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FORMATION OF BIOFILMS ON DAIRY EQUIPMENT AND THE INFLUENCE OF DISINFECTANTS ON THEM

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Визначено доцільність вивчення формування мікробних біоплівок на молочному обладнанні. Виявлено, що на обладнанні утворюються біоплівки високої і середньої щільності. На поверхні з шорсткістю 0,16 мкм утворюються біоплівки нижчої щільності, порівняно з поверхнею із шорсткістю 0,63–0,95 мкм. Встановлено, що для визначення ефективності дезінфектантів необхідно перевіряти вплив на бактерії у біоплівках

Ключові слова: бактерії, адгезія, біоплівки, матрикс, молочне обладнання, нержавіюча сталь, шорсткість, дезінфікуючі засоби

Определена целесообразность изучения формирования бактериальных биопленок на молочном оборудовании. Выявлено, что на оборудовании образуются биопленки высокой и средней плотности. На поверхности с шероховатостью 0,16 мкм образуются менее плотные биопленки по сравнению с поверхностью с шероховатостью 0,63–0,95 мкм. Установлено, что для определения эффективности дезинфектантов необходимо проверять влияние на бактерии в биопленках

Ключевые слова: бактерии, биопленки, адгезия, матрикс, молочное оборудование, нержавеющая сталь, шероховатость, дезинфицирующие средства

1. Introduction

The key task of dairy industry is the production of a sufficient amount of quality and safe dairy products. The main factor that reduces the terms of storage and safety of dairy products is the micro-organisms [1–3]. Quantitative and qualitative composition of microflora of the products depends on the compliance with hygienic conditions of production and effective sanitation of technological equipment [4–6]. According to data of WHO, the most significant source of microbial contamination of food products during production is the technological equipment [7]. About 40 % of the food poisoning of people in the world are caused by microorganisms that penetrate raw materials and finished products from processing equipment [8]. Microflora mostly survives on the surfaces of equipment during sanitation in the so-called "dead zones" (bends, joints, gaskets, valves, cracks, scratches) due to the formation of a biofilm [9–12]. According to data in [9], the equipment on which at least one plankton bacteria was detected carries about 1,000 microorganisms formed in the biofilms.

Thus, a detailed study of the microflora on dairy equipment, the mechanisms of survival of bacteria during sanitation, the sources of penetration of microorganisms into milk products is a relevant task in the dairy industry.

2. Literature review and problem statement

A microbial biofilm is the formation that consists of one or more species or genera of bacteria attached to the biogenic or abiogenic surface and surrounded by a self-producing ma-

trix [13, 14]. The matrix (extracellular polymeric substance) is a complex of biopolymers (polysaccharides, peptides, nucleic acids, exoferments and other substances) synthesized by the microorganisms that form a biofilm, which protects bacteria from factors of the environment [15, 16].

Formation of biofilms is a complex process that consists of the following stages: adhesion (attachment) of bacteria to the surface, growth of microbial mass, formation of cell clusters, products of the polymeric extracellular matrix [14, 17–20]. Microbial adhesion depends on numerous factors:

– type of bacteria (not all organisms have the same adhesion capability) [21, 22];

– physical and chemical properties of the surface (roughness, chemical composition, surface free energy, hydrophilicity or hydrophobicity of the material) [23–26];

– environmental parameters (osmolarity, pH, temperature, oxygen partial pressure, the presence of antimicrobial substances, etc.) [27–30].

Following the attachment of microorganisms to the surface, there starts the process of development of a biofilm. Density of the biofilm subsequently grows through the reproduction of bacteria and the synthesis of the matrix. Upon reaching a critical quantity of bacteria in the biofilm, the cells closest to the adhesive cell surface die due to lack of nutrients, oxygen and a change in pH. The rest of bacteria in the biofilm remain in anabiotic state.

Next, the deepest layers of the biofilm begin to produce planktonic cells that leave the biofilm and colonize other surfaces [31].

Studies show that the microbial biofilms formed on the surfaces of dairy equipment negatively impact safety of the finished product and constitute a danger to the health of people since the composition of biofilms, in addition to saprophytic microflora, may contain pathogenic microorganisms [1, 9, 32]. The biofilms formed by *E. coli*, *Listeria spp.*, *Yersinia enterocolitica*, *S. aureus*, *Salmonella spp.*, *Pseudomonas spp.*, *Bacillus cereus* and others were detected on dairy equipment [33–36]. The biofilms created by bacteria of the genera *Streptococcus*, *Staphylococcus*, *Shigella*, *Escherichia*, *Enterobacter, Bacillus* – on the surfaces of pasteurizers at dairy plants [6, 37–39].

