Почвознание агрохимия и екология, 56, 3/2022
Bulgarian Journal of Soil Science Agrochemisty and Ecology, 56, 3/2022

Investigation of the effect of anolyte for surface disinfection

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Citation: Petrova, T., & Popova, T. (2022). Investigation of the effect of anolyte for surface disinfection. Bulgarian Journal of Soil Science, Agrochemistry and Ecology, 56(3), 24-32.

Abstract

The effect of an anolyte prepared by electrolysis of water with 0.3% NaCl for disinfection of surfaces after 10 min of exposure compared to that of 1% chlorhexidine was studied. With sterile swabs, wash samples were taken from the surface of laboratory workbenches and from the laboratory sink trough (10 cm^2 area) after 10 minutes of exposure to the antimicrobials used, applied by spraying the surface. For control, wash samples were taken without treatment with detergent. The sample materials were cultured on Müeller-Hinton agar for 24 h under aerobic conditions at 37°C. Species and quantitative testing of bacteria was performed using paper disks for detection of pathogenic bacteria HiDtectTM - Rapid Identification Discs (Universal Enviro for detection of *E. coli, S. aureus, P. aeruginosa, E. faecalis, S. Typhimurium and UTI for E. coli, S. aureus, P. aeruginosa, E. faecalis, K. pneumoniae, P. mirabilis*) as prescribed by the manufacturer. The results obtained show a very similar antimicrobial effect of the two tested agents, and that the anolyte can be used for disinfection of such surfaces with the same success as the broad-spectrum disinfectant chlorhexidine.

Key words: electrochemically activated water, anolyte, chlorhexidine, washes samples, disinfection

Introduction

Effective sterilization and disinfection of various contact surfaces play an important role in the prevention of infections and the spread of pathogenic microorganisms. Chemical disinfectants such as chlorine, organic acids, hydrogen peroxide, ozone, etc. are usually used in various fields to achieve a microbiologically safe environment. However, conventional disinfectants are not effective for biofilms, especially when they are multi-species. In addition, the use of certain disinfectants is banned in many countries due to the potential for the formation of carcinogenic halogenated by-products that are hazardous to human health and the environment. In recent years, many studies have focused on the search for alternative, effective disinfectants that are safe for humans, animals, food and the environment. Weakly acidic electrochemically activated water is a new, affordable and inexpensive disinfectant produced by electrolysis of a diluted solution of sodium chloride or hydrochloric acid (or both) in an electrolyte cell with or without a separation membrane. Its use as a disinfectant for food products such as fruits, vegetables, eggs and more reduces the incidence caused by bacteria that can be transmitted through these foods, such as

Salmonella enterica, Escherichia coli, Yersinia spp., Listeria monocytogenes, Clostridium spp., Candida albicans and others. Research shows that the anolyte inactivates 100% of bacteria and fungi on a variety of hard materials and surfaces, as well as in liquid media, which is more effective than conventional chemical disinfectants such as benzalkonium chloride and povidone-iodine solutions. The anolyte is effective in reducing bacterial contamination of surfaces in contact with food without residual disinfection products (Popova et al., 2018; Popova, 2019 a; Popova, 2019 b; Ignatov et al., 2020; Possas et al., 2021).

A number of studies have shown that work surfaces in the food industry and medical equipment are a potential source of food contamination and transmission of pathogens to hospitalized patients. Electrolyzed water could be an effective and alternative disinfectant with a number of advantages. When used for surface disinfection in food and healthcare facilities, it has the potential to prevent cross-contamination and transmission of infections. Studies by Vorobjeva et al. (2004) confirmed the bactericidal action of electrolyzed oxidizing water on nosocomial pathogens within 30 s to 5 min. It can be successfully used for disinfection of medical devices such as endoscopes and hemodialysis systems. Anolyte is a strong acid different from hydrochloric or sulfuric in that it is not corrosive to skin, mucous membranes or organic materials. It is easy to work with it and is suitable for cleaning various surfaces, including food decontamination. Glutaraldehyde is considered one of the best means for endoscopic disinfection. However, it poses a high risk to medical staff as well as the environment. The strongly acidic anolyte shows a potent bactericidal effect, sparing medical action and environmental safety when disinfecting endoscopes and colonoscopes, showing a better effect than glutaraldehyde (Sakurai et al. 2002). Hospitals are facing increasingly resistant strains of microorganisms. Some work tools, such as individual pieces of electronic angiology equipment, sets for computer tomography, scans and magnetic resonance imaging scanners, are very difficult to disinfect. Neutral electrolyzed oxidizing water could be an effective biocide for disinfection of diagnostic rooms and equipment with the help of an aerosolization device as a reduction of microbial contamination between 78.99-92.50% has been registered. The use of anolyte for disinfection of air and hard surfaces could significantly reduce microorganisms and the possibility of nosocomial infections without possible damage to open devices or personnel (Pintaric et al., 2015).

