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ELECTROCHEMICALLY ACTIVATED AQUEOUS SOLUTIONS – ESSENCE, ACTION AND SOME ASPECTS OF APPLICATION

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ABSTRACT

The main characteristic of the electrochemically activated aqueous solutions" (ECAAS) is that if they are subjected to unipolar electrochemical influences with certain characteristics, in them occur metastable, i. g. electrochemically activated substances giving them unique ecological, chemical, physical, biological and, in particular, microbiological properties, which all the currently authorized chemicals (biocides, cosmetics and pharmaceuticals) do not possess. The anolyte and has reduced electron activity and pronounced oxidant properties, and the catholyte has enhanced electron activity and reductor properties.

Because of their low oxidant content, the anolytes have very little chemical buffering, hence their environmental safety. The qualities of the ECAAS have provided grounds for their broad application both in the human and in the veterinary field, in the military field, the food industry, for the disinfection of ships, wagons, airplanes, etc. We conducted tests to determine the effect of an anolyte containing Cl^- obtained by electrochemical activation of sterile distilled water with 3% NaCl on depleted sludge from a municipal wastewater treatment plant, on bovine manure and on *Clostridium perfringens*. The results show that the anolyte added in equal proportion to the manure resulted in a reduction in the total number of microorganisms 20 times ($2.6x10^5$ CFU/ml) compared to the untreated control manure ($7.5x10^6$ CFU/ml), and upon addition of a doubly lesser amount of anolyte, the total number of microorganisms decreased 10 fold ($4.5x10^5$ CFU/ml) relative to the control. After 24 hours influence of anolyte on depleted sludge, there was a decreasing in the amount of microorganisms from all the groups studied. The decreasing was statistically significant in the total number of microorganisms and that of the Gram-negative aerobes in comparison with the untreated sludge, more significant in the sludge treated with equal quantity anolyte than that, treated with twice smaller amount of anolyte. The anolyte added in equal amounts to a suspension of *C. perfringens* at a density of $2.0x10^6$ CFU/ml after 10 minutes of exposure, caused a three-fold decrease in the amount of viable cells in the suspension.

Key words: electrochemically activated aqueous solutions, antimicrobial action.

Introduction

Public health and the protection of the environment are global problems. Undoubtedly, they also are a key task of the European science strategy. The growing prevalence of strains of pathogenic bacteria, resistant to antibiotics and rapidly developing resistance to commonly used disinfectants, is a serious problem on a global scale (Alibert-Franco et al., 2009; Nallathamby et al., 2010; Popova, 2016). On the other hand, pollution of the environment with chemical agents used to combat microorganisms in all spheres of human activity is increasing. This leads to undesirable changes in ecological equilibrium and biodiversity in nature.

In 1972, a discovery was made in the USSR, which became the forerunner of the modern science of electrochemical activation of the so-called. "Diluted" – less than 0.1 mol/l of aqueous solutions known as "electrochemical activation", and solutions – as "electrochemically activated aqueous solutions" (ECAAS), commonly referred to as "live" and "dead" water. When subjected to unipolar electrochemical effects with certain characteristics, in them are formed metastable, i. e. electrochemically activated substances, giving them unique ecological, biological and, in particular,

microbiological, chemical and physical properties, which the currently authorized chemicals such as biocides, cosmetics and pharmaceuticals do not possess. In recent years, sodium chloride ECAAS have been reported to be broad-spectrum and environmentally safe biocidal products. Their range of action includes not only bacteria but also spores, viruses and fungi. Bacteria are successfully affected even when are in the form of a biofilm. There is evidence that the antimicrobial effect of these solutions is higher than that of the alcohol and is commensurate with the effect of the sodium base. They can be used for disinfection of surfaces, floors, worktops, tools, packs, hands, etc., and their action is effective and without undesirable effects (RADICAL WATERS, 2015).

