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FORMATION OF MICROBIAL BIOFILMS ON STAINLESS STEEL WITH DIFFERENT SURFACE ROUGHNESS

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ABSTRACT

The physical essence of the formation and influence of bacteria on the surface of technological equipment in the dairy industry is considered as an essential factor leading to contamination of dairy products and is a major hygienic problem. The ability of microorganisms on the surfaces of technological equipment to form biofilm forms and requirements for steel grade, relief, and its roughness were analysed. The effect of surface roughness on promoting or preventing adhesion and reproduction of biofilm forms of bacteria, which reduce the efficiency of sanitary processing of dairy equipment and thereby increase the microbial contamination of dairy products with shortened shelf life, is substantiated. Research about the process of bacterial adhesion to the surface of metals with different roughness depending on the size and shape is presented. It is found that on the surface of stainless steel with roughness 2.687 ±0.014 micron film formation process in *Escherichia coli* and *Staphylococcus aureus* are similar from 3 to 24 hours and does not depend on the size of the bacteria, and accordingly allows us to argue that rod-shaped and coccid bacteria attach freely in the hollows of the roughness are the beginning of the process of the first stage of biofilm formation. It is found that on the surface of stainless steel with roughness 0.95 ±0.092 micron film formation process in S. aureus is more intense than in *E. coli*. Thus, within 3 hours of incubation, the density of biofilms formed *S. aureus* was 1.2 times bigger than biofilms *E. coli*, by the next 15 hours of incubation formed biofilms *S. aureus* were, on average, 1.3 times denser. It is established that *S. aureus* due to its spherical shape is able to fit in the hollows of the roughness 0.95 ±0.092 μm and faster to adhere to the surface at the same time. *E. coli*, due to its rod-like shape, with such surface roughness, can adhere to the cavities only over its entire length. It is proved that by surface roughness 0.63 ±0.087 μm film intensity *S. aureus* was, on average, 1.4 times faster than *E. coli*, for roughness 0.16 ±0.018 micron film formation process took place equally for *S. aureus* and *E. coli*, but biofilms were lower in density than those formed on roughness 0.63 ±0.087 micron. Studies suggest that the use of equipment in the dairy industry with a roughness of less than 0.5 microns will reduce the attachment of microorganisms to the surface and reduce the contamination of dairy products.

Keywords: microbial adhesion; bacterial biofilm; adhesion; sanitary treatment; ejector; mathematical model

INTRODUCTION

With the widespread introduction into food production of modern automated, complex mechanized lines, when the processing speed of non-Newtonian food masses has increased significantly and new structural materials are widely introduced, there is always a need to study the adhesion strength and its effect on the passage of processes. Compliance with technological processes is directed to the separation zone taking into account both the type and condition of the surface of technological equipment and the structural and mechanical properties of the food masses. The main factor that reduces the shelf life and safety of food are microorganisms **(Kukhtyn et al., 2017; Shaheen et al., 2010; Verran et al., 2010)**. Is believed bacterial adhesion to the surface to be a complex physicochemical process that depends on surface properties such as topography, roughness, hydrophobicity, chemical composition, and surface energy; from the initial number of microorganisms, their size, temperature and pH of the environment, etc. **(Whitehead and Verran, 2007; Shaheen et al., 2010; Verran et al., 2010)**. However, among the many factors that influence the adhesion process are researchers **(Whitehead and Verran, 2007)** consider that surface properties play a major role. The presence of bacteria on the surfaces of technological equipment in the dairy industry is considered as an important factor that can lead to contamination of dairy products and is considered as an important hygienic

problem **(Kukhtyn et al., 2017)**. At the same time, microorganisms survive on the surfaces of process equipment due to their ability to form biofilm forms. In this regard, certain hygienic requirements are placed on the surface of the processing equipment used in the dairy industry, especially as regards steel grade, relief and roughness. Based on the interactions between the surface and the environment, the authors **(Stadnyk et al., 2019)** reveal the features of adhesion are given. The adhesion is increase or decrease in accordance with laws of Ammonon-Coulomb friction, the Euler relation, the terms as friction angle and friction cone, the friction coefficient. This is due to the fact that surface roughness can promote or hinder the adhesion and reproduction of biofilm forms of bacteria **(Vlková et al., 2008)**. Also, the development of biofilm helps to reduce the efficiency of sanitation of dairy equipment and thereby increases the microbial contamination of dairy products and shortens their shelf life. Consequently, studies of the formation of biofilms by bacteria of different shapes and sizes, depending on the roughness of the stainless-steel surface, are relevant in the dairy industry. Research in this direction will allow to scientifically substantiate the surface roughness parameters of dairy equipment, which would minimize the microbial adhesion process. Such studies may provide a basis for developing a method for evaluating stainless steel for the presence of adhesive properties.