The anolyte simultaneously destroys microorganisms and volatile organic compounds. Its efficiency in this respect on surfaces, handles of waste bins and hands of sanitary workers is 22.7% - 84.1%. The anolyte also effectively reduces the content of bacteria and fungi in the air. After spraying with an anolyte Guo et al. (2021) reported a reduction in the concentrations of most volatile organic compounds by 21.4% - 88.3%, as well as carcinogens. Our previous studies show that the anolyte can be used successfully for disinfection of fruits and vegetables (Popova & Petrova, 2018; Popova, 2019 c).

Despite thorough washing and disinfection, laboratory tables and sinks in classrooms and training laboratories, especially those in microbiology, are at particular risk of microbial contamination and the formation of multi-species biofilms. The constant use of chemical disinfectants has adverse side effects due to toxicity to personnel. Therefore, in the present study we aimed to examine the disinfection effect of an environmentally friendly antimicrobial agent - electrochemically activated anolyte with 0.3% NaCl, comparing its effect with the broad-spectrum disinfectant chlorhexidine at 1% concentration.

Materials and Methods

Anolyte (activated water). The effect of an anolyte obtained by electrochemical activation for 15 minutes of tap water with added NaCl (0.3% or 3g NaCl in 1 l of tap water) in an Aschbach 2.0 kettle applied at a concentration of 100% by spraying was tested. The antibacterial action of the anolyte was tested immediately after its preparation (fresh anolyte). The physical parameters pH, oxidative redox potential (ORP) and temperature of the studied electrochemically activated anolyte were determined using the Manual multi-parameter analyzer Consort C1010 (Consort bvba, Belgium).

Control. The disinfectant chlorhexidine gluconate 40 mg/ml (HIBISCRUB®) used at a final concentration of 1% was used (http://scdn.phabcart.co.uk/pdf/7840.pdf).

Surveyed surfaces. The research was performed on worktables lined with faience tiles, faience sinks in a hall for practical classes in microbiology for students of veterinary medicine and a countertop in an university microbiology laboratory.

Nutrient media. Müeller-Hinton agar (BUL BIO NCIPD - Sofia, Bulgaria) was used. HiDetect rapid identification discs (HiMedia Laboratories Pvt. Limited, Mumbai, India) for species and quantitative examination of bacteria were performed by using paper disks for rapid detection and identification of pathogenic bacteria - DT015 HiDetectTM Universal Enviro Identification Discs (for determination of *E. coli, S. aureus, P. aeruginosa, E. faecalis, S. Typhimurium*) and DT001 HiDetectTM UTI Identification Discs (for *E. coli, S. aureus, P. aeruginosa, E. faecalis, K. pneumoniae, P. mirabilis*).

Experimental staging. From the tested surfaces with sterile swabs soaked in 0.5 ml of sterile saline, swab samples were taken from an area of 100 cm^2, 3 times from each. Anolyte was then sprayed on part of each of the surfaces and chlorhexidine on another. After 10 minutes of exposure to disinfectants, swab samples were taken from an area of 100 cm^2, 3 from each treated surface. Samples were inoculated by applying the material taken with each swab on the agar surface in a Mueller-Hinton agar Petri dishes. They were cultured at 37°C for 24-48 hours under aerobic conditions. A paper disk for the detection of pathogenic bacteria was then placed on the surface of each of the resulting cultures for 30 s. The discs were transferred to sterile Petri dishes with lids and incubated at 37°C for 3-4 hours according to the manufacturer's instructions. Then, according to the color and morphology of the colonies' prints formed on the disks, the types of bacteria and the number of colonies forming units (CFU/100 cm^2) were determined.