It is reported that the process of biofilm formation on the surfaces of technological lines of dairy equipment has its own peculiarities that distinguish them from the biofilms formed on the medical equipment [12, 16, 27]. This is due to the presence of large number of bends, joints, a considerable length of dairy equipment, automatic washing [11]. That is why the surface relief, its structure and roughness exert significant impact on the process of biofilm formation, which require detailed comprehensive study.

Even though there is a significant quantity of commercially available means for sanitary processing of dairy equipment, not all of them are sufficiently effective [1, 9]. Recent research [40–42] indicate that disinfectants and antibiotics do not always act on bacteria in biofilms. It is reported that resistance of bacteria in a biofilm depends mainly on the composition of the matrix, which is different in different genera of bacteria [19, 29]. That is why disinfectants, which are effective for the biofilms of one genera of bacteria may be inefficient for others.

Thus, there are not enough studies in the dairy industry that would highlight effect of disinfectants on plankton- and biofilm-related forms of bacteria. The experiments to be conducted in this field could make it possible to identify the most effective means of sanitization. This would help to prevent the formation of stable microbial biofilms on dairy equipment and microbial contamination of finished products.

3. The aim and objectives of the study

The aim of present work was to explore the features of formation of the microflora on dairy equipment and in the finished products, the microorganisms' capability to form biofilms and to determine effectiveness of disinfectants.

To achieve the set aim, the following tasks had to be solved:

– to perform identification of microorganisms isolated from dairy equipment and the products received from milk processing plants;

– to determine density of the biofilms formed by bacteria isolated from dairy equipment;

– to examine formation process of the biofilm *Escherichia coli* on the surface of stainless steel with different surface roughness;

– to determine the impact of antibacterial preparations used for sanitizing milk equipment on the plankton and biofilm forms of microorganisms.

4. Materials and methods for exploring the microflora of dairy equipment, biofilms, and effectiveness of disinfectants

4. 1. Examined materials and equipment used in the experiment

The samples of raw milk, milk washings from dairy equipment, tanks-coolers, packing machines and finished products were selected at three milk processing plants in Ternopil and Lviv oblasts (Ukraine). Milk washings were taken from the equipment after sanitization before and half-way through the technological process of production. Washing and disinfection of the equipment was mostly carried out automatically using the CIP-plants (Cleaning In Place). We used the following disinfectants for sanitization: chlorine-based (P3-ansep CIP, Eco chlor, Medicarine); based on hydrogen peroxide and peracetic acid (P3-oxonia active-150); containing quaternary ammonium salts (Maxidez); based on silver nanoparticles (Argenvit). The samples were delivered to a laboratory in the refrigerator bag at a temperature of 4–6 °C within 1–3 hours.

The equipment used in the experiment, as well as the techniques for determining the microbiological indicators of microflora of the equipment, are described in detail in paper [43].

5. Results of studying the microorganisms of dairy equipment and biofilms

It is well known that the raw milk, which is supplied to milk processing plants, is not sterile; the milk of the highest quality, extra grade, may contain, in line with DSTU 3662-97, microorganisms in the amount of 10^5 cfu/cm^3. This microflora is formed while receiving milk, its initial treatment, cooling and transportation. Accordingly, the microorganisms of raw milk create microflora of the technological equipment at milk processing plants, despite the application of rigorous sanitization with modern disinfectant agents.

Table 1 gives results of the research into isolation of microorganisms from raw milk, technological equipment, and

finished products at the milk processing plants in Ternopil and Lviv oblasts. Milk washings were selected from the equipment after sanitization before and half-way through the technological process of production.

