VI. MAN AND BIOSPHERE

BIOCIDAL ACTION OF ANOLYTE

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Abstract. The anolyte, obtained during electrochemical activation of low mineralized water solutions possesses a strong biocidal action due to its physical-chemical properties. This study presents a brief report for the effect of anolyte application to samples of different kind of microorganisms. An idea of the physical-chemical processes taking place in the anode chamber of an electrolyzer is present. The changes in the parameters pH, ORP and the presence of active chlorine during the time and at different storage conditions of the anolyte were examined. The experiments showed their stability and preservation of biocidal activity for a period longer than 9 months. Experimental results from investigations of the biocidal effect of anolyte carried out in different laboratories are present.

Keywords: electrochemical activation, anolyte, pH, ORP (Oxidation Reduction Potential), biocidal effect, pathogens.

1. INTRODUCTION

Different chemical and physical methods are applied for the protection and preservation of the human health and the health of domestic animals. However, some of them are of low effectiveness and may damage the vital environment of living things. The tendency in development consists in the application of methods that are possible to simultaneously increase the antimicrobial efficacy and diminish the side effects on human end environment. Along with this the continuously growing resistance of pathogens to the applied antimicrobial means becomes a serious world problem [47]. To solve it, alternative approaches have to be developed. In this direction during the last decades the attention of the investigators has been attracted by the anomalous properties of water solutions, since the water plays a vital role in the biochemical and metabolic processes occurring in cells, being a universal polar solvent. It acts as a reagent for many chemical reactions like hydrolysis and oxidation-reduction. In chemical processes water, due to its high ionizing ability, possesses strong amphoteric properties, and can act both as an acid and a base in reactions of chemical exchange.

It was noticed that outside influence alters the water structure, thus changing its behaviour during the interaction with other stuff and living matter. Water molecules undergo significant structural deformation and obtain new qualities referring to physical properties and chemical reaction.

Among the possible factors of influence, the most serious attention is paid to the electrochemical activation (ECA) of water. The interest is determined by the ability of the activated water to stimulate the vital processes in the living matter from one hand, and act as an effective, cheap and harmless disinfectant from the other hand. Also, it was noticed that it preserved the antimicrobial effect at minimal concentration of active compounds, and diminished or fully eliminated the destructive action on the processed objects, i.e. a significant side effect did not exist.

In this direction episodic investigations have been noticed in the middle of the last century by Russian and Japanese scientists, but a serious work started at about 1990's when scientists from Russia, Japan, China and some European countries started with systematic investigation of the properties of the activated water and its influence on the processes in the living matter [1, 5, 26, 27, 41, 42, 45]. The destructive action on some pathogens that excludes the possibility for resistance development was of extreme interest, as well.

The paper presents a brief report of results from scientific investigations regarding the biocidal effect of the anolyte on *E. coli*, Classical swine fever (CSF), pathogens of digestate (effluent of the biogas reactors) and human viruses. Major chemical processes taking place in the anode chamber of an electrolyzer device are described.

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2. ANOLYTE PRODUCTION AND BASIC CHARACTERISTICS

For the production of ECA water a device called electrolyzer is used where the chemical process of electrolysis takes place. Its principal scheme is shown in Fig. 1. It consists of a container of water divided into two chambers by a semipermeable membrane. Titanium or platinum electrodes playing the role of cathode and anode are placed in the chambers, respectively, and a direct current is applied to them.