Statistical analysis. The results were processed mathematically and the average values (AV) and standard deviation (SD) were found. Student's t-test analysis for independent samples was applied to test the statistical dependence and reliability of the results. Significance of the differences was defined at significance level $P<0.05$. Microsoft®Office Professional Plus Excel 2013 (15.0.4569.15060) was used for the calculations, with rights from the University of Forestry, Sofia.

Results and Discussion

The physical indicators - pH, ORP and temperature of the studied anolyte are presented in table 1.

The results of the studies performed to determine the effect of anolyte and chlorhexidine for disinfection of workbenches in the hall for practical classes in microbiology, immediately after the students' work, are summarized in table 2.

As can be seen from the data in the table, the number of microorganisms detected was significant, but only *E. coli* and *E. faecalis* have been identified. *P. aeruginosa* and *S. aureus* were not isolated from these surfaces. The results show that the effect of the anolyte for decontamination of the work tables was very high, close to that of the control disinfectant chlorhexidine. The differences in the effect of both tested agents in *E. coli* were not significant ($P>0.05$), but in *E. faecalis* the effect of chlorhexidine was higher than that of the anolyte ($P<0.05$). E. coli was more sensitive to the action of anolyte and chlorhexidine than *E. faecalis.*

Table 3 presents summarized data from studies performed to determine the effect of anolyte and chlorhexidine on countertop disinfection in a microbiological laboratory. Some of them can also be seen in figures 1 and 2.

The summarized data in the table show that the number of detected microorganisms is not high, as it is significantly less than on the work tables in the practice room. In addition, *E. coli, E. faecalis* and *P. aeruginosa* were isolated from this plot, but not *S. aureus*. These differences are

Table 1. Physical indicators of the used anolyte

Initial composition		pH	ORP, mV	t°C
Water solution of NaCl 0.3%	Before electrolysis	8.92 ± 0.5	218 mV±5	22.1°C ±3
	Anolyte	2.42 ± 0.2	1000 mV ±6.5	20.7°C ±2

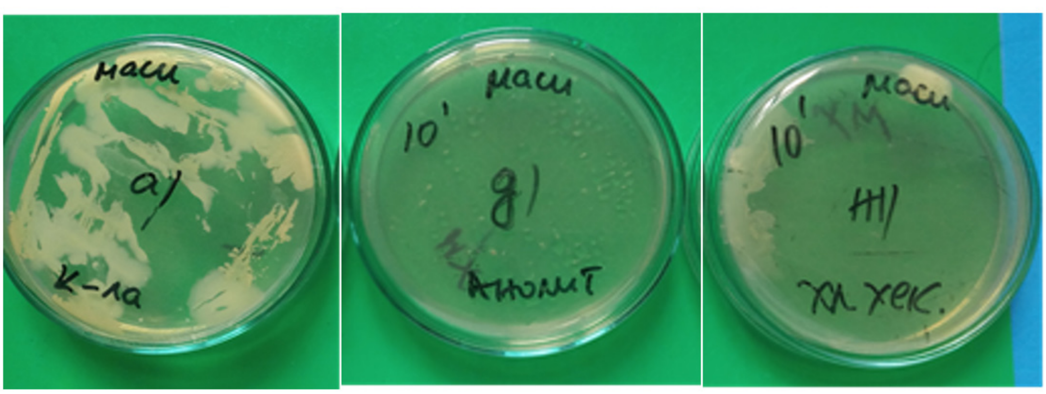
ORP – oxidative-redox potential

Table 2. Results of disinfection for 10 minutes on work tables in a room for practical classes in microbiology

Bacterial species	Amount of bacteria (CFU/10 cm^2)		
	Before disinfection	Anolyte	Chlorhexidine
E. coli	433.33 + 80.55	3.33 + 1.25	3.0 + 1.25
P. aeruginosa	0	0	0
E. faecalis	716.67 + 86.54	52.67 + 10.21	22.67 + 4.49
S. aureus	0	0	0
Total	1150.00 ± 83.55	56.00 ± 5.73	25.67 ± 2.87