Data in Table 1 show that the microflora of the raw milk was most often isolated from tanks-coolers with the most widely spread bacteria being the genus *Bacillus* and *Lactobacillus*, which were present in the examined samples in 100 % of cases. Such dairy equipment as bactofuge units, pasteurizers, homogenizers and cheese baths are contaminated with microflora almost to the same degree; bacteria were isolated from these surfaces in 51.3–9.1 % of cases. Packaging machines yielded the least number of microorganisms; such common genera of bacteria as *Bacillus* and *Lactobacillus* were isolated in 37.1–11.3 %, respectively, while other species did not exceed 10 % by isolation frequency. A similar tendency was noted also when examining the finished products, out of which most often we isolated representatives of the genus *Bacillus* and *Lactobacillus,* 44.4–28.5 %, while bacteria of the family *Enterobacteriaceae* were found in one third of the investigated samples.

In order to find what properties help microorganisms survive on technological equipment during sanitization, we studied density of the microbial biofilms in the isolated bacteria (Table 2).

Data in Table 2 show that the major genera of microorganisms, which are isolated from the dairy equipment, form microbial biofilms of high and medium density. In 100 % of cases, high-density biofilms were formed by bacteria *Bacillus spp.* and *Enterococcus faecalis*. Staphylococci, Escherichia coli and pseudomonades formed mainly high-density biofilms in 74.3 to 86.8 % of cases. Streptococci, in addition to middle- and high-density biofilms, formed low-density biofilms in 14.7 % of cases. Bacteria of the genus *Lactobacillus spp.* almost equally formed biofilms of high and medium density.

In the food industry, equipment is most commonly made of stainless steel of the following brands AISI 316, AISI 321, AISI 329, AISI 409, AISI 410 [23]. These brands of steel can have different surface roughness. According to the criteria for equipment hygiene, the surface of steel should have a roughness of less than 0.8 μm [44–46] because effectiveness of cleaning and disinfection depends on the magnitude of surface roughness.

Microphotographs of the plates made of stainless steel of brand AISI 321 with different surface roughness are shown in Fig. 1.

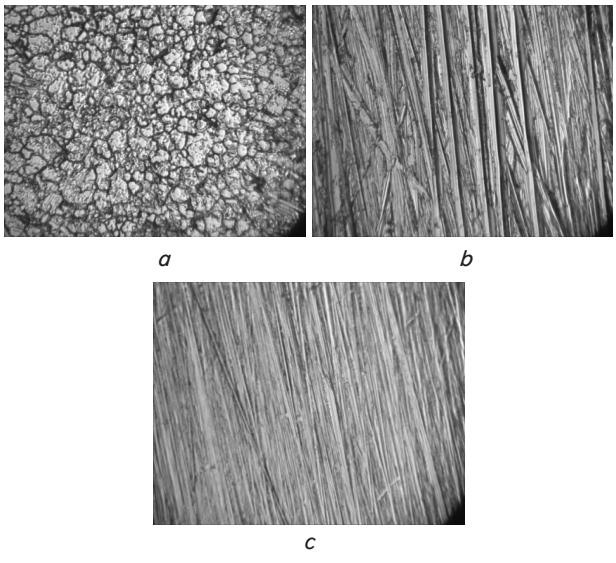

Fig. 1. Physical appearance of plates made of stainless steel of brand AISI 321 with different surface roughness under a microscope (magnification ×1500): *a* — roughness (0.955±0.072) μm; *b* — roughness (0.63±0.087) μm; *c* — roughness (0.16±0.65) μm

Fig. 1. *a–c* shows that the surface of steel with a higher roughness has deeper cavities and significant protrusions compared to the surface with less roughness.

The capability to form biofilms by the strain *Escherichia coli* on the surface of steel of brand AISI 321 with a surface roughness of 0.16±0.072 μm, 0.63±0.087 μm and 0.955±0.065 μm at temperature 17±1 °C over 24 hours is given in Table 3.

Data in Table 3 show that the surface roughness of stainless steel exerts an influence on the process of adhesion and biofilm formation by E. coli. We observed formation of biofilms with lower density on the surface of steel with a roughness of 0.16±0.065 μm, compared to the surface with a roughness of 0.63±0.087 and 0.955±0.072 μm. This pattern is observed at a temperature of 17 °C during period from 6 to 24 hours, with the subsequent formation of a biofilm with high density regardless of the surface roughness. In other words, over 24 hours of incubation, at a temperature of 17±1 °C, the matrix of the biofilm *Escherichia coli* fills up all cavities and protrusions of the steel surface with its roughness no longer important for adhesion.

