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RESEARCH ARTICLE

Activity of Disinfecting Biocides and Enzymes of Proteases and Amylases on Bacteria in Biofilms

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Abstract

The presence of microbial biofilms on the surfaces of medical instruments, operating equipment, prostheses, catheters, technological lines in the food industry is a fact that contributes to the infection of the macroorganism and contamination of raw materials and products. The aim of the work was to investigate the effect of disinfecting substances Vantocilu TG and Catamine AB and their combination with enzymes on bacteria in biofilms. In the experiments, we used disinfecting substances Vantocil TG (Arch Biocides LTD, Great Britain) and Catamine AB (Intersintez, Ukraine). Enzymes: Everlase 16 L and Termamyl 300 L (Novozymes, Denmark). It was found that bacteria in biofilms withstood the minimum bactericidal concentration of Vantocil and Catamine, which was set on their planktonic forms. From one mL of wash from the biofilm after exposure to Vantocil were isolated from 1.9×10^3 to 4.3×10^3 microbial cells, and after treatment with Catamine from 5.6×10^3 to 1.7×10^4. At the same time, after treatment of biofilms with Vantocil and Catamine together with enzymes, a decrease in the number of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* cells was observed, on average by two orders up to 10^1 CFU/mL, compared with treatment with biocides only. That is, there is a clear synergy of enzymes and biocides, which ultimately has a more detrimental effect on bacteria in biofilms.

Keywords: *Vantocil TG, Catamine AB, Enzymes, Biofilm degradation*

Dezenfektan Biyositler ve Proteaz ve Amilaz Enzimlerinin Biyofilmlerdeki Bakteriler Üzerine Aktivitesi

Öz

Gıda endüstrisinde tıbbi cihazların, ameliyat ekipmanlarının, protezlerin, kateterlerin, teknolojik alanların yüzeylerinde mikrobiyal biyofilmlerin varlığı, makroorganizma enfeksiyonlarına ve hammadde ve ürünlerin kontaminasyonuna katkıda bulunan bir gerçektir. Bu çalışmanın amacı, dezenfektan maddeler Vantocilu TG ve Catamine AB'nin ve bunların enzimlerle kombinasyonlarının biyofilmlerdeki bakteriler üzerine etkisini araştırmaktır. Deneylerde, Vantocil TG (Arch Biocides LTD, İngiltere) ve Catamine AB (Intersintez, Ukrayna) dezenfektan maddeleri ile Everlase 16 L ve Termamyl 300 L (Novozymes, Danimarka) enzimlerini kullandık. Biyofilmlerdeki bakterilerin, planktonik formlarına karşı uygulanan Vantocil ve Catamine'nin minimum bakterisidal konsantrasyonlarına dayanıklılık gösterdiği saptandı. Vantocil ile sağaltımdan sonra bir mL biyofilm yıkantısından $1.9\text{x}10^3$ ile $4.3\text{x}10^3$ arası mikroorganizma ve Catamin ile sağaltımdan sonra $5.6\text{x}10^3$ ile $1.7\text{x}10^4$ arası mikroorganizma izole edildi. Aynı zamanda, biyofilmlerin enzimlerle birlikte Vantocil ve Catamine ile sağaltımından sonra, *Staphylococcus aureus, Escherichia coli* ve *Pseudomonas aeruginosa* bakteri sayılarında sadece biyositlerin kullanıldığı sağaltım ile kıyaslandığında ortalama olarak 10^1 CFU/mL'ye kadar iki kat bir azalma gözlendi. Sonuçta, biyofilmlerdeki bakteriler üzerine daha hasar verici bir etkiye sahip açık bir enzim ve biyosit sinerjisi mevcuttu.

Anahtar sözcükler: *Vantocil TG, Catamine AB, Enzimler, Biyofilm degradasyonu*

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INTRODUCTION

Disinfection, as a component of all hygienic measures, in medical, veterinary and food industries is aimed at the destruction of opportunistic and infectious pathogen microorganisms to prevent infection of humans, animals and to produce safe food. Therefore, the pharmaceutical industry is constantly working to create ideal disinfectants that have a wide range of antimicrobial action in minimal concentrations, and not cause resistance in bacteria, are non-toxic, non-corrosive, non-allergenic, cheap, etc. [1-4]. However, despite the large number of disinfectants on the market, an ideal drug does not exist, as microorganisms adapt quite quickly to new antibacterial substances [5-7].

Bacterial resistance to biocides may be associated with their presence in the biofilm [8-16]. The modern generalized term "biofilm" is used to define the set of bacteria and products of their metabolism at the interface between solid and liquid phases attached to the surface in an aqueous or water-saturated medium [14,17]. Today, most scientists recognize that a significant number of micro-organisms in natural and artificial environments exist in the form of structured, attached to the surface formations-biofilms [18-20]. Bacteria in the biofilm are surrounded by their own producing matrix (EPS), which consists of poly-saccharides, proteins, uranium acid and humic sub-stances [21-23]. It is due to the matrix, which acts as a barrier that protects bacterial cells inside, many antimicrobial agents cannot penetrate the biofilm [18,24,25].