The activated solutions have properties that make them effective in the treatment of a number of diseases (Atanasov et al., 2014, Karadzhov et al., 2014, Ignatov et al., 2015). The anolyte is a bright, clear solution with chlorine odor. It has antiseptic, anti-allergic, anti-inflammatory, anti-edematous and anti-itching properties, destroys bacteria and many viruses, fungal flora, has anti-inflammatory, anti-allergic and anti-edematous action. It shows a local healing effect by acting on the bacteria or on the inflammatory hearth only in case of direct contact. Unlike the catolyte, the anolyte keeps its properties longer. It can be stored in closed glassware for months (Ashbah, 2008; Popova et al., 2016 b). For the first time Ashbah (2008) established the selective antibiotic properties of the anolyte by conducting chronic tonsillitis anolyte treatment (rinsing and washing of the tonsil furrows). By bacteriological studies they found that the anolyte selectively destroys the pathogenic microflora (*S. haemolyticus*, *S. aureus*, etc.) but does not affect or destroy microorganisms that are not involved in the process of throat inflammation (micrococci, nonhemolytic streptococci). This gives an enormous advantage to the antibiotics, which, by destroying the pathogenic flora, also destroys the normal bacterial environment, which leads to the appearance of some secondary diseases – candidiosis and other fungal infections, dysbacteriosis, impairment of immune and fermentative functions, etc. For disinfection of water, for rinsing and washing in tonsillitis and treatment of trophic ulcers and others different anolytes can be used, the properties of which depend on the redox potential and the content of chlorine or iodine ions in it. As a result of the electrolysis of the aqueous salt solution, strong oxidants accumulate in the anode: chlorine radicals – chlorine dioxide, hypochlorous acid, hypochlorite ions and oxygen radicals – atomic oxygen, ozone and hydrogen peroxide. This composition, as well as the high redox potential, determines the properties of the anolyte (Ashbach, 2008; Benyaeva et al., 2009; Gluhchev et al., 2018).

The catholyte or "living water" is a solution of alkaline properties obtained from water in the electrolysis process. Its taste, smell and color practically do not differ from the water but differs from it by several parameters. The most important for explaining its healing properties are the redox – potential, acidity / alkalinity – pH and the presence of active micro – and macro – elements. It has now been found that catholyte has antioxidant and immunostimulating properties and accelerates the regeneration of tissues, and at introduction of certain minerals it helps against diabetes, hypertension, osteoporosis and other diseases. It is an accessible and simple remedy of maintaining the balance between acid-forming and alkali-forming products, since it has a pH of 7 to 12, depending on activation (Ashbach, 2008; Gluhchev et al., 2015; Ignatov et al., 2015).

Because of their low oxidant content, the anolytes have very small chemical buffering, hence their environmental safety, both in their production and application, and after their intended use. The qualities of the ECAAW have provided grounds for their broad application both in the human and in the veterinary field. A wide range of their applications are also found in the military, food and beverage industry for the disinfection of ships, wagons, aircraft, etc.

These data allowed us to focus on conducting preliminary studies to assess the possibilities of using an anolyte as an antimicrobial agent in some organic materials, which was the purpose of this work.

Materials and methods

Anolyte (activated water). The effect of an anolyte containing Cl^- obtained by electrochemical activation of sterile distilled water with 3% NaCl was tested.

Sludge. A sample of mixed sludge, dehydrated by the belt filters from the urban waste water treatment plant near Sofia, was investigated.

Bovine manure. An aged fertilizer of dairy cows was used.

Nutrient media. Selective media for microorganisms of different basic groups, manufactured by the company Antisel – Sharlau Chemie S.A., Spain, were used for isolation and culturing. Respectively, Eosin Methylene Blue agar and Endo agar for Gram-negative aerobic and facultative anaerobic bacteria, Salmonella-Shigella agar for *Salmonella enterica*, Cetrimide Agar for *Pseudomonas* species, Chapman Stone agar for those of the genus *Staphylococcus* and Sabouraud agar for fungi have been selected. Agar of Mueller Hinton (Antisel – Sharlau Chemie S.A., Spain), selective differentiating agar for *Clostridium perfringens* (Merck-Bio Lab, Bulgaria) and selective agar for enterococci (BUL BIO NCIPD LTD – Bulgaria) were also used.

Experimental staging

The antimicrobial activity of the anolyte was investigated 24 hours after mixing with the *sludge* in two variants: 1 part sludge and 1 part anolyte (1:1) and 1 part sludge and 0.5 parts anolyte (2:1). As a control, untreated sludge was examined.