Table 1

Frequency of isolation of microorganisms from raw milk, technological equipment, and finished products at milk processing plants, % M±m, n=77

Examined object	Frequency of isolation of microorganisms						
	Bacillus	*Lactobacillus*	*Enterococcus*	*Staphylococcus*	*Streptococcus*	*Pseudomonas*	*Enterobacteriaceae*
Raw milk	100	100	100	100	100	100	100
Tanks-coolers	100	100	78.5±4.9	71.3±2.6	52.7±3.4	68.8±4.5	77.2±4.7
Bactofuge units	51.3±3.6	33.7±2.2	36.5±2.3	24.3±1.1	9.1±0.5	11.2±0.7	22.7±1.3
Pasteurizers, homogenizers	64.5±4.7	44.8±2.6	42.5±2.4	12.4±0.5	2.3±0.1	3.2±0.2	37.7±2.2
Cheese baths	77.4±5.6	69.3±3.1	24.6±1.5	17.3±1.1	7.5±0.4	4.7±0.3	38.5±2.5
Packaging machines	37.1±1.9	11.5±0.7	4.6±0.2	2.3±0.1	4.5±0.2	0	8.9±0.5
Dairy products	44.4±2.8	28.5±1.5	11.7±0.7	8.9±0.4	3.2±0.1	2.7±0.1	31.5±0.2

Table 2

Formation of biofilms by the microorganisms isolated from technological equipment at milk processing plants, %, $M \pm m$, n=180

Microorganisms	Quantity of microorganisms, which formed a biofilm of density		
	low	medium	high
Bacillus spp.	0	0	100
Lactobacillus spp.	0	42.7±2.8	57.3±3.1
Enterococcus			
– faecalis	0	0	100
– faecium	0	21.4±1.5	78.6±3.9
Staphylococcus			
– coagulase (positive)	0	13.2±0.8	86.8±4.2
– coagulase (negative)	0	21.4±1.5	78.6±3.4
Streptococcus spp.	14.7±0.8	56.8±2.3	28.5±1.7
Pseudomonas spp.	0	25.7±1.1	74.3±4.8
Escherichia coli	0	17.6±1.3	82.4±4.7

Table 3

Density of the *Escherichia coli* biofilms on the surface of stainless steel of brand AISI 321 at 17±1 °C, units

Formation time of a microbial biofilm, hours	Surface roughness of stainless steel		
	0.16±0.065 μm	0.63±0.087 μm	0.955±0.072 μm
3	0.213±0.002	0.214±0.002	0.217±0.002
6	0.418±0.002	0.426±0.002	0.467±0.002
9	0.462±0.003	0.508±0.003	0.572±0.003
12	0.585±0.003	0.680±0.004	0.708±0.004
18	0.634±0.004	0.712±0.004	0.746±0.004
24	0.863±0.004	0.987±0.005	1.217±0.006

Results of electron-microscopic studies of bacteria, which were isolated from technological equipment at the milk processing plants, and are in the formed biofilm, are shown in Fig. 2.

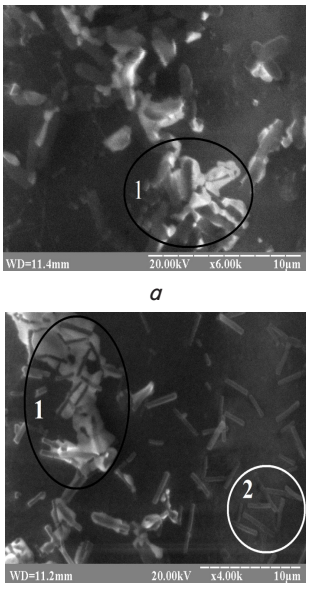

Fig. 2. Microphotographs of microorganisms formed in a biofilm on dairy equipment: *a* – *Escherichia coli; b* – *Pseudomonas fluorescens;* 1 – bacteria in biofilm; 2 – bacteria without biofilm

An analysis of electron-microscopic images, which are shown in Fig. 2, revealed that the microorganisms that are in a biofilm have a physical appearance of solid clusters. By forming cell clusters, bacteria in a biofilm acquire better capabilities to survive under adverse action of detergents and disinfectants.