The presence of bacteria in the biofilm creates serious problems with infection of various surfaces in human and veterinary medicine and the food industry [26,27]. Bacteria in biofilms are much more difficult to destroy with antimicrobial drugs, which can potentially lead to the accumulation and spread of dangerous pathogens. It is reported that the concentration of biocide, which is necessary to kill microbial cells in the biofilm, should be several times higher than the working for this agent [5,28,29]. Therefore, efforts are constantly being made to improve the performance of existing disinfectants or to develop new ones to affect microorganisms in the biofilm state.

Studies found that disinfection with chlorine dioxide and chlorine-containing agents reduced the number of planktonic bacteria in a good way, but had little effect on the content of bacteria in biofilms [18,30]. Perumal et al.[31] found that disinfectants based on hydrogen peroxide in working concentrations did not affect clinical isolates of *Acinetobacter spp., Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which were in biofilms and were isolated in medical institutions. However, planktonic forms of these bacteria were sensitive to these biocides. The authors argue the need to test the effectiveness of disinfectants on biofilm bacteria, rather than planktonic, as this poses a threat for the use of such agents to control the spread of these pathogens.

Therefore, given the role of the matrix in protecting microbial cells from the action of biocides, researchers are looking for different methods for its destruction [32,33]. One such method is the use of enzymes to destroy the extracellular matrix of the biofilm. Studies have shown that enzymes have been significantly effective in reducing the density of *P. aeruginosa* biofilm and its degradation from various surfaces [8,34]. In particular, there were used synthetic polysaccharides to destroy the matrix of biofilms formed by pseudomonads [34-37], used microbial amylase and proteases for destruction of biofilms of gram-positive and gram-negative bacteria. However, researchers are inclined to the opinion that due to the heterogenicity of the composition of the biofilm matrix, the use of mono-enzymes has a limiting potential.

Therefore, for the effective use of enzyme agents in practice, it is necessary to comprehensively study the process of growth and development of biofilm in a particular object with knowledge of the approximate composition of possible microflora. In addition, it is advisable to combine different classes of enzymes with biocidal substances for better contact of the latter with bacterial cells. Therefore, the use of enzymes in combination with antibacterial substances to degrade the biofilm and reduce the content of microorganisms is promising and important in many sectors of the economy. The purpose of the study was to investigate the effect of disinfectants Vantocil TG and Catamine AB and their combination with enzymes on bacteria in biofilms.

MATERIAL AND METHODS

The study contained disinfectants Vantocil TG-20%-an aqueous solution of polyhexamethylenebiguanidine hydro-chloride (Arch Biocides LTD, UK) and Catamine AB - a solution containing 49-51% of alkyldimethylbenzylammonium chloride (Intersynthesis, Ukraine), proteolytic enzyme-Everlase 16 L and amylolytic enzyme-Termamyl 300 L (Novozymes, Denmark), strains of test cultures of *Escherichia coli* (055K59 No.3912/41), *Staphylococcus aureus* (ATCC 25923) and *P. aeruginosa* (27/99). Stainless steel plates of the AISI 321 brand in the size of 30×30 mm for cultivation of biofilms.

The minimum bactericidal concentration of disinfectants was determined by the standard suspension method [3].

The density of microbial biofilms and the effect of disinfectants and enzymes on them were determined according to the guidelines [16]. Briefly: Biofilms of bacterial test cultures were grown on sterile stainless-steel plates in petri dishes for 24 h in plain broth with 1% glucose concentration. The plates with biofilms were then washed three times with sterile phosphate buffer to remove planktonic cells and the plates were dried. Disinfectants or enzymes were added to petri dishes with plates and kept for 15 min. The plates were removed, washed with phosphate buffer and the biofilms were fixed with 96° ethyl alcohol for 10 min. Then the biofilms were stained with a solution of crystalline violet for 10 min. After that, the plates with biofilms were washed three times with phosphate buffer to remove paint residues. Then 5.0 cm^3 of 96° ethyl alcohol was added to a petri dish with a plate and left for 20-30 min, shaking periodically. The optical density of the alcohol solution was measured spectrophotometrically at a wavelength of 570 nm. At the optical density of the washing solution up to 0.5 units, the density of the formed biofilm was considered low, from 0.5 to 1.0 units -average and at a density of solution more than 1.0 units the density of the formed biofilm was considered high [19].

To determine the number of bacteria in the biofilm after exposure to biocides and enzymes, washes were removed from the plates using a sterile swab. Ten-fold dilutions of the wash were then prepared and 1.0 cm^3 of each dilution was sown in petri dishes, plated with plain broth and incubated at 37°C for 24-48 h. Before use, the enzymes were dissolved in 0.1 M phosphate buffer, pH 8.3.

Statistical Analysis

Statistical processing of the results was carried out using methods of variation statistics using the program Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the standard error of the mean (SE) were determined. The difference between the comparable values was considered to be significant for $P<0.05$.

Results

At the first stage of the study, we determined the minimum bactericidal concentration of Vantocil TG and Catamine AB in the suspension method on planktonic forms of bacteria during 15 min of action at a solution temperature of 20±1°C. It was found *(Table 1)* that Vantocil TG showed a better antimicrobial effect on gram-negative bacteria *(E. coli* and *P. aeruginosa),* compared with gram-positive bacteria *(S. aureus)*. In particular, the minimum bactericidal concentration of Vantocil against *S. aureus* was 4.5 times higher, compared with test cultures of *E. coli* and *P. aeruginosa*.