Experiments were carried out to determine the antimicrobial activity of the anolyte 24 hours after mixing with aged *bovine manure* in two variants: 1 part fertilizer and 1 part anolyte (1:1) and 1 part fertilizer and 0.5 parts anolyte (2:1). Untreated fertilizer was examined as a control.

Clostridium perfringens, isolated from bovine manure, also was examined. To suspension at a density of $2.0x10^6$ CFU/ml, an equal amount of anolyte was added. Its impact was determined after 10 minutes. An untreated suspension was used as a control.

Quantification of microorganisms was performed by the classical method in serial 10-fold increasing dilutions of the test materials in sterile saline solution. Of these, seedlings in amounts of 0,1 ml were made on Mueller Hinton agar and on the selective nutrient media for microorganisms from different groups, three for each dilution. After incubation at 37° C for 24 – 72 hours under aerobic and anaerobic conditions (with Anaerocult® A mini-Merck-Bio Lab, Bulgaria) the mean arithmetic number of developed colonies was determined and the amount of colony forming units (CFUs) in 1 ml or 1 g of the starting material was established.

Statistical analysis. The results were processed statistically by Student-Fisher's classic method.

Results

The results of the studies of the quantitative changes of the microorganisms in the sludge from the urban sewage treatment plant after application of anolyte are presented in Table 1.

Table 1: Inactivation of microorganisms in sludge from urban sewage treatment plant 24 hours after anolyte treatment

Material	Total number of bacteria	Gram-negative aerobes	*Pseudo-monas* spp.	*Staphylo-coccus* spp	*Entero-coccus* spp
Untreated sludge	$4.0x10^{6}$* ±2.45**	$2.7x10^{4}$ ±1.2	$2.1x10^{4}$ ±1.5	$3.2x10^{4}$ ±1.5	$3.2x10^{3}$ ±1.0
Sludge and anolyte 2:1	$4.2x10^{5}$ ±0.96	$6.5x10^{3}$ ±2.4	$4.7x10^{3}$ ±1.9	$2.3x10^{4}$ ±0.7	$2.9x10^{3}$ ±1.4
Sludge and anolyte 1:1	$2.6x10^{5}$ ±0.98	$3.9x10^{3}$ ±1.5	$2.3x10^{3}$ ±0.8	$2.1x10^{4}$ ±0.5	$1.6x10^{3}$ ±0.3

** Average in CFU/ ml. ** Standard deviation.*

Salmonella enterica. and fungi were not isolated from the sludge.

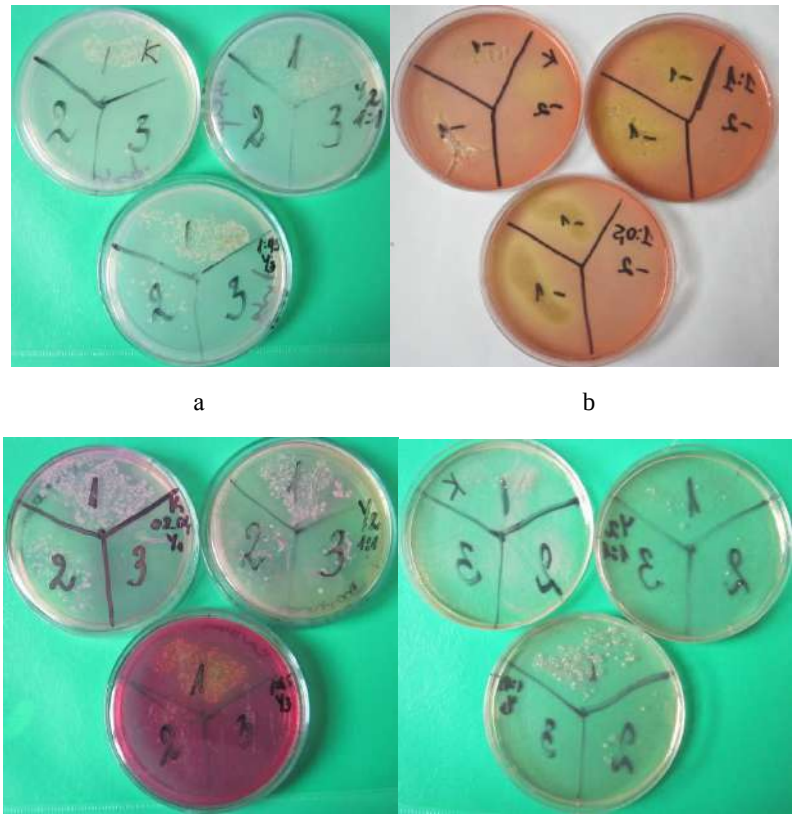
As can be seen from the results in the table, a 24-hour impact of anolyte couses a decrease in the amount of microorganisms from all the studied groups. The decrease in the total number of microorganisms compared to the untreated sludge is statistically reliable ($P<0.001$) in the treatment of the sludge with an equal amount of anolyte and $P<0.01$ at the application of a double less anolyte. Even more significant is the decrease in the number of Gram-negative aerobic and facultative anaerobic microorganisms and of the species of the genus *Pseudomonas*. Differences with untreated sludge are of high statistical significance ($P<0.001$) for both variants of treatment.