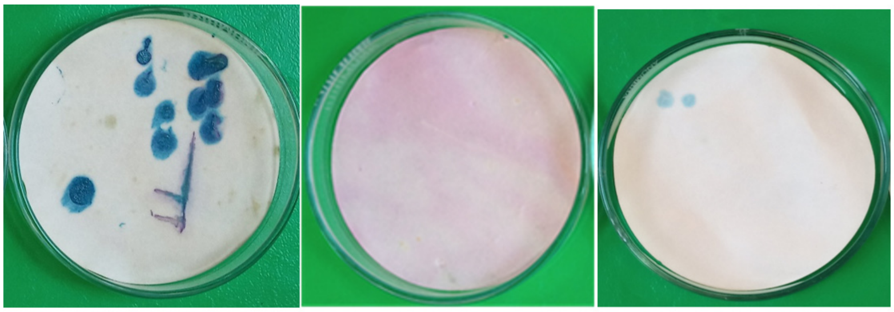
Table 3. Results of disinfection (10 min exposure) on a countertop in a microbiological laboratory

Bacterial species	Amount of bacteria (CFU/10 cm^2)		
	Before disinfection	Anolyte	Chlorhexidine
E. coli	23.33 + 80.55	0	0
P. aeruginosa	42.33 + 29.78	2.60 + 1.40	3.00 + 0.82
E. faecalis	7.00 + 2.94	3.00 + 1.63	4.00 + 2.16
S. aureus	0	0	0
Total	72.66 ± 37.76	5.60 ± 1.52	7.00 ± 1.49



A B C

Fig. 1. Bacterial growth on Müeller-Hinton agar of washing samples from the surface of worktops (A - control; B - after 10 minutes of action of anolyte; C - after 10 minutes of action of chlorhexidine)



A B C

Fig. 2. Species and quantitative study of bacteria (from laboratory countertop flushing samples) with paper discs HiDtectTM - Universal Enviro for determination of *E. coli, S. aureus, P. aeruginosa, E. faecalis, S. Typhimurium* (A - control, B - after 10 minutes of action of the anolyte, C - after 10 minutes of action of chlorhexidine)

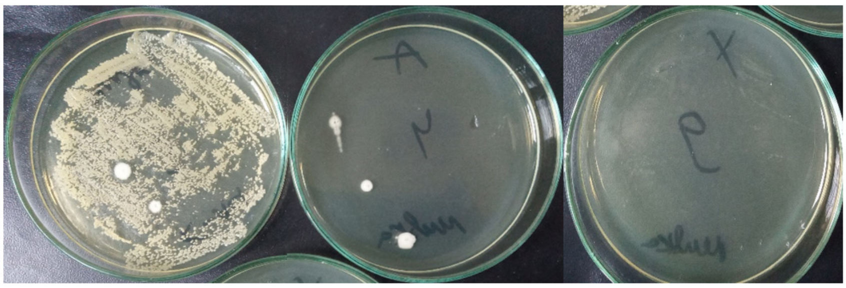
probably due to the presence of many students in the practical study room, the import and use of various tools that may be carriers of different microorganisms, as well as their work with different materials during classes. In addition, the nature of the experiments and materials in the laboratory differs somewhat from that in the hall for practical classes. The work desks are lined with faience tiles. Probably during cleaning some of the microorganisms are retained in the joints between the tiles. The surfaces in both rooms are cleaned and disinfected daily. The effect of the anolyte on decontamination of the countertop in the microbiological laboratory turned out to be higher than that of chlorhexidine. The differences in the effect of both tested agents compared to the isolated bacteria were not significant ($P>0.05$), which indicates that the anolyte can be used for disinfection of such surfaces with the same success as chlorhexidine. A great advantage of the anolyte is that it is completely safe for people, treated materials and surfaces, and to the environment. Obviously, with lower microbial contamination, the anolyte is very efficient, comparable to that of the most active disinfectants. Gram-negative bacteria *E. coli* and *P. aeruginosa* turned out to be more sensitive to the action of anolyte and chlorhexidine than *E. faecalis*.