At the same time, Catamine AB had a better effect on gram-positive microflora than on gram-negative. The minimum bactericidal concentration of Catamine relative to test cultures of *S. aureus* was 2.0 times lower compared to the cultures of *E. coli* and 4.0 times compared to *P. aeruginosa*.

It was also found that Vantocil TG acts bactericidal in much lower concentrations compared to Catamine. In particular, the minimum bactericidal concentration of Vantocil relative to test cultures of *S. aureus* was 6.9 times lower than that of Catamine. To inhibit *E. coli* and *P. aeruginosa* cells, the minimum bactericidal concentration of Vantocil was 62 and 125 times lower, respectively, than the concentration of Catamine.

It is believed that the planktonic state of bacteria is intended for the colonization of other surfaces or substrates, and microorganisms are mainly in the biofilm state in the synthesized matrix, which performs a protective function. In fact, the presence of bacteria in the peptide glycolytic matrix of the biofilm and in the depressions of the surface roughness prevents the penetration of disinfectants into the cells [16,18]. Therefore, for effective antimicrobial action of biocides, it is necessary to destroy the bacterial biofilm and ensure maximum contact of the microbial cell with the disinfectant [25]. Given this phenomenon, the next step in our work was to investigate the effect of disinfectants Vantocil TG and Catamine AB in combination with enzymes on bacteria in biofilms. Vantocil TG and Catamine AB were used in concentrations that provided a bactericidal effect on planktonic bacteria *(Table 1)*. Enzymes were used at a concentration that provided maximum proteolytic and amylolytic activity at a temperature of +20±1°C for 15 min of exposure.

The results of studies of the effect of Vantocil TG and enzymes on biofilms formed by *S. aureus* are shown in *Fig. 1*.

It was found that under the action of Vantocil the matrix of the *S. aureus* biofilm was destroyed, which is indicated by a 1.5-fold decrease ($P<0.05$) in the optical density of the biofilm washing solutions. However, the biofilm was still of high density - more than 1.0 unit. Treatment of the biofilm with the proteolytic enzyme Everlase 16 L more intensively destroyed the matrix compared to Vantocil, as the density decreased by 2.4 times ($P<0.05$), i.e. to medium density. This indicates the presence in the matrix of the biofilm of a significant number of peptide components. The effect on biofilms with amylase Termamyl 300 L also significantly destroyed the matrix, its density decreased by 2.1 times ($P<0.05$) relative to the average density. However, the degradation of the biofilm under the influence of Vantocil in combination with the enzymes Everlase 16 L and Termamyl 300 L was the most intensive - the optical density of the washing solutions decreased 4.1 times ($P<0.05$) and the biofilm was considered of low density (less than 0.5 units).

Table 1. *Minimum bactericidal concentration of Vantocil TG and Catamine AB on test cultures of S. aureus, E. coli, P. aeruginosa at an exposure of 15 min and a solution temperature of 20±1°C*

Test Cultures	Concentration of Solutions, %	
	Vantocil TG	Catamine AB
S. aureus	0.009	0.062
E. coli	0.002	0.125
P. aeruginosa	0.002	0.250
n =15		

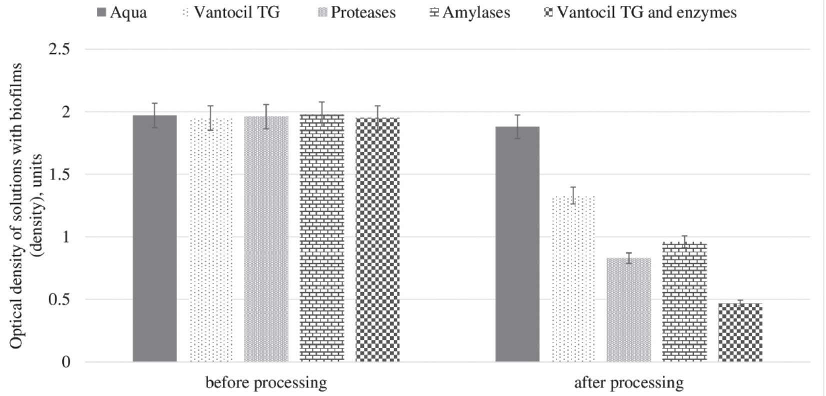


Fig 1. The effect of Vantocil TG and enzymes on biofilms formed by *S. aureus* (action for 15 min at a solution temperature of 20±1°C)

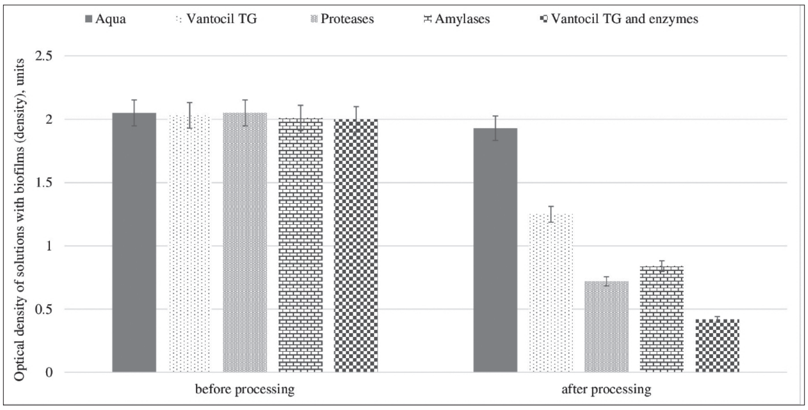


Fig 2. The effect of Vantocil TG and enzymes on biofilms formed by *E. coli* (action for 15 min at a solution temperature of 20±1°C)