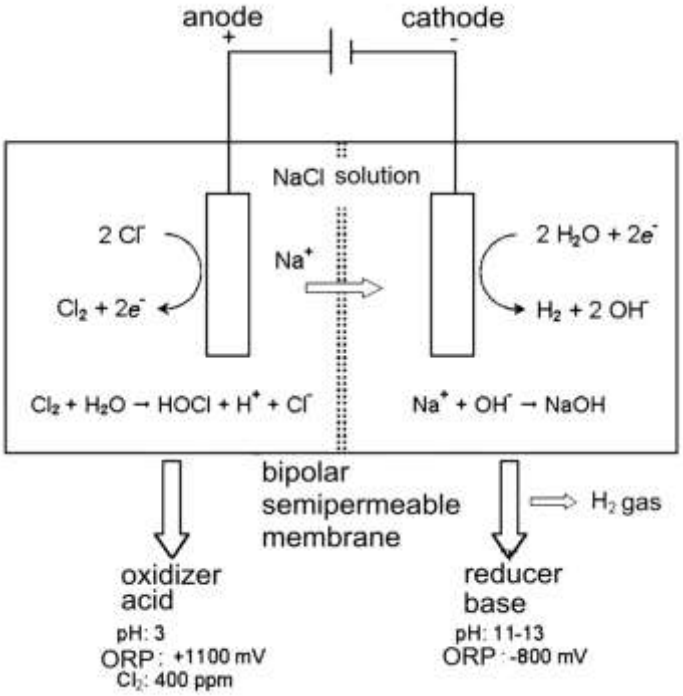

Fig. 1. Laboratory setting for a membrane electrolysis method for the preparation of an acid (anolyte) and alkali (catholyte) solution in electrochemical activation of sodium chloride

The electrolysis process induces essential changes in the water contents and the structure of the end compounds leading to its ionization, restructuring and energy. As a result, two fraction are created in the chambers: acidic one (anolyte) with pH ≈ 3.0 and ORP > 400 mV in the anode chamber, and reducing one (catholyte) with pH > 9.0 и ORP < -100 mV in the cathode chamber.

The higher ORP the stronger is its oxidizing ability. ORP determines the speed and direction of all electrochemical processes in the body, and even their development in general.

There is still a lack of full understanding about the processes in the electrolyzer and their influence on the possibility for storage of the obtained anolyte and catholyte in practice.

Since the paper is focused on the properties of the anolyte and the effects of its application, following chemical processes taking place in the anode chamber are described in the next chapter.

3. CHEMICAL PROCESSES IN THE ANODE CHAMBER

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 oxidation reduction reactions on the surface of the electrodes. As a result, new chemical compounds and different intermolecular interactions are being formed. Due to the electrolysis following reactions can take place at the anode [33].

$$OH^- \rightarrow O^* + 2e^- + H^+ \quad (1)$$

$$O^* + O^* \rightarrow O_2 \quad (2)$$

$$OH^- \rightarrow OH^* + e^- \quad (3)$$

$$OH^* \rightarrow O^* + e^- + H^+ \quad (4)$$

$$2OH^* \rightarrow H\text{-}O\text{-}O\text{-}H \rightarrow (H_2O_2) \quad (5)$$

In bulk oxidative processes products of electrolysis of water – oxygen (O_2), hydrogen peroxide (H_2O_2) and hydrochlorine acid (HClO) play a special role.

During the electrolysis, an extremely reactive compound H_2O_2 is formed [25]. Its formation occurs due to the hydroxyl radicals (OH*) which are products of the discharge of hydroxyl ions (OH^-) at the anode:

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

where OH^* is the hydroxyl radical.

The chlorine-anion is transformed to Cl_2:

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

As a result of these chemical processes a solution of highly active oxidants Cl_2O, ClO_2, HClO, Cl^-, O_2, O_3, HO_2, H_2O_2, OCl^-, Cl_2, H^+ is produced [16, 19, 41, 49]. The oxygen atom in the compound is in a negative first oxidation state. Also, the surface tension and electrical conductivity is increased [14, 15, 17]. The metal electrodes provoke significant changes in the orientation and position of the ions [6]. This water has high ORP and is in active metastable state [41].

The values of pH, ORP, electrical conductivity and viscosity depend on different factors including the ratio of water volumes in the two electric chambers, the parameters of the electrodes, NaCl concentration, temperature, electric voltage and processing time.

The obtained new properties of the anolyte are basic preconditions for its application in medicine, agriculture, stock breeding and industry. [6, 23, 31, 51, 52].