The results of the studies performed to determine the effect of anolyte and chlorhexidine for disinfection of a sink in a room for practical classes in microbiology are presented in table 4, and some of them - in figures 3 and 4.

The sink in the practice room is washed and disinfected daily. Probably for this reason, the total number of microorganisms in it was significantly less than that on the countertop in the hall. However, the species composition of the isolated bacteria was higher. The presence of *P. aeruginosa* is understandable. It inhabits wet objects and is very resistant to chemical antimicrobial agents, but it turned out to be very sensitive to the anolyte, as well as to the applied control disinfectant. *S. aureus* was also found in the sink, which also showed high sensitivity to anolyte. The application of the anolyte for decontamination of the sink was very efficient, as the result was close to that of the control disinfectant. Following administration of anolyte and chlorhexidine for 10 minutes, the most viable *E. faecalis* cells were detected. Of the studied Gram-positive and Gram-negative bacteria, this species was the least susceptible to disinfection with anolyte and chlorhexidine, and the effect of the latter in this case was slightly higher than that of the anolyte. The effect of the anolyte for decontamination of the sink was very similar to that of chlorhexidine. The differences in the effect of the two tested agents on the isolated bacteria were not significant ($P>0.05$), which indicates that the anolyte can be used for disinfection of

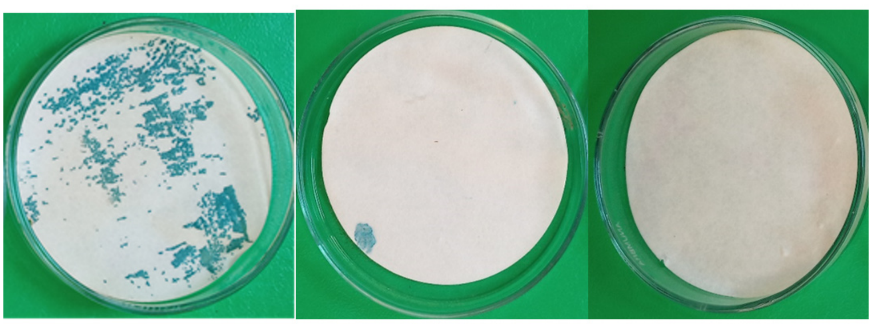
Table 4. Results of disinfection for 10 minutes on the sink in a room for practical classes in microbiology

Bacterial species	Amount of bacteria (CFU/10 cm^2)		
	Before disinfection	Anolyte	Chlorhexidine
E. coli	313.33 + 32.99	4.67 + 4.11	4.00 + 2.92
P. aeruginosa	90.67 + 22.00	6.00 + 4.32	4.75 + 3.27
E. faecalis	239.33 + 158.07	21.67 + 4.19	15.25 + 10.03
S. aureus	36.33 + 10.40	1.00 + 0.82	0
Total	679.66 ± 55.87	33.34 ± 3.36	24.00 ± 5.41



A B C

Fig. 3. Bacterial growth on Müeller-Hinton agar of washing samples from the surface of a laboratory sink (A - control; B - after 10 minutes of action of anolyte; C - after 10 minutes of action of chlorhexidine)



A B C

Fig. 4. Species and quantitative study of bacteria (from laboratory sink flushing samples) with paper discs HiDtectTM - UTI for detection of pathogenic bacteria E. *coli, S. aureus, P. aeruginosa, E. faecalis, K. pneumoniae, P. mirabilis* (A – control; B - after 10 minutes of action of the anolyte; C - after 10 minutes of action of chlorhexidine)

sinks with the same success as other widely used disinfectants such as chlorhexidine.