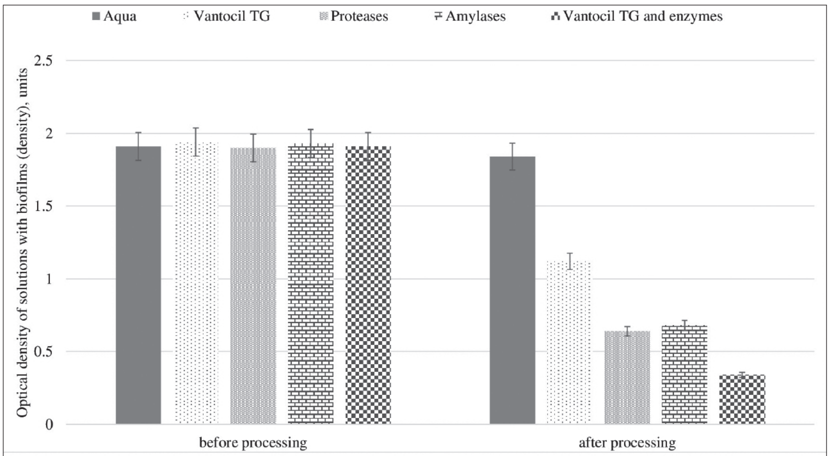


Fig 3. The effect of Vantocil TG and enzymes on biofilms formed by *P. aeruginosa* (action for 15 min at a solution temperature of 20±1°C)

The study of the effect of Vantocil TG and enzymes on biofilms formed by *E. coli* is shown in *Fig. 2*.

A more intensive degradation process of *E. coli* biofilm under the influence of Vantocil and enzymes than *S. aureus* biofilm was revealed. In particular, under the action of Vantocil, the optical density of the biofilm decreased 1.6 times (P<0.05), and under the influence of enzymes Everlase 16 L and Termamyl 300 L 2.8 and 2.4 times (P<0.05), respectively. In this case, after the action of enzymes, the biofilms became of medium density. However, the greatest degradation of the matrix of the biofilm of *E. coli* was observed under the simultaneous influence of Vantocil and enzymes - the optical density of solutions from the biofilm decreased by 4.8 times (P<0.05) and the biofilms became of low density.

The effect of Vantocil TG and enzymes on biofilms formed by *P. aeruginosa (Fig. 3)* showed a similar pattern as the effect on biofilms of *S. aureus* and *E. coli*. However, the matrix of the biofilm of *P. aeruginosa* was more susceptible to destruction than *S. aureus* and *E. coli*. In particular, under the influence of Vantocil, the optical density of biofilm solutions decreased 1.7 times (P<0.05), and under the action of proteolytic and amylolytic enzymes 3.0 and 2.8 times (P<0.05), respectively. However, biofilms of *P. aeruginosa* became of low density only when simultaneously treated with Vantocil and enzymes - 0.34±0.2 units.

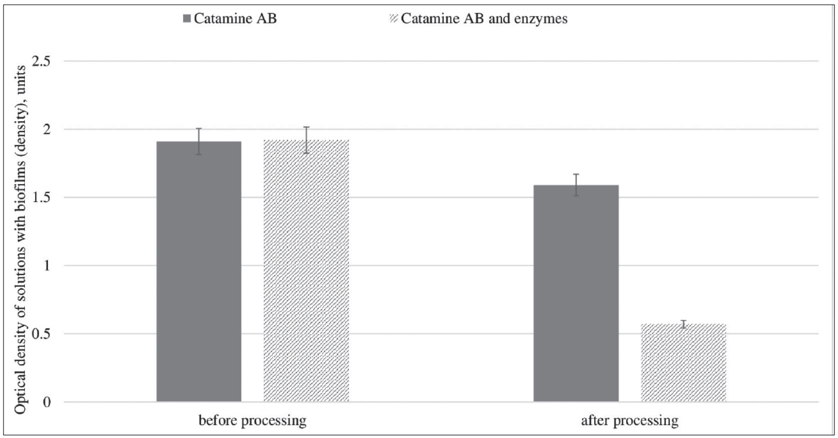
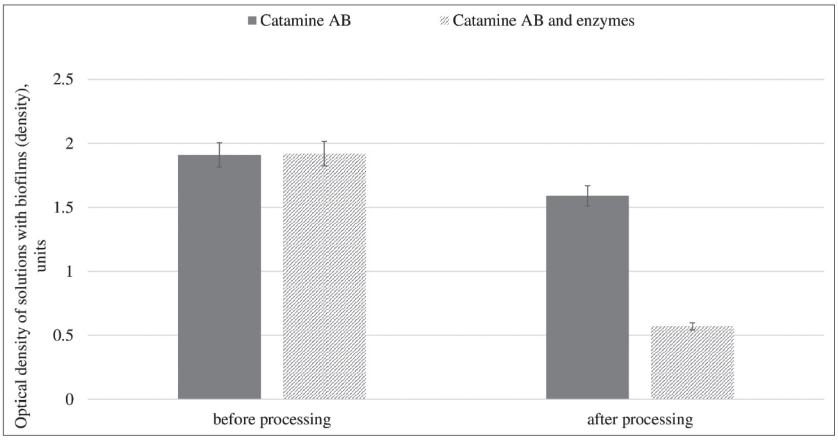