Analysis of studies of adhesive bonds of biofilms

As mentioned above, microbial adhesion is a complex biological process that is influenced by the physiological features of the microorganisms, the environmental properties and the physicochemical properties of the surface **(Yi et al., 2004; Moons and Michiels, 2009; Whitehead and Verran, 2007; Götz, 2002)**. The results of the studies indicate **(Monds and O'Toole, 2009)**, that the nutrients for the microorganisms of the biofilm are contained in the solution, so the composition of the nutrient medium, its ionic energy, pH, temperature also affect the adhesion intensity of the microorganisms to the base. The formed biofilm consists of an inhomogeneous structure, resulting in a gas concentration gradient, in particular, a decrease in the amount of oxygen from the periphery to the depth, pH gradients and nutrients. These gradients of biofilm functioning provide physiological variability between individual biofilm cells - they grow more slowly at the depth of the cell than at the periphery of the film, leading to phenotypic stability and a dramatic change in environmental factors. The formation of the biofilm and the strength of its adhesion to the surface are influenced by the hydrophobic properties of the surface of the microorganisms, the presence of their villi and flagella. Most bacteria have a negatively charged surface that contains hydrophobic external components that cause hydrophobic interaction with the substrate. The presence on the surface of biofilms of polysaccharides or proteins helps bacteria to adhere to the surface, thereby providing a competitive advantage in the formation of biofilms for specific cells of the microbial association **(Yi et al., 2004)**. In addition, the structure of the biofilm is influenced by the presence in its composition of microorganisms, which in the course of their growth and development release the gaseous substances of metabolism. This leads to a decrease in the density of the biofilm and the uneven flow of nutrient substrates **(Götz, 2002)**. Among the many factors that influence the adhesion process, researchers highlight the role of surface properties, which is defined as the most significant **(Zogaj et al., 2003; Kania et al., 2007)**. As a result, three theories of microbial adhesion to the surface were proposed: thermodynamic, DLVO theory and XDLVO **(Kolari, 2003; Langsrud et al., 2016)**. The thermodynamic theory is based on the fact that when the microorganisms are attached to the surface there is a change in the total free energy of Gibbs, Van der Waals forces. Theory DLVO is based on the fact that the colloidal particles of the lyophobic dispersion system can move closer to each other without interruption until their liquid diffuse shells come into contact. Theory XDLVO is based on thermodynamic and DLVO theory **(Langsrud et al., 2016)**. However, researchers believe that all three theoretical models designed to reveal the essence of microbial adhesion to the surface have been designed for the perfect colloidal system. Under production conditions, microbial adhesion is a much more complicated process and attachment of microorganisms can occur in different ways.

Analysis of recent research.

In the dairy industry stainless steel corrosion-resistant steels of the following brands are most often used for the equipment AISI-304, AISI-316, AISI-321 **(Jullien et al., 2003; Dantas et al., 2016)**. These steel delivery conditions can have different surface roughness from 0.2 – 3.2 mm. Studies to investigate the effect of terrain and surface roughness on microbial adhesion are not straightforward. According to research **(Dantas et al., 2016),** there is a correlation between surface roughness and bacterial adhesion, the attachment of microorganisms to the surface increases with increasing roughness. From the analysis of works **(Kukhtyn et al., 2016)** found that the formation of biofilm was much slower on the surface with a roughness of up to 0.4 μm, compared with a roughness greater than 0.8 μm. Using electron microscopy, the researchers **(Dantas et al., 2016; Kukhtyn et al., 2016)** found that the primary adhesion of microbial cells occurs along the hollows of the surface roughness, since under these conditions the area of contact of the microbial cell with the surface increases. However, other studies indicate that there is virtually no correlation between stainless steel surface roughness and microbial adhesion. There are also various data on the effect of surface wettability on microbial adhesion. It was found that the number of adherent bacteria decreased with increasing surface hydrophobicity, and microorganisms that attached to hydrophobic surfaces were more easily removed by increasing the flow force during fluid circulation **(Dou et al., 2015)**. However, other researchers **(Kolari, 2003)**, indicate that there is no correlation between surface wettability and microbial adhesion. It is investigated that formed biofilms on surfaces with high roughness reduce the efficiency of heat transfer in condenser heat exchangers by about 15%. Thus, the hygienic quality and cleanliness of the processing equipment in the dairy industry after sanitation can be closely linked to relief and roughness. Therefore, research into the process of film formation on stainless steel with different roughness over

time and using different shapes and sizes of bacteria is promising. These studies will allow us to understand more deeply the process of the formation of microflora on the equipment and, accordingly, the contamination of food products. Also, finding out the effect of surface roughness on microbial adhesion will help to improve and modify the surfaces that interfere with adhesion.

The purpose of the work was to determine the effect of different surface roughness of stainless steel on the process of microbial adhesion and film formation, depending on the physiological and morphological features of the microorganisms that contaminate the equipment.

To achieve this goal, the following tasks were set:

- to experimentally investigate the process of film formation by rod-shaped and coccoidal bacteria in stainless steel with different surface roughness at a temperature of 25 °C;
- simulate the process of film formation on stainless steel with different surface roughness.

Scientific hypothesis

The successful formation of microbe biofilms is not possible without microorganisms' surface adhesion. The scientists have separated five main stages of biofilms formation and development on any surface. Bacteria surface adhesion depends on the initial number of microorganisms, their shape, temperature, environment pH, size and etc. The primary adhesion of microbial cells is taking place along the cavities of the surface roughness as under such conditions the contact area of the microbial cell and the surface is getting bigger. Nevertheless, any process of each biofilm formation has specific features that are regulated by both genetic, biochemical properties of the bacterial cell and environmental factors as well. We may claim that a biofilm is a microbial combination formed by the cells attached to the surface and to one another and are in the matrix of synthesized extracellular substances. The most serious biofilm hazard on the milk processing equipment is that the biofilm extracellular matrix protects the bacteria from the disinfection agents' actions. The survived microorganisms occupy the new surfaces and milk products. Thus, while studying the microbial adhesion and biofilms formation under laboratory conditions one can not always take into consideration the impact of production factors and physiological specific features of microbial cells. Conducting the research on studying the process of film formation on the stainless steel of different surface roughness during a certain period of time and using different bacteria forms and size will allow us to understand deeper the process of microbial flora formation on the equipment and also food contamination. To find out the impact of surface roughness on microbial adhesion means to facilitate the improvement and modification of the surfaces which resist the adhesion.