The high bactericidal effect of the anolyte is due to the available chlorine compounds, including ClO^-, HClO and Cl_2. To prevent the loss of chlorine, which is one of the main contributing factors to its antimicrobial activity, it should be stored in closed containers or used immediately after receipt (Cui et al., 2009). An anolyte derived from aqueous saline solution has a strong oxidative potential and electron deficiency, which gives it the ability to oxidize and sterilize (Hati et al., 2012). In the process of microbial inactivation, oxidized water damages cell membranes, disrupts cellular metabolic processes and thus kills the cell and destroys biofilms (Hua et al., 2019; Okanda et al., 2019). High ORP adversely affects bacterial metabolism and ATP production, possibly due to changes in electron flow in microbial cells. The low pH also destabilizes the bacterial outer membrane, leading to the entry of hypochlorous acid into the bacteria, which kills them by inhibiting glucose oxidation by certain enzymes (Yoo & Lee, 2017). Another active element in the anolyte, namely nascent oxygen, was recently proven by a Bulgarian research team (Vassileva et al., 2022).

The results of our study are consistent with those of other authors. Deza et al. (2007) investigated the efficacy of neutral anolyte water in reducing populations of *E. coli, P. aeruginosa, S. aureus,* and *L. monocytogenes* on plastics and wooden kitchen cutting boards. The study shows that the efficacy of the neutral anolyte in the studied treatment is comparable to that of NaClO, with the advantage that it is environmentally safe and has a longer shelf life. Handojo et al. (2009) investigated residual bacteria and different types of food left on table objects after various washes and sanitary protocols. The authors found that the neutral and acidic anolyte is as effective as other tested chemical disinfectants for food contact surfaces against E. coli and Staphylococcus epidermidis.

Electrolyzed water has been found to provide excellent results in cleaning and disinfection compared to traditional chemicals. On-site anolyte production requires few environmentally friendly and safe ingredients (Tango et al., 2019). In surface wiping, the average bacterial killing rate for 5 minutes after disinfection with anolyte has been found to be 99.32%, which is higher than 97.74% of chlorine solution. The immediate bactericidal effect of a weakly acidic anolyte is better than chlorine disinfection, and it also has the advantage of greater safety for workers and the environment. As the surface can be easily re-contaminated when used in clinical settings, the frequency of disinfection should be increased (Wang et al., 2012).

There is evidence in the scientific literature that the anolyte destroys biofilms formed by both Gram-negative (*Vibrio parahaemolyticus*) and Gram-positive bacteria (*L. monocytogenes*) by inactivating isolated cells, a potential source of secondary contamination (Han et al., 2017). Okanda et al. (2019) found that a weakly acidic anolyte completely inactivates P. aeruginosa in the biofilm, even when immersed at 15°C for 5 min. Acidic electrolyzed water successfully disinfects a two-species biofilm formed by *Salmonella Enteritidis* and *Pseudomonas fluorescens* (Hua et al., 2019), and Liu et al. (2020) reported the successful use of slightly acidic electrolyzed water alone to wash a milking system. The anolyte with appropriate parameters can achieve the same or even better hygienic effects compared to chemical commercial detergents. Yitian et al. (2017) investigated a weakly acidic anolyte and identified it as an ideal and environmentally friendly disinfectant for the prevention and control of bacterial infections on farms in the inactivation of microbes in disinfection channels. The anolyte is significantly more effective than bromide and glutaraldehyde in inactivating *S. Enteritidis*, and it does not cause respiratory side effects due to the lack of Cl_2 release of both bromide and glutaraldehyde and prevents potential dangers to workers' health.

Our research shows that the anolyte prepared by electrolysis of water with 0.3% NaCl has a disinfectant effect comparable to that of the modern disinfectant chlorhexidine, but the process needs to be optimized for individual applications of electrolyzed oxidized water. Analytic acidic water has great potential to compete with current antimicrobials. Anolyte treatment can be used as an effective method to reduce microbial contamination on various surfaces. An important advantage is that the anolyte is produced on site when direct use is required, thus reducing the risk to workers' health and the environment by eliminating the use of concentrated chemicals. The use of electrolyzed water is a new technology with a huge scope for further research and development and with potential opportunities not only to compete with current practices, but also to surpass them in many respects.

Conclusions

1. Anolyte prepared by electrolysis of water with 0.3% NaCl is an effective disinfectant for worktops and tables in microbiological training laboratories.

2. The anolyte shows high efficiency in disinfection of laboratory sinks.

3. The effect of the anolyte is similar to that of chlorhexidine in the disinfection of tables and sinks.

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