Fig 4. The effect of Catamine AB and its combination with enzymes on biofilms formed by *S. aureus* (action for 15 min at a solution temperature of 20±1°C)

Fig 5. The effect of Catamine AB and its combination with enzymes on biofilms formed by *E. coli* (action for 15 min at a solution temperature of 20±1°C)



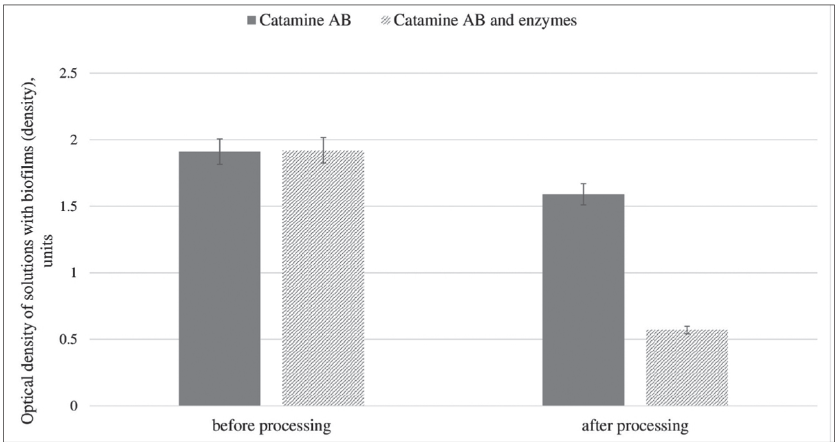


Fig 6. The effect of Catamine AB and its combination with enzymes on biofilms formed by *P. aeruginosa* (action for 15 min at a solution temperature of 20±1°C)

Therefore, the obtained experimental data indicate that the disinfectant Vantocil TG weakly destroys the matrix of biofilms formed by bacteria *S. aureus, E. coli* and *P. aeruginosa*. At the same time, the simultaneous use of Vantocil with proteolytic and glycolytic enzymes leads to significant degradation of the biofilm in the studied bacteria.

In addition to disinfectants based on polyhexamethylenebiguanide hydrochloride, drugs, containing quaternary ammonium compounds, in particular Catamine AB, are widely used in Ukraine and abroad. Therefore, the next part of the work was to determine the effect of Catamine and its action with enzymes on microbial biofilms. The results of the study are shown in *Fig. 4, 5, 6*.

It was found that Catamine in the minimum bactericidal concentration for planktonic cultures to a lesser extent destroyed the biofilms of *S. aureus, E. coli* and *P. aeruginosa*, compared with Vantocil. It was found that biofilms of *S. aureus* were more intensively degraded by Catamine than biofilms of *E. coli* and *P. aeruginosa*. In particular, the optical density of solutions from *S. aureus* biofilms after Catamine treatment decreased 1.4 times ($P<0.05$), and in *E. coli*

Table 2. *Influence of disinfectants and enzymes on the quantitative content of microbial cells in biofilm (action for 15 min at a solution temperature of 20±1°C)*

Studied Bacteria	Bacterial Status	Bacterial Count in 1 cm³ Suspension from Biofilm, CFU				
		Control	Vantocil	Vantocil with Enzymes	Catamine AB	Catamine AB with Enzymes
S. aureus	plankton	$1.1±0.1×10^7$	0	0	0	0
	biofilm	$5.2±0.2×10^8$	$4.3×10^{3*}$	$5.1×10^{1*}$	$5.6×10^{3*}$	$4.4×10^{1*}$
E. coli	plankton	$3.4±0.2×10^7$	0	0	0	0
	biofilm	$4.9±0.1×10^8$	$2.5×10^{3*}$	$1.7×10^{1*}$	$8.2×10^3$	$7.8×10^{1*}$
P. aeruginosa	plankton	$2.8±0.1×10^7$	0	0	0	0

** – P< 0.05 – concerning control*

and *P. aeruginosa* biofilms 1.3 and 1.2 times, respectively. In addition, the biofilms of all bacteria sampled after Catamine treatment remained of high density.

The combination of the action of Catamine with enzymes Everlase 16 L and Termamyl 300 L significantly increased the degradation of the biofilm in both gram-positive and gram-negative bacteria. In particular, under this effect on the biofilms of *S. aureus*, the optical density of the washing solutions decreased 4.1 times (P<0.05) and the biofilms became of low density (0.47±0.2 units). Biofilms of gram-negative bacteria *E. coli* and *P. aeruginosa* degraded less even under the influence of Catamine with enzymes than biofilms of *S. aureus*. The decrease in the optical density of solutions from biofilms in these bacteria was 3.7 and 3.4 times, respectively (P<0.05). The density of biofilms was on the border between low and medium - 0.54-0.57 units, respectively.

In general, the obtained data show that Catamine has a weaker effect on the matrix of the biofilm of *S. aureus, E. coli* and *P. aeruginosa*, compared with Vantocil. However, when combining disinfectants Vantocil TG, Catamine AB with proteolytic and glycolytic enzymes, synergism is manifested in more intensive degradation of biofilms of gram-positive and gram-negative bacteria, their density decreases from high to low.