MATERIAL AND METHODOLOGY

In the dairy industry, stainless steel corrosion-resistant steels that can have a different surface roughness from 0.2 – 3.2 microns are most commonly used for equipment. Surface quality is judged by geometrical parameters and condition of the surface layer, which is determined by the physical, mechanical properties and structure. The characteristics of the geometric properties of the surface include macro- and microgeometric parameters. Stainless steel corrosion-resistant nickel-chromium austenitic steel plates were used for the study AISI 321 (standard of the American Institute of Steel and Alloys) size 30 × 30 mm and 4 mm thick, with surface roughness R_a = 2.687 ±0.014 microns, R_a = 0.95 ±0.092 microns, R_a = 0.63 ±0.087 microns, R_a = 0.30 ±0.065 microns, R_a = 0.25 ±0.035 microns, R_a= 0.24±0.026 microns and R_a = 0.16 ±0.018 microns. The process of forming the biofilm was carried out by strains *Escherichia coli ATCC 25299* and *Enterococcus faecalis ATCC19433* on brand stainless steel surface AISI 321 with different roughness over time.

Research methods

Experimental studies were carried out using modern standard and conventional methods: technical (determination of surface roughness of stainless steel), microscopic (light and electron microscopy of the process of film formation and degradation), spectrophotometric (optical density), biofilm, microbiology, microbiological parameters of dairy equipment and dairy products. Investigation of the process of film formation by microorganisms on the surfaces of metals with different roughness was carried out in a sterile Petri dish. The sterile stainless steel plates with appropriate surface roughness were placed in the cup and sterile meat-peptone broth was introduced into the cupand the appropriate test culture (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* or any other dedicated dairy equipment) in concentration to 1 cm^2 plate area accounted for from 1 to 50 thousand cells. The incubation was carried out at temperatures from +17 °C, +25 °C to +37 °C for 3, 6, 9, 12, 18 and 24 hours, depending on the purpose of the experiment. After incubation, the plates were removed from the Petri dishes, washed three times with planktonic (unattached) microorganisms with phosphate buffer. Recorded the formed microbial biofilms on the plates with 96% ethyl alcohol. After fixing, the biofilms were stained, for this purpose the plates were immersed in 0.1% crystalline violet aqueous solution. After staining, the plates were washed three times with phosphate buffer and dried. After drying, each plate was individually poured into 7.0 cm^3 of 96% ethyl alcohol and left for 20 min. After 20 min exposure, 5 cm^3 of the wash solution was removed from biofilms and the optical density t 570 nm was determined spectrophotometrically on the KFK-3 photometer (Ukraine) (Figure 1).

Electron microscopic studies of the process of forming biofilms on stainless steel were performed on an electron raster microscope (REM 106 I, Ukraine) with magnification from 2000 to 5000 times (Figure 2). In addition, the adhesion of microorganisms in the cavities or roughness projections was visually assessed using a microinterferometer MII-4Y4.2 with magnification in 1500 times (Figure 3).

Figure 1 Photometer (KFK-3).

Figure 2 Electron raster microscope (REM 106 I).

Figure 3 Microinterferometer MII-4Y4.2.

To determine the parameters of the surface layer, the following technique was used:

- scanning the surface with a 3D scanner David Laser Scanner SLS-1 using the software David Laser Scanner Professional Edition;
- processing the scanned surface using the program PowerShape;
- determining the required geometric characteristics of the scanned part of the surface.

A large number of dots were merged into the surface when scanned. To determine the roughness and waviness of the surface layer, we cut out a scanned area of 10 x 10 mm in software PowerShape, which is shown in Figure 4. We draw two two perpendicular planes (Figure 5) that will cut the plot. A fragment of a longitudinal profile with points (vertices and cavities) is shown in Figure 6. Similar operations were performed with a transverse profile, a fragment of which with vertices and cavities is shown in Figure 7. Determining the lengths of perpendiculars from cavities or vertices to the center line using the formula:

$$R_a = \frac{\sum_i^n |Y_i|}{n},$$

Where:

n – the number of points (in our case 107); $\sum_i^n |Y_i|$ – the sum of the lengths of all perpendiculars (in our case 2038 μm).

So,

$$R_{a_1} = \frac{203.8}{107} = 1.905 \text{ (microns).}$$

$$R_{a_2} = \frac{104.7}{64} = 1.6 \text{ (microns).}$$

Accordingly, the arithmetic mean, is:

$$R_a = \frac{R_{a_1} + R_{a_2}}{2} = \frac{1.905 + 1.635}{2} = 1.77 \text{ (microns).}$$

Using the standard table of accepted values of Ra, we can determine that the test area has a roughness within Ra 1.6 and Ra 2.0.