It is believed that the effective concentration of disinfectants for biofilm forms of bacteria is several times higher than that which acts on the planktonic microorganisms [35, 40, 42]. We determined sensitivity of the bacteria formed in a biofilm to six disinfectants used for sanitizing dairy equipment at milk processing plants. The method of determining sensitivity of planktonic bacteria to the given means served as a control. In the experiments, we used the means in a concentration and at temperature according to the manufacturer's instructions.

Table 4

Sensitivity of planktonic and biofilm forms of bacteria to disinfectants for sanitizing dairy equipment

Examined microorganisms	Form	Quantity of bacteria per 1 cm^3 in a suspension or washing, cfu						
		Control	Argenvit	P3-oxonia active-150	Eco chlor	Medicarine	P3-ansep CIP	Maxidez
Staphylococcus aureus	planktonic	$1.3\pm0.1\times10^7$	$8.5\pm0.6\times10^5$	0	0	0	0	0
	biofilm	$2.3\pm0.2\times10^8$	$1.8\pm0.7\times10^7$	0	$2.1\pm0.1\times10^3$	$4.3\pm0.2\times10^2$	$2.2\pm0.2\times10^2$	$3.8\pm0.2\times10^1$
Streptococcus spp.	planktonic	$1.0\pm0.1\times10^7$	$5.4\pm0.3\times10^3$	0	0	0	0	0
	biofilm	$3.2\pm0.2\times10^5$	$6.2\pm0.4\times10^3$	1.0×10^2	$1.2\pm0.1\times10^3$	$3.1\pm0.1\times10^2$	$1.1\pm0.1\times10^1$	$0.9\pm0.1\times10^1$
Enterococcus faecalis.	planktonic	$1.4\pm0.1\times10^7$	$9.8\pm0.7\times10^5$	0	0	0	0	0
	biofilm	$4.1\pm0.2\times10^7$	$1.7\pm0.1\times10^7$	0	$1.0\pm0.1\times10^3$	$2.3\pm0.1\times10^2$	$2.0\pm0.2\times10^1$	$7.6\pm0.4\times10^2$
Lactobacillus spp.	planktonic	$2.1\pm0.2\times10^7$	$2.4\pm0.2\times10^3$	0	0	0	0	0
	biofilm	$9.8\pm0.6\times10^6$	$4.8\pm0.3\times10^4$	$2.0\pm0.1\times10^2$	$4.1\pm0.3\times10^2$	$1.0\pm0.1\times10^2$	$4.1\pm0.2\times10^1$	$9.0\pm0.2\times10^1$
Escherichia coli	planktonic	$1.1\pm0.1\times10^7$	$1.5\pm0.1\times10^5$	0	0	0	0	0
	biofilm	$3.8\pm0.2\times10^8$	$3.2\pm0.2\times10^7$	0	$6.1\pm0.4\times10^2$	$5.2\pm0.3\times10^2$	$9.0\pm0.6\times10^2$	$1.2\pm0.1\times10^2$
Pseudomonas aeruginosa	planktonic	$1.3\pm0.1\times10^7$	$1.1\pm0.1\times10^5$	$2.5\pm0.1\times10^1$	$7.3\pm0.5\times10^2$	0	$5.0\pm0.3\times10^2$	0
	biofilm	$5.9\pm0.4\times10^6$	$4.2\pm0.2\times10^5$	$5.0\pm0.2\times10^2$	$9.9\pm0.7\times10^3$	$1.1\pm0.1\times10^2$	$6.8\pm0.4\times10^3$	$2.0\pm0.2\times10^3$
Pseudomonas fluorescens	planktonic	$1.0\pm0.1\times10^7$	$7.0\pm0.5\times10^3$	0	0	0	0	0
	biofilm	$3.5\pm0.2\times10^5$	$8.2\pm0.7\times10^3$	0	0.9×10^1	$3.5\pm0.2\times10^1$	$0.8\pm0.1\times10^1$	0
Bacillus spp.	planktonic	$1.3\pm0.1\times10^7$	$9.1\pm0.7\times10^5$	0	0	0	0	0
	biofilm	$3.0\pm0.2\times10^8$	$4.7\pm0.1\times10^7$	$2.2\pm0.1\times10^1$	$4.6\pm0.3\times10^2$	$6.2\pm0.1\times10^2$	$4.1\pm0.2\times10^1$	$7.7\pm0.5\times10^2$

Results of research into determining sensitivity of the planktonic and biofilm forms of bacteria to disinfectants are given in Table 4.