It is believed that the concentration of antibacterial substance, required for the destruction of bacteria in the biofilm, should be several times higher than the minimum bactericidal value determined on planktonic bacteria. It was important to investigate the effect of disinfectants at the minimum bactericidal concentration found on planktonic bacteria and in combination with enzymes on the quantitative content of microorganisms in the biofilm. The research results are given in *Table 2*.

It was found that bacteria in biofilms withstood the minimum bactericidal concentration of Vantocil and Catamine, which was established on their planktonic forms. From $1.9×10^3$ to $4.3×10^3$ microbial cells were isolated from one ml of biofilm wash after exposure to Vantocil, which is almost five orders less than in the control. At the same time, after the action of Vantocil with enzymes, a decrease in the number of *S. aureus, E. coli* and *P. aeruginosa* cells was observed, on average by two orders up to $5.1×10^1$ CFU/mL, compared with treatment with Vantocil alone.

After treatment of biofilms with Catamine, slightly more bacteria were isolated than after treatment with Vantocil, in particular, the content of *S. aureus* cells was 1.3 times higher (P<0.05), *E. coli* 3.3 times (P<0.05), and *P. aeruginosa* by almost one order ($1.7×10^4$ CFU/mL of wash). The simultaneous action of Catamine with enzymes caused a decrease in the number of bacteria in the biofilm by two orders, compared with the action of Catamine alone. However, 10^1 microbial cells were isolated from *S. aureus* and *E. coli* biofilms and 10^2 from *P. aeruginosa* biofilms, indicating less destruction of the biofilm matrix by disinfectant and enzymes and protection of cells from contact with the biocide.

Discussion

The presence of microbial biofilms on the surfaces of medical instruments, operating equipment, prostheses, catheters, production lines in the food industry is an obvious fact that contributes to microorganism infection and contamination of raw materials and products [19,26,27]. Therefore, the use of biocides is aimed at the destruction of planktonic and biofilm forms of microorganisms on various surfaces [1,3,4,8]. However, successful control of microorganisms, present in biofilms, is possible with the use of disinfectants that destroy the exopolysaccharide matrix and promote closer contact of bacteria with the biocide [34]. Among the significant range of disinfectants, a significant part of them contains as active substances - biguanides and quaternary ammonium compounds. In this study, we determined the effect of disinfectants Vantocil TG and Catamine AB and enzymes Everlase 16 L and Termamyl 300 L on the degradation of biofilm matrix. It was found that Vantocil TG in the minimum bactericidal concentration, which was determined on planktonic bacteria, reduced the density of the biofilm of *S. aureus* by 1.5 times, *E. coli*-1.6 times and *P. aeruginosa*-1.7 times, comparing with the control before processing. This indicates that the exopolysaccharide matrix of biofilms contains components that are poorly degraded by this biocide. At the same time, treatment of biofilms with proteolytic and amylolytic enzymes significantly reduced their density. In particular, after treatment with enzyme Everlase 16 L, the density of the biofilm of *S. aureus* decreased 2.4 times, *E. coli* - 2.8 times and *P. aeruginosa* - 3.0 times. Matrix degradation was less effective with Termamyl 300 L biofilms than with Everlase 16 L. In particular, the density of *S. aureus, E. coli,* and *P. aeruginosa* biofilms decreased 2.1, 2.4, and 2.8 times, respectively. This indicates the heterogeneous chemical composition of the biofilm in different bacteria and for their destruction it is necessary to use enzymes of different classes [34,35]. According to [14,17,21-23] the composition of the biofilm matrix depends on many factors, the availability of nutrients, species composition of microflora, pH of the medium, type of surface, etc. Due to this, the protective function of even one species of bacteria in the biofilm will be different. In addition, a study [36] reported that the degradation of the biofilm of *P. aeruginosa* by the Savinase enzyme was stronger than with Alphamylase treatment, with better proteolytic enzyme matrix destruction. When treating biofilms with Vantocil with enzymes revealed a synergism of action, in particular, the optical density of solutions from biofilms of *S. aureus, E. coli* and *P. aeruginosa* decreased by 4.1, 4.8, 5.6 times compared with the control, and the biofilms became of low density. Synergism of different enzymes in the fight against heterogeneous biofilms was reported [21-23,34]. Despite the fact that Catamine in the minimum bactericidal concentration for planktonic cultures destroyed the biofilms of *S. aureus, E. coli* and *P. aeruginosa* to a lesser extent, compared with Vantocil, the general patterns of exposure to biofilms of Catamine with enzymes were the same as for treatment with Vantocil.

During the study of the effect of disinfectants on the quantitative content of microorganisms in the biofilm, it was found that from one mL of wash from the biofilm after exposure to Vantocil were isolated from 1.9×10^3 to 4.3×10^3 microbial cells, and after treatment with Catamine- from 5.6×10^3 to 1.7×10^4. The results confirm the data of many researchers [18,28-31] that the determined minimum bactericidal concentration on planktonic bacteria does not have a bactericidal effect on biofilm forms. At the same time, after treatment of biofilms with Vantocil and Catamine together with enzymes, a decrease in the number *of S. aureus, E. coli* and *P. aeruginosa* cells was observed, on average by two orders to 10^1 CFU/mL, compared with treatment with biocides only.