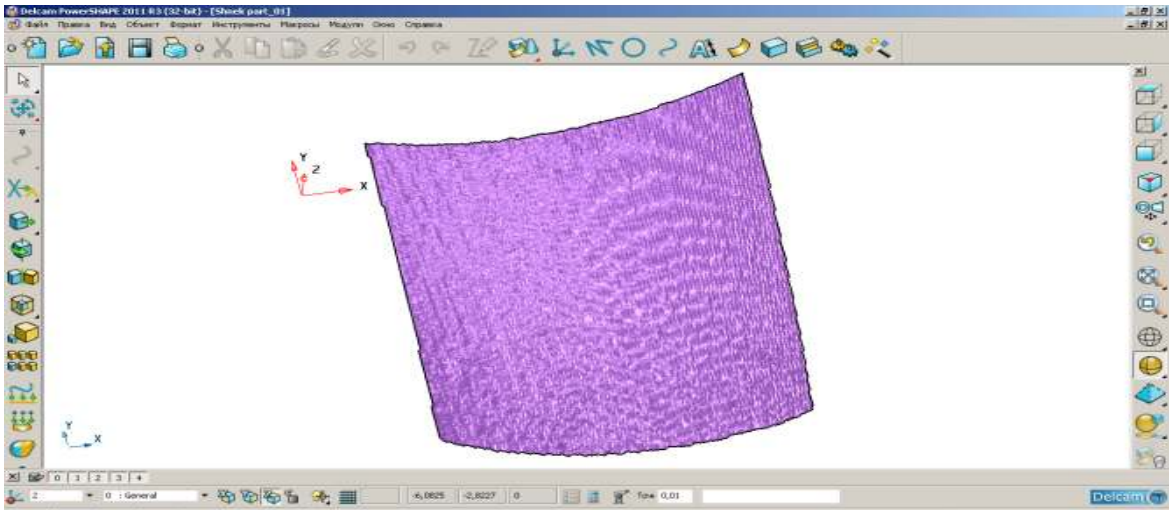

Figure 4 Fragment plot measuring 10 x 10 mm.

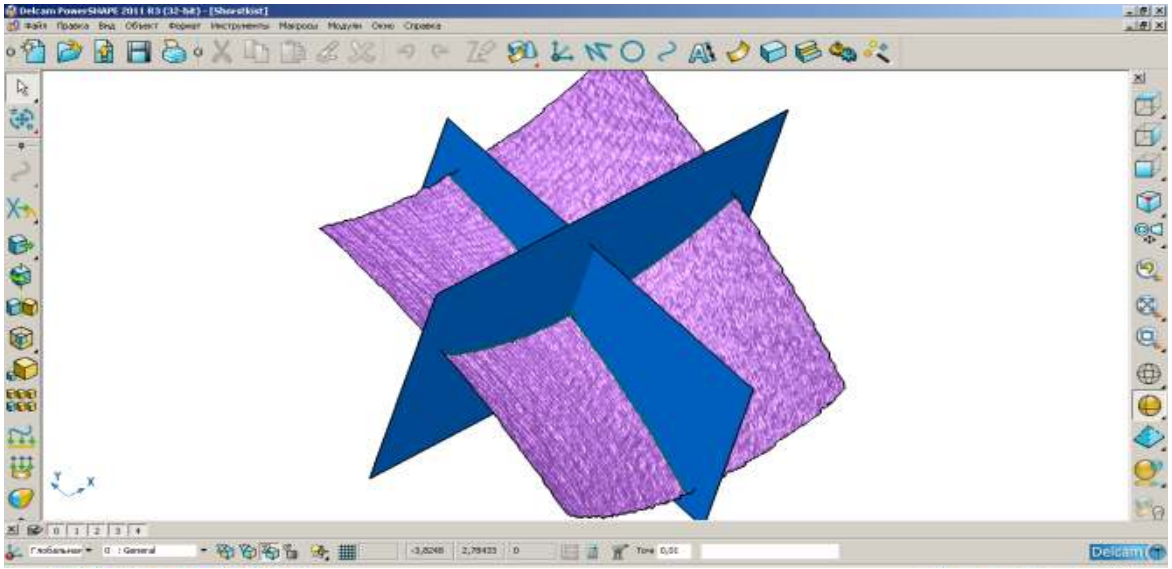

Figure 5 The section of the plot perpendicular to the planes.

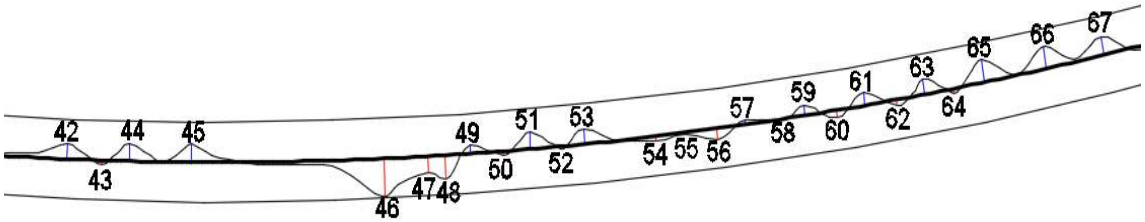

Figure 6 Fragment of a longitudinal profile with points of vertices and cavities.

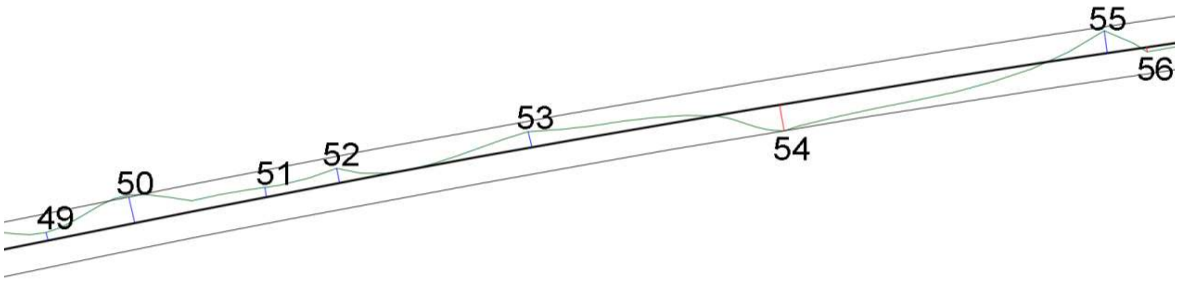

Figure 7 Fragment of transverse profile with vertices and cavities.