At trace of changes in the quantities of *Staphylococcus* species also show a decrease compared to the untreated sludge, especially in the sample using an equal amount of anolyte, but the differences are not statistically reliable ($P>0.05$) for both variants of treatment. Similar results were also found in quantitative studies of *Enterococcus* species. The reduction in the number of enterococci was statistically reliable ($P<0.01$) for the sludge treated with an equal amount of anolyte and unreliable for the sample treated with double less anolyte ($P>0.05$).

Some of the results of the research carried out with the sludge from the urban sewage treatment plant are also presented in Fig. 1.

The studies with aged bovine manure after treatment with anolyte are preliminary and the changes in the total number of microorganisms are determined. Some of the results are shown in Fig. 2 a. The data show that anolyte added in equal amounts quantitative ratio to bovine fertilizer after 24 hours results in reduction of the total number of microorganisms in it 20 times ($2.6x10^{5}$ CFU/ml) compared to the untreated control manure ($7.5x10^{6}$ CFU/ml). When adding a double less anolyte amount, after 24 hours the total number of microorganisms decreases 10 times ($4.5x10^{5}$ CFU/ml) relative to the control.

Clostridium perfringens was isolated on selective differentiating agar from aged dairy cow manure (Fig. 2 b). After 10 minutes of action of anolyte added to a suspension of *C. perfringens* at a density of $2.0x10^{6}$ CFU/ml, a three-fold decrease in the number of colonies on selective agar compared to the control ($6.2x10^{5}$ CFU/ml) was detected.



a b

c d

Figure 1: Growth of microorganisms at various dilutions of the tested samples (1 - 10^{-1}, 2 - 10^{-2}, 3 - 10^{-3}) on: a) Mueller Hinton agar; b) Endo agar; c) Chapman Stone agar and d) selective agar for enterococci. In each figure, the Petri dishe on the left is the control (untreated sludge); the Petri dishe on top right - sludge and anolyte in a 2:1 ratio; Petri dishe below - sludge and anolyte 1:1.

a b

Figure 2: Growth of microorganisms: a) after treatment of bovine manure with anolyte in ratio of 1 part manure and 1 part anolyte (top left, dilution 1: 100) and 1 part manure and 0.5 parts anolyte (upper right, dilution 1: 100), control - unprocessed manure (below, dilution 1: 10000); b) colonies of *Clostridium perfringens* isolated from bovine manure on selective differentiating agar.

Discussion

It is known that the use of antibiotics in productive animals leads to selection of bacteria resistant to antimicrobials used in human medicine. They can be spread with animal fertilizers as well as among people through food and can cause infections in humans, which is the reason for banning the use of such agents as growth stimulants. Also, more often observed combination of antimicrobial polyresistance and increased virulence of the agents of communicable diseases requires vigilance in epidemiology and creativity in the development of prophylactic and therapeutic options (Popova, 2013). The anolyte could be a reliable prospect in this aspect. Upon contact with the microbial cell, the anolyte causes its destruction mainly by way of disrupting the integrity of its cell wall, leakage of intracellular components, ribosome disruption, cytoplasm coagulation, etc., these processes being analogous to the processes used by macroorganism in the fight against bacteria, viruses, as well as foreign and cancer cells (Ashbach, 2008; Bakhir, 2009 a, b, c).

