess antibacterial effect on the strain of *E. coli DH5a*, however it is obvious that the catholyte has a stronger bacteriocidal effect than the anolyte.

However, it should be noted that the pharmacological studies of electrochemically activated solutions

of water and their virucidal effects and toxicity have not yet been completely evaluated.

4. Conclusion

The electrochemical water treatment has several advantages compared to alternative chemical methods for disinfection of water. These advantages are the efficiency, stability, controllability and convenient automatic control of the electrolysis processes, as well as simplicity of the construction equipment. The devices for the electrochemical water treatment are compact, have a high level of reliability, easy operation and demand, and may be fully automated. On the other hand, in the electrochemical treatment of water increases the expenditures of the energy consumption. Therefore, the electrochemical treatment is usually more advantageous for devices with low or average productivity (up to 1020 m³/h). In multi-stage schemes to improve the water quality and to carry out its disinfection, the electrochemical treatment can conveniently be combined with other water treatment methods.

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