Statistic analysis

To obtain mathematical dependencies when comparing the results of the study of optical density by the factor of time and roughness for different temperatures and the initial bacterial count, regression equations of optical density and bacterial development were proposed.

RESULTS AND DISCUSSION

Obviously, in some cases the search for geometric connections to provide film formation by microorganisms on metal surfaces can be significantly simplified if the initial surface synthesis with the number of microorganisms are solved. However, the instability of the adhesion values and external conditions of operation of the surfaces, as well as variations in the film´s structured parameters, lead to the need to search for non-standard approaches to establish their bonds. The situation is complicated by significant differences in the initial surface areas, features of the microbial film, etc. According to research **(Dantas et al., 2016)**, there is a correlation between surface roughness and bacterial adhesion, with the attachment of microorganisms to the surface increases with increasing roughness. An analysis of the work **(Kukhtyn et al., 2016)** found that the formation of biofilm was much slower on the surface with a roughness of up to 0.4 μm, compared with a roughness greater than 0.8 μm. The theory of adhesion interactions of microorganisms with the the rough surface was based on research. The study of the phenomena of adhesion of microorganisms with a rough surface is conventionally divided into two stages: the application of the number of microorganisms on the surface, considering the time and temperature of the formation of the film, and when the relative formation of the film occure. Both cases occur in viscous materials transport systems whose properties are limited by support deformations, compression, and velocities. Studies have shown that the surface of stainless steel with roughness 2.687 ±0.014 microns, the process of film formation in *E. coli* and *S. aureus* passed over with the same from 3 to 24 hours of incubation and did not depend on the size and shape of the bacteria, but on the surface with roughness 0.95 ±0.092 microns, the process of film formation in *S. aureus* was more intense than in *E. coli*. Therefore, during the first 3 hours of incubation, the optical density of the formed biofilms *S. aureus* was 1.2 times higher compared to the density of biofilms formed *E. coli.* The next 15 hours of incubation formed biofilms *S. aureus* were, on average, 1.3 times denser, and no significant difference was found between 18 and 24 hours of incubation. This gives reason to believe that *S. aureus* due to its spherical shape, is able to be positioned in the roughness depressions of 0.95 ±0.092 microns and faster to adhere to the surface. Simultaneously *E. coli,* due to the rod-like shape, at such a surface roughness, adheres to the hollows only longitudinally and form biofilms. However, was found that the intensity of the film-forming process in bacteria on the surface with a roughness of 0.95 ±0.092 mm depends on the shape and size of the bacteria only up to 18 hours of incubation. The obtained data have corresponded **(Verran et al., 2010)** with the research which prove that *L. monocytogenes* adhesion on the stainless steel of the roughness less than 0.8 mkm was slower than on the surface of roughness 30 mkm. Although, we have found that apart from surface roughness the biofilm formation is also influenced by the form and size of microorganism cells. This can be explained by the fact that coccus forms of microorganisms at 0.95 and 0.63 mkm surface roughness was forming biofilms more intensely than the string formed bacteria. We agree with scientists idea **(Hočevar et al., 2014)**, that this phenomenon takes place due to the increased area of bacteria-surface contact. When used in experiments stainless steel with a roughness of less than 0.8 microns, as recommended in the food industry according to hygienic standards **(Council directive 93/43/EEC; Whitehead and Verran, 2007)** it is found that at a surface roughness of 0.63 ±0.087 microns, the intensity of film formation *S. aureus* was on average in 1.4 times faster than for *E. coli*, up to 18 hours of incubation. At the same time, at a roughness of 0.16 ±0.018 μm, the film-forming process proceeded equally for *S. aureus* and *E. coli,* but the formed biofilms were lower in density than those formed at a roughness of 0.63 ±0.087 μm. Figure 8 presents a schematic model of the formed biofilm in the depressions and on the projections of the roughness.

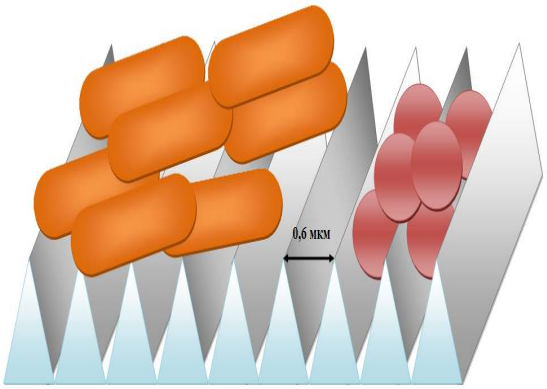

Figure 8 Schematic model of the formed biofilm in the depressions and at the projections of the roughness.

The technique of investigation of the optical density of the biofilm is developed, based on the use of the plan of experiment realization in the software complex Microsoft Excel, which allows to obtain a mathematical model of the film formation process in the form of regression equations. The function of the response of the obtained equation is the biofilm density from roughness and time. The optimization parameter used is the change in the number of bacteria during the process. What we don't know is the relationship between the input parameters and the number of bacteria, but we have a model that can be represented as:

$$Y = f(Ra, \tau),$$

Where:

Y – change in biofilm density, thousands; τ – time, hour; Ra – surface roughness of the equipment; Y_1 – *E. Coli*; Y_2 – *E. faecalis.*

Table 1a *E. coli,* T = 37 °C, Ra = 0.955 microns.

Initial bacterial count	0 Control	3	6	9	12	18	24
Up to 1 thousand	0.091	0.105	0.139	0.235	0.634	1.287	1.602
2 – 10 thousands		0.121	0.247	0.342	1.223	1.671	1.769
20 – 50 thousands		0.181	0.374	0.463	1.304	1.716	1.804

Table 1b *E. faecalis,* T = 37 °C, Ra = 0.955 microns.