4. BIOCIDAL PROPERTIES

The anolyte has strong antibacterial, antiviral, antifungal, anti-allergic, anti-inflammatory, antiedematous and antipruritic effect [5, 7, 13, 15, 17, 19, 22, 23, 28, 31, 34, 35, 41, 42, 48]. The most active oxidants like chlorine dioxide, ozone, nascent oxygen, hydrogen peroxide and others are among the substances created during the electrolysis [43]. Together with this the values of pH and ORP are of great importance. ORP is fundamental for the microorganism's vitality. Its change leads to destruction of their integrity and as result to their death. However, these values are different for different species. The vital surroundings are between +200mV and +700 mV for the aerobes, and between -700mV and -100mV for the anaerobes. The high concentration of oxidants and free radicals leads to ultra-structural changes in the bacteria cell membrane and to significant morphometric changes of the contour and the size, and to lesions. [22].

There are proofs for the antibacterial effects on Gram-neagtive bacteria [33], *Staphylococus aereus* [38], *Pseudomonas aeruginosa* [39, 45], *E. coli* [45, 46]. For the fungi there are evidences for effects on *Candida albicans* [40, 45]. Other studies show effects on different pathogens [4, 21].

Experiments confirm its bactericidal action and absence of bacterial adaptation and resistance. A biocidal activity against *E. coli, P. aeruginosa, S. aureus, Clostridium difficile, Bacillus subtilis, Helicobacter pylori, vankomicin-45, resistant Enterococcus species, C. albicans* has been reported. [12]. No harmful influence on biological tissues has been observed [46]. It was successfully applied in dermatology, for wound treatment and suppression of the cold and herpes viruses [26, 32], used as a disinfectant in hospitals [5, 22], food industry [21, 51, 52], and as well as for disinfection of the environment [29].

The mechanism of its action could be explained with the destruction of the microbial cell proteins due to the oxidative stress on the one hand, and high ORP, on the other hand, creating non-balanced osmosis between the ions concentration in the solution and in the cell. Thus, the membrane structure will be damaged [12].

Investigation on E. coli

Number of scientists have studied the influence of the anolyte on of *E. coli* strains. Anolyte Sterilox obtained from electrolysis of diluted NaCl solution with ORP > +1100 mV and pH = 5.5 was used by Zinkevich et al. [53]. The authors treated planktonic cells of *E. coli* JM109. Using atomic force microscope (AFM) they noticed that all cells inflated and burst within 5 min of treatment. Also, experiments have shown a full destruction of proteins, DNA and RNA. It is supposed that the anolyte enters the cells inducing structural and functional damages on the cell membrane and cell wall.

Similar work was done by Kumar et al. [24]. They evaluated the inactivation efficacy of anolyte of pH = 2.7 and ORP = + 1100 mV on *E. coli* O157:H7, *Salmonela enteritidis* and *Lusteria monocytogenes*. The following five strains of *E. coli* E06 (milk), E08 (meat), E10 (meat), E16 (meat) and E22 (calf feces) were used. All patogens have been significantly reduced (7.0 logCFU/ml) or fully destroid (8.0logCFU/ml) after 2 to 10 minutes activation in the whole temperature range from 4℃ to 23℃. It was supposed that the low pH of the anolyte made sensitive the outer cell membrane thus helping the hypochlorous acid to enter the cell and destroy it.

It is known that the characteristic feature of the above strong oxidants is their extreme activity towards the environment due to which they very fast reduce and inactivate. This predetermines their reactivity and antimicrobial action respectively for a short time. The majority of the known literature supports this standpoint [29, 50], while others [10, 33, 37] claim a few months stability after the production. In [33] a standard technology was applied for the production of anolyte with known concentration of active chlorine, values of ORP and pH, and consecutively measured them. The results have shown insignificant change in the values of pH and ORP. Even some chlorine still persisted in the solution.