There is a clear synergy of enzymes and biocides, which ultimately has a more detrimental effect on bacteria in biofilms. In this case, it can be argued that enzymes destroy the matrix of the biofilm, which promotes better contact of antibacterial substances with target cells. Therefore, we believe that the combination of antibacterial substances with enzymes is a good prospect in the fight against bacteria in biofilms on the surfaces of various materials. When choosing a disinfectant, it is necessary to evaluate its effectiveness against bacteria in biofilms under conditions close to production.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MK, VK and VH conceived and executed the idea, designed experiments, analyzed results and a deep revision of the manuscript. ZM, YH, TY and SK collected samples, performed experiments, contributed to tand implementation of the research. All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

References

1. Kovalenko VL, Ponomarenko GV, Kukhtyn MD, Paliy AP, Bodnar OO, Rebenko HI, Kozytska TG, Makarevich TV, Ponomarenko OV, Palii AP: Evaluation of acute toxicity of the "Orgasept" disinfectant. *Ukr J Ecol,* 10 (4): 273-278, 2020. DOI: 10.15421/2020_199

2. Kovalenko AM, Tkachev AV, Tkacheva OL, Gutyj BV, Prystupa OI, Kukhtyn MD, Dutka VR, Veres YM, Dashkovskyy OO, Senechyn VV, Riy MB, Kotelevych VA: Analgesic effectiveness of new nanosilver drug. *Ukr J Ecol,* 10 (1): 300-306, 2020.

3. Kovalenko VL, Kovalenko PL, Ponomarenko GV, Kukhtyn MD, Midyk SV, Horiuk YV, Garkavenko VM: Changes in lipid composition of *Escherichia coli* and *Staphylococcus areus* cells under the influence of disinfectants Barez, Biochlor and Geocide. *Ukr J Ecol,* 8 (1): 547-550, 2018.

4. Jin M, Liu L, Wang D, Yang D, Liu W., Yin J, Yang H, Wang H, Qiu Z, Shen D, Shi D, Li H, Guo J, Li J: Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. *ISME J*, 14, 1847-1856, 2020. DOI: 10.1038/s41396-020-0656-9

5. Davin-Regli A, Pagès JM: Cross-resistance between biocides and antimicrobials: An emerging question. *Rev Sci Tech*, 31 (1): 89-104, 2012. DOI: 10.20506/RST.31.1.2099

6. Berhilevych OM, Kasianchuk VV, Kukhtyn MD, Lotskin IM, Garkavenko TO, Shubin PA: Characteristics of antibiotic sensitivity of *Staphylococcus aureus* isolated from dairy farms in Ukraine. *Regul Mech Biosyst*, 8 (4): 559-563, 2017. DOI: 10.15421/021786

7. Kozlovska IM, Romanjuk NY, Romanjuk LM, Kukhtyn MD, Horiuk YV, Karpyk GV: The effect of antimicrobial agents on planktonic and biofilm forms of bacteria that are isolated from chronic anal fissures. *Regul Mech Biosyst*, 8 (4): 577-582, 2017. DOI: 10.15421/021789

8. Thallinger B, Prasetyo EN, Nyanhongo GS, Guebitz GM: Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. *Biotechnol J*, 8 (1): 97-109, 2013. DOI: 10.1002/biot. 201200313

9. Romling U, Balsalobre C: Biofilm infections, their resilience to therapy and innovative treatment strategies. *J Intern Med*, 272 (6): 541-561, 2012. DOI: 10.1111/joim.12004

10. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ: Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*, 13 (5): 269-284, 2015. DOI: 10.1038/nrmicro3432

11. Chapman JS: Biocide resistance mechanisms. *Int Biodeterior Biodegradation*, 51 (2): 133-138, 2003. DOI: 10.1016/S0964-8305(02)00097-5

12. Zhang L, Mah TF: Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *J Bacteriol*, 190 (13): 4447-4452, 2008.

DOI: 10.1128/JB.01655-07

13. Patel R: Biofilms and antimicrobial resistance. *Clin Orthop Relat Res*, 437, 41-47, 2005. DOI: 10.1097/01.blo.0000175714.68624.74

14. Costerton W, Veeh R, Shirliff M, Pasmore M, Post C, Ehrlich G: The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest*, 112 (10): 1466-1477, 2003. DOI: 10.1172/JCI20365

15. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F: Resistance of bacterial biofilms to disinfectants: A review. *Biofouling*, 27 (9): 1017-1032, 2011. DOI: 10.1080/08927014.2011.626899

16. Kukhtyn M, Kravcheniuk K, Beyko L, Horiuk Y, Skliar O, Kernychnyi S: Modeling the process of microbial biofilm formation on stainless steel with a different surface roughness. *East-Eur J Enterp Technol*, 2 (98) 14-21, 2019. DOI: 10.15587/1729-4061.2019.160142

17. Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, Qiu J, Guan X, Huang T: Beyond risk: Bacterial biofilms and their regulating approaches. *Front Microbiol*, 11:928, 2020. DOI: 10.3389/fmicb.2020.00928

18. El-Azizi M, Farag N, Khardori N: Efficacy of selected biocides in the decontamination of common nosocomial bacterial pathogens in biofilm and planktonic forms. *Comp Immunol Microbiol Infect Dis, 47*, 60-71, 2016. DOI: 10.1016/j.cimid.2016.06.002