Initial bacterial count	0 Control	3	6	9	12	18	24
Up to 1 thousand	0.091	0.112	0.156	0.307	0.672	1.295	1.670
2 – 10 thousands		0.136	0.263	0.395	1.246	1.683	1.774
20 – 50 thousands		0.196	0.392	0.467	1.415	1.743	1.851

Table 2 Optical density (number) by *E. coli* bacterial factors**.**

	37* <1	37* 2 – 10	37* >50	25* 2 – 10	17* 2 – 10
Y	-0.049	-0.181	-0.169	-0.027	0.08
y1	0.171	0.281	0.217	0.151	-0.028
y2	0.014	0.063	0.08	0.018	3.239*10^-3
y3	9.522*10^-3	4.579*10^-3	8.186*10^-3	0.01	0.01
y4	-0.055	-0.084	-0.058	-0.054	5.657*10^-3
y5	1.671*10^-3	3.785*10^-4	-2.485*10^-4	1.237*10^-3	7.219*10^-4

According to the results of the experiment (Table 1a and 1b), we obtain the regression quadratic equations of the law of change of density from surface roughness and time, which are shown graphically in Figure 9 for *E. Coli* (a) and *E. faecalis* (b) for 18 hours at Ra = 0.63 ±0.87 microns. Thus, from the obtained dependencies in Figure 9 it can be noted that the process of formation of biofilms *E. Coli* and *E. faecalis* on stainless steel in addition to surface roughness, the initial bacterial count is significantly affected. On steel plates with a roughness of 0.95 ±0.018 microns, the film formation process of *E. coli* is slower compared to *E. faecalis* (Figure 9). According to the data obtained and the dependence construction, it was found that the film density depends on the size of the bacteria and on the initial number of microbial cells on the steel surface That is why we consider that the surface maximum roughness for the most efficient sanitary treatment of milk processing equipment should be 0.5 mkm. Such a treatment is the best decision to prevent film formation both by coccus and string formed bacteria.

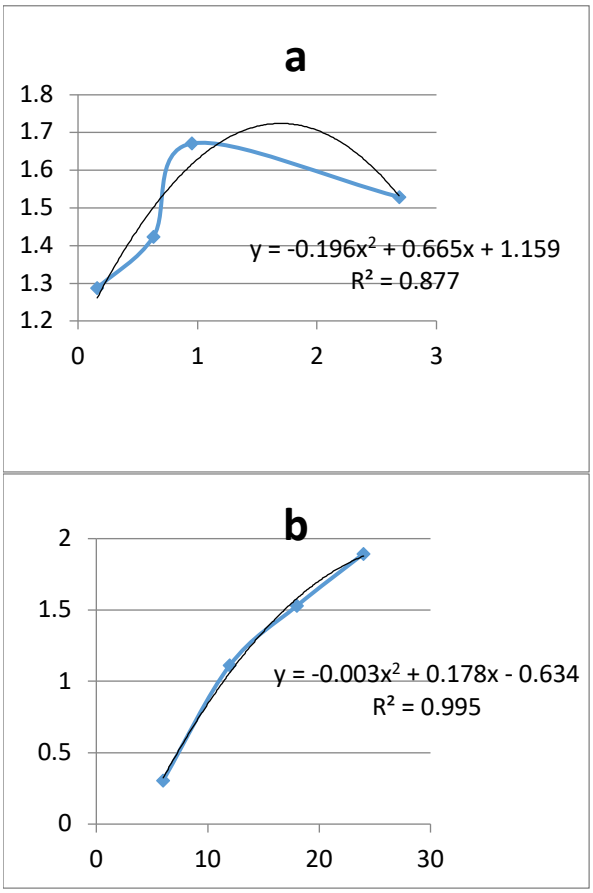

Figure 9 Change in optical density *E. coli* (a) and *E. faecalis* (b) for time 18 hours.

For bacteria E. coli

Optical density regression equation (quantity) **Y** by time factors and roughness **r** for different temperature ranges T °C and the initial bacterial count according to Table 2 will be:

$$Y_{k,i} := y_0 + y_1 \cdot r_k + y_2 \cdot t_i + y_3 \cdot r_k \cdot t_i + y_4 \cdot (r_k)^2 + y_5 \cdot (t_i)^2$$

The regression equation of the rate of change of quantity **Z** by time factors tand roughness **r** for different temperature ranges T °C and the initial bacterial count according to Table 3:

$$Z_{k,i} := z_0 + z_1 \cdot r_k + z_2 \cdot t_i + z_3 \cdot r_k \cdot t_i + z_4 \cdot (r_k)^2 + z_5 \cdot (t_i)^2$$

By comparing population intensities *E. coli* for T = 37*I(n<1), I(2<n<10), I(20<n<50) got that $Y(2<n<10)_{k,I} = s1_{k,i}* Y(n<1)_{k,I}$, $Y(20<n<50)_{k,I} = s2_{k,i}* Y(2<n<10)_{k,I}$,

Where:

$$s1 = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1.056 & 1.139 & 1.152 & 1.252 \\ 1.167 & 2.075 & 1.777 & 1.346 \\ 1.201 & 1.435 & 1.455 & 1.554 \\ 1.698 & 1.776 & 1.929 & 1.252 \\ 1.625 & 1.472 & 1.298 & 1.087 \\ 1.278 & 1.112 & 1.104 & 1.09 \end{pmatrix}$$

$$s2 = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1.053 & 1.165 & 1.496 & 1.396 \\ 1.889 & 1.198 & 1.514 & 1.56 \\ 1.6 & 1.178 & 1.354 & 1.226 \\ 1.138 & 1.085 & 1.066 & 1.363 \\ 1.051 & 1.125 & 1.027 & 1.199 \\ 1.05 & 1.089 & 1.02 & 1.133 \end{pmatrix}$$

k = 0 – 6; i = 0 – 3.
Thus, the initial number of bacteria is taken into account as a factor.