Investigation on the classical swine fever (CSF)

The classical swine fever (CSF) is prevalent in different regions of the world inflicting heavy economic losses. It is caused by enveloped viruses belonging to the genus *Pestivirus* of the family *Flaviviridae*. 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℃, and over 6 weeks at 4℃. Its thermal resistance may vary depending on the strain type, but the inactivation depends mostly on the medium containing the virus. Although the CSF virus loses its infectivity in cell cultures at 60℃ for 10 min, it is able to withstand at least 30 min at t = 68℃ 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.

The resistance and inactivation of the CSF virus is a subject of extensive research [8, 11, 20, 23]. However, studies about the virucidal effect of anolyte are scarce and of insufficient depth, making research on the possibilities of applying 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*, a challenging problem.

Such a study has been carried out by Nikolova et al. [33]. Experiments were conducted with the anolyte obtained by the electrolysis apparatus "Wasserionisierer Hybrid PWI 2100". The device was equipped with four titanium electrodes coated with platinum. Chemically pure sodium chloride (NaCl) with 0.3 % solution of in distilled water was used. The anolyte had pH = 3.2±0.03, ORP = +1070±10.7 mV and electrical conductivity 1471±14.7 μS/cm. The parameters of control sample water were pH = 7.4±0.07, ORP = +255±2.6 mV, and electrical conductivity 114.2±1.1 μS/cm.

The infected cell cultures and suspensions sample from spleen, kidney and lymph node have been treated with anolyte diluted with distilled water at different degree and different concentration of the cell culture. The obtained results have shown that in case of non-diluted anolyte and independently of concentration, the virus was fully destroyed, while at dilution of 1:1 and higher its development was suppressed. 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. Also, the virucidal effect of non diluted anolyte on organ suspension containing CSF virus is proved. This effect is probably due to destructive influence of anolyte on the envelope proteins of the CSF virus because of the content of O_2 and reactive oxygen spieces H_2O_2, and others in it.

Action on pathogens of digestate

Experiments have been carried out at the Institute of Microbiology of BAS and aimed at the suppression of pathogens in digestate, obtained during the process of biogas production from plant waste. For this electrolyzer СТЭЛ 6М11 and 0.5% of NaCl solution were used. The strain *E. coli WF+* and meticillin - resistant strain *S. aureus* 1337 (MR*SA*) were investigated. The treatment effect has been registered on isolated from microbe populations of Gram-positive and Gram-negative bacteria, as well as on monocultures of MR*SA* and *E. coli.* The results have shown a full destruction of the microbe population in case of preliminary dilution of the digestate in ratio 1:100 or 1:1000.

The MR*SA* and *E. coli* bacteria have been fully destroyed in *in vitro* experiments.

Also, the experiments have shown that the effect on a bacteria culture was not dependent on the duration of treatment. [9] proved that the anolyte with ORP +700 mV destroyed all cells of *E. coli, P. aeruginosa, S. aureus*, on the 5^{th} min. The cells of *Basillus subtilis* were an exception but their number was reduced at about 100 times. According to Zinkevich et al. [53] this might be attributed to the

destruction of cell proteins. The latter results are supporting such an assumption. Also, the experiments have shown that the value of pH was not relevant for the antibacterial activity of the anolyte.

Analysis of pH and ORP has shown small change of their values despite the time of storage. Nevertheless, of its reactive ability the chlorine was preserved to some extent which explains the preservation of its biocidal activity after a period of more than 9 months in case of normal conditions of storage.

Antiviral effect on human viruses

The antiviral effect of anolyte has been tested in the Institute of Microbiology of BAS. Investigation has been carried out for human adenovirus HAdV-5 and human respiratory syncytial virus HRSV. Electrolyzer HYBRID PWI-2100 was used, producing anolyte with pH = 2.5 and ORP =800 mV.

No effect was observed upon the interaction of the sample with the extracellular versions of HAdV-5 up to 60 min and HRSV at 15 min. Low activity against HAdV-5 was manifested after 90 min (Δlog = 0.3). Same low virucidal effect against HRSV virions was observed but for a shorter period of interaction (at 30 min) and without change till 120 min between the sample and the virions.

