Effect of Electrochemically Activated Water on Spore-Forming Bacteria

Nataliya Pankiv, Liubov Palianytsia,
Nataliya Berezovska, Ruslana Kosiv

Technology of Organic Products Department, Lviv Polytechnic National University, UKRAINE, Lviv, S. Bandery street 12,
E-mail: n.pankiv@i.ua, liubapal@ukr.net,
NBeresovska@gmail.com, r.kosiv@online.ua

Abstract – The effect of electrochemically activated water on the viability spore-forming bacteria Bacillus and Clostridium genera is investigated.

It is established that the anolyte inhibits the growth of microorganisms, causing the death of 98% of the cells.

Key words – catholyte, anolyte, bacteria *Bacillus* and *Clostridium* genera, electrochemical activation.

I. Introduction

Microorganisms play an important role in human life. A large proportion of mineralization of organic compounds accounted for microorganisms. In biotechnological processes they are producers of food products (dairy products, yogurt, vinegar), enzymes (protease, α-amylase), food and feed additives (glutamate, vitamins), pharmaceuticals (antibiotics) and other substances, used in industry (alcohols, acids, etc.).

However, the bacteria can cause considerable damage in production processes, getting as extraneous microflora. Therefore, improvement of methods of combating these pathogens remains an actual task.

There is a large number of bacteria in raw materials that are used in biotechnological processes. It leads to its deterioration during storage and the consequent loss of raw materials and reduce the yield of the product and increase the output side agents.

There are various methods of combating extraneous microflora, namely: physical (ultrasound, electromagnetic radiation), chemical (antiseptic effect, oxidants), mechanical (ultrafiltration), thermal (boiling, sterilization). Today the common physical-chemical method of disinfection is the electrochemical activation of water and aqueous solutions.

It is known that electrochemically activated water exhibits antibacterial properties, acting differently on different groups of microorganisms. In [1] is a list of prokaryotes, eukaryotes, viruses and their range of experimental kill rates determined for acidic (pH 2–5) and neutralised (pH 5–8) electrochemically activated solution anolyte. As shown in [1] electrochemically activated solutions have an extremely wide range of antimicrobial activity on microorganisms. Acidic electrochemically activated solutions exhibit comparable bactericidal action with antiseptics, which are widely used in industry, including sodium hypochlorite, chlorhexidine gluconate, glutaraldehyde and benzalkonium chloride.

Research of Russian scientists confirms the bactericidal action of neutral anolyte (pH = 7, oxidation-reduction potential ORP = 900 mV) against the bacteria *Escherichia coli*, *Proteus vulgaris, P. mirabilis, Salmonella dublin, S. enteritidis, Morganella morganii, Pseudomonas aeruginosa, Staphylococcus aureus*. The results of these studies have shown that under influence of anolyte death of these types of bacteria except *Staphylococcus aureus*, took place after 5 minutes. *Staphylococcus* was killed after 7 minutes [2].

Other scientists from South Africa [3] found out that the undiluted and the 10^{-1} dilution of halide derived anolyte was effective in killing *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* except *Bacillus subtilis*, which exhibited the most resistance. The anolyte caused bacterial death in two ways – by complete collapse of proteins or by causing oxidative stress which resulted in protein fragmentation.

Bacterial endospores are more resistant to the effects of anolyte compared to vegetative cells [4].

To date, the mechanism of electrochemically activated water influence at the cellular level has been defined. Compounds, formed during electrochemical activation, directly affect lipid membrane, organell cells, as well as intracellular complexes and compounds. The catholyte, saturated with reducing agents and the anolyte, rich in oxidants, change cells oxidation-reduction potential on the surface and inside, thus regulating the activity of endogenous biooxidants and bioantioxidants. Thus membrane permeability is changed by electroosmosis. Penetration of electrochemically activated water into cells accelerates biochemical reactions that occur in the cytoplasm [5,6].

II. Materials and methods

Electrochemically activated water was prepared in the electrolyzer «Ekovod». Water from the municipal water supply (Lviv) served as the initial water.

For investigation spore-forming bacterial cultures genera *Bacillus* and *Clostridium*, isolated from raw grain and cultured on slope agar, were taken. At first the initial bacterial suspension in sterile water was prepared. After that, the same number of suspension in the three tubes was selected and brought to 10 cm^3 catholyte, anolyte and sterile water (control). Investigated suspension was kept for 1 hour at room temperature. Further viability of the bacteria was studied by seeding in Petri dishes on solid nutrient agar, followed by incubation at 30 ^{0}C for three days. Colonies were counted in Petri dishes and resistance of the bacteria to the influence of electrochemically activated water was determined.

pH and ORP was measured using the pH -301 instrument.

III. Results and discussion

The initial water pH and oxidation-reduction potential were 7,70 and -64,8 mV, respectively. As a result of electrochemical activation the catholyte and anolyte with the following parameters: pH 10,90 and 3,45, ORP (-249,5) and +177,9 respectively, were received. Bacteria genera *Bacillus* and *Clostridium*, soaked in sterile water with pH and ORP 6,68 -10,0 mV served as a control.

The results of calculations of colony-forming unit (CFU) in Petri dishes are presented in Table 1.

As can be seen from Table 1, lower growth of colonies in the samples bacteria genera *Bacillus* and *Clostridium* in comparison with control is observed.