For bacteria E. faecalis

Optical density regression equation (quantity) **Y** by time factors**t**and roughness **r** for different temperature ranges **T** °C and the initial bacterial count according to the Table 4:

$$Y_{k,i} := y_0 + y_1 \cdot r_k + y_2 \cdot t_i + y_3 \cdot r_k \cdot t_i + y_4 \cdot (r_k)^2 + y_5 \cdot (t_i)^2$$

The regression equation of the rate of change of quantity **Z** by time factors**t**and roughness **r** for different temperature ranges T °C and the initial bacterial count according to the Table 5.

$$Z_{k,i} := z_0 + z_1 \cdot r_k + z_2 \cdot t_i + z_3 \cdot r_k \cdot t_i + z_4 \cdot (r_k)^2 + z_5 \cdot (t_i)^2$$

By comparing population intensities *E. faecalis* for T = 37*I(n<1), I(2<n<10), I(20<n<50) got that $Y(2<n<10)_{k,I} = s1_{k,i}* Y(n<1)_{k,I}$, $Y(20<n<50)_{k,I} = s2_{k,i}* Y(2<n<10)_{k,I}$,

Where:

$$s1 = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1.056 & 1.111 & 1.214 & 1.311 \\ 1.229 & 1.759 & 1.686 & 1.57 \\ 1.186 & 1.279 & 1.202 & 1.405 \\ 1.899 & 1.794 & 1.854 & 1.338 \\ 1.585 & 1.486 & 1.3 & 1.077 \\ 1.293 & 1.144 & 1.062 & 1.09 \end{pmatrix}$$

$$s2 = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1.042 & 1.133 & 1.441 & 1.729 \\ 2.007 & 1.459 & 1.49 & 1.404 \\ 2.039 & 1.259 & 1.266 & 1.361 \\ 1.118 & 1.084 & 1.136 & 1.307 \\ 1.104 & 1.129 & 1.036 & 1.203 \\ 1.051 & 1.061 & 1.043 & 1.128 \end{pmatrix}$$

k = 0,..,6; i = 0,..,3.

According to the regression equation, we construct response surfaces (Figure 10, 11 and 12).

Table 3 Changes in the number of *E. coli* bacterial factors.

	37* <1	**37* 2 – 10**	**37* >50**	**25* 2 – 10**	**17* 2 – 10**
Z0	-0.031	-0.056	-0.051	-0.033	-0.014
Z1	0.013	0.039	0.027	0.023	-3.509*10^-5
Z2	0.013	0.021	0.021	0.012	6.538*10^-3
Z3	-5.267*10^-4	-8.55*10^-4	-8.591*10^-6	6.023*10^-5	-1.62*10^-4
Z4	-3.2*10^-4	-7.675*10^-3	-3.668*10^-3	-5.458*10^-3	3.285*10^-3
Z5	-3.325*10^-4	-6.006*10^-4	-6.451*10^-4	-2.947*10^-4	-1.947*10^-4

Table 4 Optical density (number) by *E. faecalis* bacterial factors.

	37* <1	**37* 2 – 10**	**37* >50**	**25* 2 – 10**	**17* 2 – 10**
y0	-0.058	-0.177	-0.161	-0.065	0.074
y1	0.189	0.245	0.161	0.143	-0.028
y2	0.016	0.069	0.092	0.038	5.594*10^-3
y3	0.011	5.343*10^-3	8.453*10^-3	6.702*10^-3	0.012
y4	-0.061	-0.068	-0.034	-0.047	4.798*10^-3
y5	1.56*10^-3	1.715*10^-4	-6.781*10^-4	9.417*10^-4	7.161*10^-4

Table 5 Changes in the number of *E. faecalis* bacterial factors.

	37* <1	**37* 2 – 10**	**37* >50**	**25* 2 – 10**	**17* 2 – 10**
z0	-0.032	-0.027	-0.053	-0.032	-0.027
z1	0.028	0.021	0.05	0.033	0.021
z2	0.013	8.004*10^-3	0.02	0.013	8.004*10^-3
z3	-9.673*10^-4	-4.898*10^-4	-1.185*10^-3	-1.942*10^-4	-4.898*10^-4
z4	-3.618*10^-3	-2.157*10^-3	-6.644*10^-3	-8.725*10^-3	-2.157*10^-3
z5	-3.21*10^-4	-2.354*10^-4	-5.768*10^-4	-3.279*10^-4	-2.354*10^-4