The results obtained in our research are consistent with those of other authors. Peev (2017) found that an anolyte obtained by electrochemical activation of an aqueous solution of sodium carbonate and sodium chloride has a well-expressed antibacterial effect in 48 hours exposure to lagoons for storage of bovine manure. Apparently, the longer duration of action of the anolyte and the low-protein aqueous medium is a prerequisite for a higher antimicrobial effect compared to our treatment variants. Also in our country Dimitrova et al. (2013) in the study of the bactericidal effect of ECAAS with different pH, % NaCl and oxidation-reduction potential on compost in the ratio 1: 3 and an exposure time of 10 min and 2 h, testify to results showing that the exposure time and the pH of the ECAAS did not significantly affect their bactericidal activity. The solution with highest ORP (1100 mV) inhibits the total number of bacterial cells in the microbial population of the compost with log 2 and the number of Gram negative bacteria with log 4, which results are explained by high ORP and oxidative stress for cell membrane structures and their disability. In our experiments, we also found a higher effect of anolyte on Gram-negative bacteria, even in high-protein environments. Tasheva et al. (2010) reported that electrochemically activated aqueous solutions of sodium carbonate and sodium chloride exhibit high antifungal activity. These anolytes inactivate *Candida albicans* field strains in less than 15 minutes.

In beekeeping, the use of an anolyte for disinfection of inventory and crops, as well as for prophylaxis and treatment of sick bee families, can provide effective control of diseases (including American rot, ascosferosis, nosematosis, etc.), enhancement immune protection of the honeybee and honey production. Gurgulova et al. (2010) demonstratea the complete biocidal and sporocidal action of anolytes received with a combination of sodium carbonate and sodium chloride and sodium chloride only with ORP of 800-1200 mV at 15 min exposure on the strain of the American rot – *Paenibacilus larvae* and causative agents of the European rot on the beehive *Paenibacillus alvei* and *Melissoccocus pluton*. In a comparative study of the disinfecting effect of anolytes with a starting composition of combination of sodium carbonate and sodium chloride and sodium chloride alone with an ORP of 800-1200 mV and the most commonly used antimicrobials in beekeeping, Gurgulova et al. (2011) reported full bactericidal effect on *S. aureus* after 5 min exposure, sporocidal and bactericidal action against *M. pluton* was recorded at 15 min exposure time, and sporocidal effect for spores of *Bacillus cereus* – at longer exposure 15 – 60 min. In our studies with *C. perfringens*, we also found that a 10-minute impact of anolyte is not sufficient for complete destruction, but a threefold reduction of viable cells in the suspension is achieved in this period.

The antimicrobial activity of the anolyte in the materials we studied is significantly less pronounced than what we found in our other studies (Popova et al., 2016 a, b, c). On the one hand, this is due to the different environment in which it operates. In an aquatic environment, its effect is many times higher and faster than in a protein environment, such as sludge and manure. On the other hand, the type of the studied microorganisms is important. Spore-forming bacteria such as *C. perfringens* appear to be less sensitive than non-sprouting bacteria such as *Staphylococci* and Gram-negative bacteria. The difference in morphology is also important for the significant difference in results. The macrocapsule of *C. perfringens* vegetative cells apparently has an effective protective action against the anolyte. With a view to assessing the practical use of an anolyte for treatment of sludge from urban waste water treatment plants, further research is needed to accurately determine its effect on the major microorganisms in the sludge, including spore-forming, added in varying amounts after different intervals of impact.

Conclusion

After a 24-hour action with anolyte, a reduction in the amount of microorganisms from all the studied groups is achieved in dehydrated sludge from a municipal waste water treatment plant. The most significant is the reduction of the total number of microorganisms and that of Gram-negative aerobes compared to the untreated sludge, in a higher degree when treating the sludge with an equal amount of anolyte.

Anolyte, added in equal proportion to aged bovine manure, after 24 hours reduces the total number of microorganisms by 20 times ($2.6x10^5$ CFU/ml) in comparison to the untreated control manure ($7.5x10^6$ CFU/ml), and adding a double lesser amount of anolyte, the total number of microorganisms was reduced 10 times ($4.5x10^5$ CFU/ml) relative to the control.

Anolyte added in equal amounts to a suspension of *Clostridium perfringens* at a density of $2x10^6$ CFU/ml caused a threefold reduction in the amount of viable cells after 10 minutes of exposure.

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