Data in Table 4 show that out of the six examined disinfectants only the preparation Argenvit has proved to be ineffective, not only for the destruction of the bacteria in biofilms, but even for the destruction of the planktonic forms. This preparation exerted weak bactericidal effect on bacteria and destroyed 74.0–99.0 % of planktonic microorganisms and 46.3–90.0 % of microorganisms formed in the biofilms.

All working solutions of the disinfectants P3-ansep CIP, Eco chlor, Medicarine, Maxidez showed bactericidal effect on the planktonic bacteria in the concentrations recommended in the instructions. The bacteria formed in biofilms demonstrated increased resistance to the given solutions of disinfectants. After the action of the means, the milk washings from the biofilm surfaces revealed from 9 to 9,900 cfu/cm^3.

The most effective disinfectant for the destruction of microbial biofilms turned out to be the P3-oxonia active-150 based on hydrogen peroxide and peracetic acid. This agent showed bactericidal effect on biofilms of the bacteria *Staphylococcus aureus, Streptococcus spp., Enterococcus faecalis* and *Pseudomonas fluorescens*. The agent decreased the number of bacteria in the biofilms formed by *Lactobacillus spp., Escherichia coli, Pseudomonas aeruginosa and Bacillus spp.* to 500 cfu per 1 cm^3 of washing. The effect of the agent is caused by the action of hydrogen peroxide, which gives off free radicals during reaction, acting on the biofilm's matrix. *Pseudomonas aeruginosa* proved the most resistant to disinfectants. Only the planktonic forms *P. aeruginosa* were sensitive to the preparations Medicarine, Maxidez, while biofilm forms were resistant to all the agents used in the experiment.

6. Discussion of results of studying the formation of microbial biofilms on dairy equipment and the action of disinfectants

A presence of microbial biofilms on the surfaces of dairy equipment is regarded as a danger to the health of consumers of products, because the biofilms can contain, in addition to the saprophytic, the pathogenic micro-organisms [1, 9, 34, 35]. It is also obvious that bacteria from the biofilms penetrate dairy products and reduce the time of their shelf life [32, 33]. The studies found that even after standard sanitization using modern washing and disinfectant agents, dairy equipment is not sterile. The microorganisms are isolated from its surfaces that subsequently form the microflora of finished products. The most common bacteria on equipment are those of the genus *Bacillus, Lactobacillus* and the family *Enterobacteriaceae*, which are isolated in 77.2–100 % of cases from raw milk and tanks-coolers, as well as in 22.7–77.4 % of cases from other dairy equipment. This allows us to assume that after the disinfection of dairy equipment its surfaces contain only those bacteria that have the capability to produce films of high and medium density. Thus, in 100 % of cases, high-density biofilms were formed by the bacteria *Bacillus spp.* and *Enterococcus faecalis. Staphylococcus spp., Escherichia coli* and *Pseudomonas spp.* formed high-density biofilms in 74.3–86.8 % of cases. Data from the scientific literature indicate [6, 38, 39] that microbial biofilms protect bacteria during sanitization and help to survive on equipment. That is why, even under condition of using automatic CIP-plants, a constant microbiological control over effectiveness of the conducted sanitization must be put in place at enterprises. Reliable control over this process will ensure the production of dairy products that are safe in terms of microbiological indicators, as well confidence in their quality during storage.

An important factor during formation of biofilms is the process of initial attachment of bacteria to the surface. This stage affects the rate and further growth of biofilms on dairy equipment. Data in the scientific literature indicate that the adhesion of microorganisms depends on numerous factors, including the important role of the surface roughness [23–26]. Stainless steel, which is used for dairy equipment, should have surface roughness less than 0.8 μm [46] since the efficiency of washing the equipment depends on this magnitude. It was found that on the surfaces of stainless steel of brand AISI 321, with a surface roughness of 0.16±0.065 μm, there occurs the process of formation of the *Escherichia coli* biofilms of lower density compared to the surface with a surface roughness of 0.63±0.087 and 0.955±0.072 μm. This pattern is observed at a temperature of 17 °C, over the period from 6 to 24 hours, followed by the formation of biofilms with high density regardless of the surface roughness. In other words, over 24 hours of incubation, at a temperature of 17 °C, the matrix of the *Escherichia coli* biofilms fills up all cavities and protrusions at the steel surface with roughness no longer important for the adhesion. This indicates that all the equipment in dairy industry must have such a surface roughness that prevents and inhibits the process of both initial adhesion of bacteria and subsequent formation of biofilms. In addition, effective sanitization of equipment should take place as soon as possible upon completion of the technological process in order to prevent formation of the high-density biofilms. The formed dense biofilms will influence the effectiveness of equipment sanitization with disinfectant agents.