Figure 10 (Y) shows that the number of bacteria *E. coli* highest value gets at roughness surface 2.68 μm after 20 hours of incubation. The speed of development (Z) *E. coli* **i**ncreases most intensively with roughness values between 0.95 μm and 2.68 μm and time, starting after ten hours of incubation. Analysis of Figure 11 shows that similar trends in bacterial counts and growth rates, as well as in temperature +17 ±1 °C. However, the number of bacteria is increasing (Y) and fills roughness (Z) from 2.68 microns to 0.63 microns at 18 hour of incubation, and at 20 hour is practically up to 0.30 microns. From the temperature data +37 ±1 °C the number of bacteria is growing rapidly (Y) and the surface roughness is filled (Z) 2.68 – 0.63 microns within 12 hours of incubation. On the basis of mathematical modeling, it was found that the adhesion and intensive process of biofilm formation *E. coli* passes in the hollows of great roughness 2.68 – 0.95 micronsand it gradually fills the hollows with less roughness 0.63 – 0.16 microns. Figure 12 shows that there is a similar pattern in the process of film formation for *E. faecalis*, as in *E. coli* at this temperature. However, the rate of growth *E. faecalis* is more intense as the roughness depressions fill from 2.68 microns to 0.63 microns as opposed to *E. coli* to 0.95 microns. The process of film formation in *E. faecalis* for temperatures +37 ±1 °C, on the surface with different roughness, was more intense compared to *E. coli*, that is, the rate of filling all the roughness depressions took 12 hours. The data obtained indicate that the biofilm density depends on the initial number of microbial cells on the steel surface. It has been shown that the more contaminated the surface with microorganisms, the faster the process of film formation and formation of dense biofilms.

CONCLUSION

It is established that in addition to surface roughness, the shape and size of cells of microorganisms influence the process of biofilm formation. This is due to the fact that at roughness of 0.95 and 0.63 microns, cocoon forms of microorganisms more intensively formed biofilms than rod-shaped ones, since this phenomenon is associated with an increase in the area of contact of bacteria with the surface. The process of forming a biofilm *E. coli* on stainless steel, depended on the surface roughness and the initial number of microbial cells on the surface. The density of biofilms formed at the initial number of cells *E. coli* up to 1 thousand per cm^2 the area was on average 1.8 – 2.2 times ($p \leq 0.05$) lower, compared to the biofilm formed on the surfaces with the initial number of microbial cells 2 – 10 thousand and 20 – 50 thousand per cm^2 area of steel. This indicates that in order to prevent the formation of high density biofilms it is necessary to carry out careful sanitary treatment of dairy equipment. It was found that at a favorable temperature *E. faecalis* for 9 – 12 hours. capable of forming medium- and high-density biofilms on stainless steel surface with a roughness of 0.955 ±0.092 μm. However, the density of biofilms at the initial cell count *E. faecalis* up to 1 thousand per cm^2 the area was an average of 1.5 – 2.1 times ($p \leq 0.05$) ower, compared to the biofilm formed in the variants with the initial cell number of 2 – 10 thousand and 20 – 50 thousand per cm^2 area of steel.

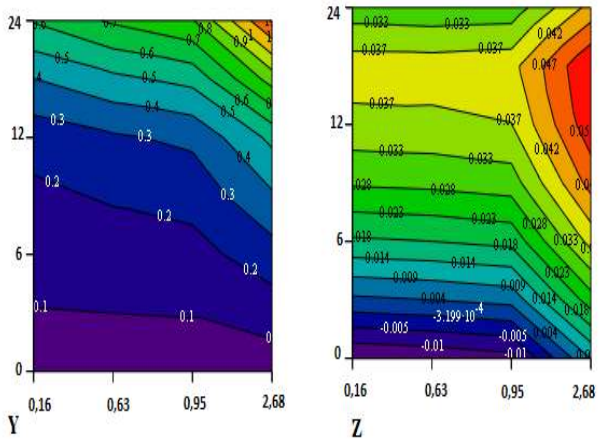

Figure 10 Charts of optical density changes (Y) and the intensity of development (Z) *E. coli* from surface roughness and incubation time in the process of biofilm formation at temperature +17 ±1 °C with the initial number of microbial cells 2 – 10 thous and sm^2 area.

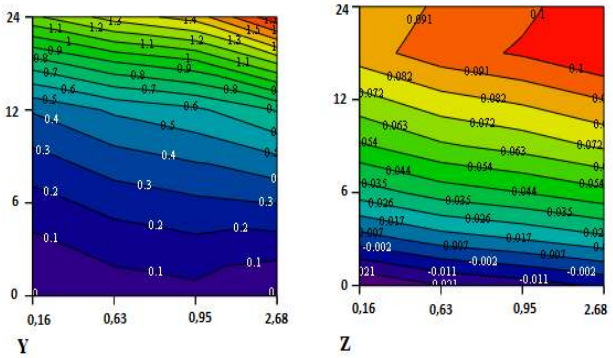

Figure 11 Charts of optical density changes (Y) and the intensity of development (Z) *E. coli* from surface roughness and incubation time in the process of biofilm formation at temperature +25 ± 1°C with the initial number of microbial cells 2 – 10 thous and sm^2 area.

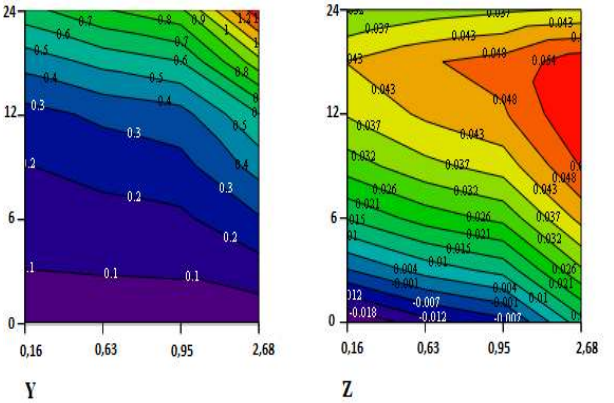

Figure 12 Diagram of change of quantity (Y) and the intensity of development (Z) *E. faecalis* from surface roughness and incubation time in the process of biofilm formation at temperature +17 ±1 °C with the initial number of microbial cells up to 2 – 10 thous and sm^2 area.

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