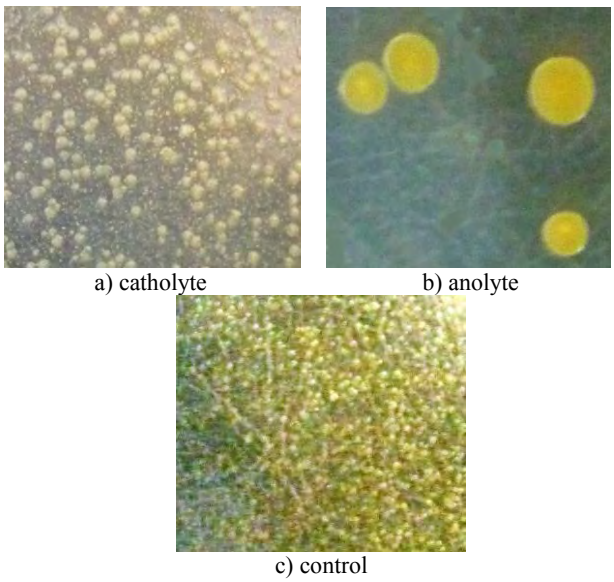
TABLE 1

THE RESULTS OF CALCULATIONS OF COLONIES IN PETRI DISHES

Bacteria genus	Number of bacteria, CFU/cm^3		
	Catholyte	Anolyte	Control
Bacillus	1567	37	2349
Clostridium	507	12	1071

Thus, in Petri dishes, in which bacteria genus *Bacillus,* that have been hold up in electrochemically activated water, were grown, the number of colonies in samples with catholyte decreased by 33.29%, and in the samples with anolyte – by 98.42% compared to the control.

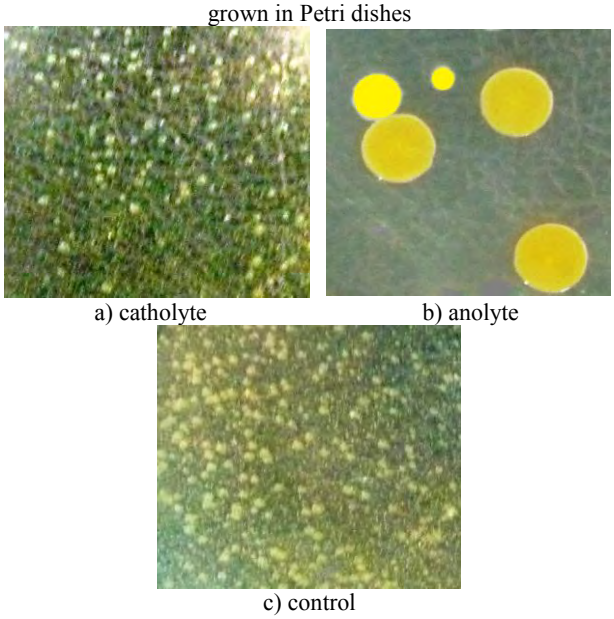
Besides, samples had different size of colonies (Fig. 1.2).



a) catholyte b) anolyte

c) control

Fig. 1 Colonies of bacteria genus *Bacillus*, grown in Petri dishes



a) catholyte b) anolyte

c) control

Fig. 2 Colonies of bacteria genus *Clostridium*, grown in Petri dishes

In Petri dishes, where bacteria genus *Clostridium*, which have been sown and soaked in the catholyte and anolyte, number of colonies decreased by 52.66% and 98.88% respectively, compared to the control.

In samples of both bacteria exposed to the catholyte, colonies were around 0,5-1 mm, in those exposed to the anolyte – 2-6 mm.

Control was characterized the smallest colonies – 0,2-0,5 mm.

Conclusion

So, the results show that electrochemically activated water affects the viability of spore-forming bacteria genus *Bacillus* and *Clostridium*, causing their death.

Thus, using of the catholyte allows to reduce the viability of these bacteria by 35-50%. The anolyte kills the spore-forming bacteria almost completely (98%).

This method of destroying microorganisms can be used in food production and in microbiological practice for destruction of extraneous microflora.

Reference

[1] R. M. S. Thorn, S. W. H. Lee, G. M. Robinson, J. Greenman, D. M. Reynolds, "Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments", European Journal of Clinical Microbiology & Infectious Diseases, 31(5), pp. 641-653, 2012.

[2] K.S. Golohvast, D.S. Ryzhakov, V.V. Chajka, A.N. Gulkov, "Perspektivy ispol'zovanija jelektrohimicheskoj aktivacii rastvorov" ["Application potential of solution electrochemical activation"], Voda: himija i ekologija – Water: chemistry and ecology. no 2, pp. 23-30, 2011.

[3] T.E. Cloete, M.S. Thantsha, M.R. Maluleke and R. Kirkpatrick, "The antimicrobial mechanism of electrochemically activated water against *Pseudomonas aeruginosa* and *Escherichia coli* as determined by SDS-PAGE analysis", Journal of Applied Microbiology, 107(2), pp. 379–384, 2009.

[4] Robinson GM, Lee SWH, Greenman J, Salisbury VC, Reynolds DM, "Evaluation of the efficacy of electrochemically activated solutions against nosocomial pathogens and bacterial endospores", Lett Appl Microbiol, 50(3), pp. 289–294, 2010.

[5] V.I. Prilutskii, V.M. Bakhir, Jelektrohimicheski aktivirovannaja voda: anomal'nye svojstva, mehanizm biologicheskogo dejstvija [Electrochemically activated water: anomalous properties, mechanism of biological action]. Moscow: VNIIIMT, 1997.

[6] B. I. Leonov, V.I. Prilutskii, V.M. Bakhir, Fiziko-himicheskie aspekty biologicheskogo dejstvija jelektrohimicheski aktivirovannoj vody [Physico-chemical aspects biological effects electrochemically activated water]. Moscow: VNIIIMT, 1999.