It was established that out of the tested disinfectants for sanitizing the dairy equipment, the silver-based preparation had no effect on the biofilm and planktonic forms of bacteria. Chlorine-based disinfectants (P3-ansep CIP, Eco chlor, Medicarine), as well as those based on quaternary ammonium compounds (Maxidez), showed bactericidal effect on the planktonic bacteria but did not act on the biofilm forms. Upon the action of these agents, we isolated bacteria from the biofilms in the amount of 9 to 9,900 cfu/cm^3. The most effective disinfectant for the destruction of microbial biofilms turned out to be P3-oxonia active-150 based on hydrogen peroxide and peracetic acid. The given agent exerted bactericidal effect on bacteria in the *Staphylococcus aureus, Streptococcus spp., Enterococcus faecalis* and *Pseudomonas fluorescens* biofilms. The agent reduced the number of bacteria in the biofilms formed by *Lactobacillus spp., Escherichia coli, Pseudomonas aeruginosa and Bacillus spp.* to 500 cfu per 1 cm^3 of washing. Data from the scientific literature also indicate that the agents containing hydrogen peroxide are the most effective for the destruction of microbial biofilms on equipment [1, 5, 12]. That is why we support scientists [35, 40] who argue that disinfectants showing bactericidal action on microorganisms under laboratory studies may prove ineffective under industrial production. Bacteria in biofilms are more resistant to disinfectants because they form a peptide-polymeric matrix and differ in the rate of development and consumption of nutrients compared with the planktonic forms of bacteria [16, 20]. That is why the established minimum bactericidal concentration of the agent

on planktonic test cultures of micro-organisms cannot be an indicator of the effectiveness of sanitization of dairy equipment. When designing and determining the effectiveness of disinfectants, it is necessary to select such a working concentration that acts not only on the planktonic forms but also on the bacteria, which populate the formed biofilms. In addition, in order to efficiently sanitize dairy equipment, it is necessary to determine adaptation capability of the isolated microflora to disinfectants, and, based on the results of experiments, to replace the agents every 6–12 months of their application.

Thus, the biofilms on dairy equipment are one of the sources of contamination of dairy products by microorganisms; to deal with them, it is required to take a comprehensive approach to solving this problem. It is necessary to carry out research in order to examine composition of the biofilms' matrix, the impact of various biocides and enzymes on them, and to design equipment with anti-adhesive properties.

7. Conclusions

1. It was established that microorganisms of the genera *Bacillus spp.* and *Lactobacillus spp.* are isolated from dairy equipment after sanitization and from the finished dairy products in 100–37.1 % of cases. Bacteria of the genera *Staphylococcus, Streptococcus, Enterococcus, Pseudomonas* and the *Enterobacteriaceae* family are isolated much less often.

2. We determined that bacteria isolated from the equipment form biofilms of high and medium density. This indicates that bacteria formed in the biofilms will survive during sanitization of dairy equipment on its surfaces.

3. It was established that the process of biofilm formation on stainless steel depends on the surface roughness. Escherichia coli forms biofilms with lower density on the surface of steel with a surface roughness of 0.16±0.065 μm, compared to the surface with a surface roughness of 0.63–0.072 μm over 24 hours at a temperature of 17 °C. After this period, e. coli fills up all cavities and protrusions of steel, with roughness no longer important for the process of biofilm formation.

4. It was found that the disinfectant Argenvit had proved to be inefficient for the biofilm and planktonic forms of bacteria. The disinfectants P3-ansep CIP, Eco chlor, Medicarine та Maxidez showed bactericidal effect on the planktonic bacteria, however, they did not act on the biofilm forms. The most effective disinfectant in terms of action on the bacteria in biofilms turned out to be the disinfectant P3-oxonia active-150 based on hydrogen peroxide and peracetic acid. Thus, in order to efficiently sanitize dairy equipment, it is required to employ disinfectants that affect bacteria in biofilms.

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