Investigations on the viruses of the cold and herpes showed positive effect in both cases. The first virus was fully destroyed after 2-3 days of treatment, while the development of the second one has been inhibited.

5. DISCUSSION

The brief survey of the results presented in the literature up to now has shown the undoubted biocidal effect of the anolyte on different microorganisms. In all of the reported cases either they have been fully destroyed or their development has been hampered. Differences in the obtained results could be related to the differences between pathogens and the experimental conditions. Very important for anolyte effectiveness are its characteristics depending on the electrolyzer, duration of the electrochemical process, the obtained physical-chemical changes affecting the bioactive components, and the initial contents of the electrolyzed water.

It is important to notice that besides pH and ORP that are measured during the experiments, other characteristics like surface tension, osmotic pressure, electro conductivity, viscosity, energy of the hydrogen bonds and distribution of water clusters according to the number of molecules in them, are not measured and might be of significant importance for the elucidation of anolyte biocidal activity. Unfortunately, some of them cannot be easily standardized and change fast in time. This may explain the different data about the duration of biocidal activity. According to some results the activation ability of the anolyte is preserved no longer than a few days [29, 50]. Other investigators [33] claim duration of about one year for pH and ORP at normal conditions (less than 10% from the initial values). Even chlorine is still present. Also, different storage conditions do not influence significantly the anolyte bioactivity which means that it can be successfully used for long.

The mechanism of action of the activated water on living organisms is not fully understood. Predominating is the opinion that the cell envelop is destroyed at direct contact, the inner liquid runs out and the cell mortifies. The anomalous properties could be related to one or other action, but a full picture of the process is still missing. A probable assumption could be assigned to the differences in osmotic pressure of catholyte, anolyte and blood plasma. This may bring to intensive run out of the oxidized stuff from the cell and increased infusion of intercellular liquid into the cell. This may explain the statement that the anolyte enters the cell and provokes inflation and burst [22]. Also, the change in electrical potentials at the cellular membrane may affect the action of potassium-sodium pump.

The explanation of the registered biocidal effects of the anolyte on the living matter stems from the processes complexity on the one hand, and the lack of confirmed theory about the water structure at all. [3].

The development of molecular and structural-chemical concepts has enabled to clarify an explanation of the ability of water molecules to form short-lived hydrogen bonds with neighboring molecules and many other chemical substances and to bond them into intermolecular associates [18, 27, 36].

It was noticed that under the influence of electric power the hydrogen bonds are easily disrupted and the cluster size is diminished to up to a few molecules which will decrease the water viscosity.

A measure called Non-Equilibrium Energy Spectrum (NES) suggested by Antonov [2] reflects the structure of water as a result of external influences.

6. CONCLUSION

The electrochemical activation of natural water or of NaCl solutions is developing as a fast and effective method for the production of disinfectants demonstrating higher effectiveness compared to the traditional biocidal means [15]. The obtained solution possess high antimicrobial capacity against a vast number of actual microbial species [30, 43]. It is worth noticing that the anolyte obtained from tap water has also a remarkable biocidal ability [10].

The investigations on the properties of the anolyte and its action on the living matter carried out by many scientists give reason for the following inferences.

1. Anolyte could be successfully used against different pathogens in the animals and human beings. The lack of side effects makes it harmless for use.
2. Its destructive action on different microorganisms allows for its use as disinfectant means at homes, offices and hospitals.
3. Its cheap production and activity preservation for long time allows for its use in agriculture and stock breeding as an effective and ecological mean in the struggle against diseases on plants and animals.
4. The fast destruction of some pathogens avoids development of resistance.
5. Anolyte is environmentally friendly, as it quickly breaks down there and passes into water.
6. The development of theory explaining the processes connected to the changes in water structure during ECA and the influence on vital processes is a basic problem.

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