19. Kukhtyn M, Berhilevych O, Kravcheniuk K, Shynkaruk O, Horiuk Y, Semaniuk N: Formation of biofilms on dairy equipment and the influence of disinfectants on them. *East-Eur J Enterp Technol*, 5/11 (89): 26-33, 2017. DOI: 10.15587/1729-4061.2017.110488

20. Hemati S, Kouhsari E, Sadeghifard N, Maleki A, Omidi N, Mahdavi Z, Pakzad I: Sub-minimum inhibitory concentrations of biocides induced biofilm formation in *Pseudomonas aeruginosa*. *New Microbes New Infect*, 38:100794, 2020. DOI: 10.1016/j.nmni.2020.100794

21. Orgaz B, Kives J, Pedregosa AM, Monistrol IF, Laborda F, SanJose C: Bacterial biofilms removal using fungal enzymes. *Enzyme Microb Technol*, 40, 51-56, 2006. DOI: 10.1016/j.enzmictec.2005.10.037

22. Leroy C, Delbarre C, Gillebaert F, Compere C, Combes D: Effect of commercial enzymes on the adhesion of a marine biofilm forming bacterium. *Biofouling*, 24, 11-22, 2008. DOI: 10.1080/08927010701784912

23. Oliveira NM, Martinez-Garcia E, Xavier J, Durham WM, Kolter R, Kim W, Foster KR: Correction: Biofilm formation as a response to ecological competition. *PLoS Biol*, 13 (8): e1002232, 2015. DOI: 10.1371/journal.pbio.1002232

24. Xavier JB, Picioreanu C, Rani SA, van Loosdrecht MCM, Stewart PS: Biofilm control strategies based on enzymatic disruption of the extracellular polymeric substance matrix- a modeling study. *Microbiology*, 151, 3817-3832, 2005. DOI: 10.1099/mic.0.28165-0

25. Walker SM, Fourgialakis M, Cerezo B, Livens S: Removal of microbial biofilms from dispense equipment: Effect of enzymatic predigestion and detergent treatment. *J Inst Brew*, 113, 61-66, 2007. DOI: 10.1002/j.2050-0416.2007.tb00257.x

26. Hoque J, Konai MM, Gonuguntla S, Manjunath GB, Samaddar S, Yarlagadda V, Haldar J: Membrane active small molecules show selective broad spectrum antibacterial activity with no detectable resistance and eradicate biofilms. *J Med Chem,* 58 (14): 5486–5500, 2015. DOI: 10.1021/acs.jmedchem.5b00443

27. Oxaran V, Dittmann KK, Lee SH, Chaul LT, de Oliveira CAF, Corassin CH, Gram L: Behavior of foodborne pathogens *Listeria monocytogenes* and *Staphylococcus aureus* in mixed-species biofilms exposed to biocides. *Appl Environ Microbiol*, 84:e02038-18, 2018. DOI: 10.1128/AEM.02038-18

28. Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib NE: Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. *Arch Microbiol*, 196 (7): 453-472, 2014. DOI: 10.1007/s00203-014-0983-1

29. Horiuk Y, Kukhtyn M, Kovalenko V, Kornienko L, Horiuk V, Liniichuk N: Biofilm formation in bovine mastitis pathogens and the effect on them of antimicrobial drugs. *Indep J Manag Prod*, 7 (10): 897-910, 2019. DOI: 10.14807/ijmp.v10i7.1012

30. Berry D, Xi C, Raskin L: Microbial ecology of drinking water distribution systems. *Curr Opin Biotechnol*, 17, 297-302, 2006. DOI: 10.1016/j.copbio.2006.05.007

31. Perumal PK, Wand ME, Sutton JM, Bock LJ: Evaluation of the effectiveness of hydrogen-peroxide-based disinfectants on biofilms formed by Gram-negative pathogens. *J Hosp Infect*, 87 (4): 227-233, 2014. DOI: 10.1016/j.jhin.2014.05.004

32. Salata V, Kukhtyn M, Pekriy Y, Horiuk Y, Horiuk V: Activity of washing-disinfecting means "San-active" for sanitary treatment of equipment of meat processing enterprises in laboratory and manufacturing conditions. *Ukr J Vet Agric Sci*, 1 (1): 10-16, 2018. DOI: 10.32718/ujvas1-1.02

33. Verkholyuk MM, Peleno R, Turko I: Resistance of *S. aureus* Atcc 25923, *E. coli* 055k59 No. 3912/41 and *P. aeruginosa* 27/99 to the Wash-disinfectant «Milkodez». *EUREKA: Health Sci*, 1, 55-60, 2020. DOI: 10.21303/2504-5679.2020.001100

34. Lequette Y, Boels G, Clarisse M, Faille C: Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling*, 26 (4): 421-431, 2010. DOI: 10.1080/08927011003699535

35. Vickery K, Pajkos A, Cossart Y: Removal of biofilms from endoscope: Evaluation of detergent efficacy. *Am J Infect Control,* 32, 170-176, 2004. DOI: 10.1016/j.ajic.2003.10.009

36. Meireles A, Borges A, Giaouris E, Simões M: The current knowledge on the application of anti-biofilm enzymes in the food industry. *Food Res Int*, 86, 140-146, 2016. DOI: 10.1016/j.foodres.2016.06.006

37. Loiselle M, Anderson KW: The use of cellulose in inhibiting biofilms formation from organisms commonly found on medical implants. *Biofouling*, 19, 77-85, 2003. DOI: 10.1080/0892701021000030142