Наведено дослідження процесу адгезії бактерій до поверхні з різною шорсткістю залежно від розмірів і форми. Встановлено, що на поверхні нержавіючої сталі з шорсткістю 2,687±0,014 мкм, процес плівкоутворення у E. coli та S. aureus проходив однаково упродовж з 3 до 24 години та не залежав від розмірів бактерій. Це дозволяє стверджувати, що паличковидні і кокові бактерії вільно прикріпляються у западинах шорсткості та розпочинається початковий процес першої стадії формування біоплівки. Під час санітарної обробки у западинах шорсткості можуть залишатися, як кокові, так паличковидні бактерії. На поверхні сталі з шорсткістю 0,95±0,092 мкм процес плівкоутворення у S. aureus проходив інтенсивніше, ніж у E. coli. Упродовж 3 год інкубації щільність сформованих біоплівок S. aureus була в 1,2 раза більша, порівняно з біоплівками E. coli. У наступні 15 годин інкубації сформовані біоплівки S. aureus були, в середньому в 1,3 раза щільніші. Це дає підставу вважати, що S. aureus завдяки кулястій формі здатний розміщуватися у западинах шорсткості 0,95±0,092 мкм і швидше адгезуватися до поверхні. Водночас E. coli, завдяки паличковидній формі, за такої шорсткості поверхні може адгезуватися у западини тільки повздовж. Доведено, що за шорсткості поверхні 0,63±0,087 мкм інтенсивність плівкоутворення S.aureus була, в середньому в 1,4 раза швидша, ніж у E. coli. Водночас, за шорсткості 0,16±0,018 мкм процес плівкоутворення проходив однаково у S. aureus і E. coli, але біоплівки були нижчої щільності, порівняно з такими, які формувалися за шорсткості 0,63±0,087 мкм.

Отже, використання обладнання у молочній промисловості з шорсткістю менше 0,5 мкм дозволить зменшення прикріплення мікроорганізмів до поверхні і зниження контамінації молочних продуктів

Ключові слова: мікробна адгезія, формування біоплівок, шорсткість поверхні нержавіючої сталі, процес плівкоутворення

UDC 664.579.674
DOI: 10.15587/1729-4061.2019.160142

MODELING THE PROCESS OF MICROBIAL BIOFILM FORMATION ON STAINLESS STEEL WITH A DIFFERENT SURFACE ROUGHNESS

M. Kukhtyn
Doctor of Veterinary Sciences, Professor *
E-mail: kuchtynnic@gmail.com
K. Kravcheniuk
Postgraduate student*
E-mail: kravchenukx30@gmail.com
L. Beyko
PhD, Associate Professor *
E-mail: beykol@ukr.net
Y. Horiuk
PhD, Senior Lecturer
Department of Infectious and Parasitic Diseases**
E-mail: goruky@ukr.net
O. Skliar
Doctor of Veterinary Sciences, Professor
Department of Therapy, Pharmacology,
Clinical Diagnostics and Chemistry
Sumy National Agrarian University
Herasyma Kondratieva str., 160, Sumy, Ukraine, 40021
E-mail: sklyar1956@gmail.com
S. Kernychnyi
PhD, Associate Professor
Department of Veterinary Obstetrics,
Internal Pathologic and Surgery**
E-mail: serhii.kernychnyi@gmail.com
*Department of Food Biotechnology and Chemistry
Ternopil Ivan Puluj National Technical University
Ruska str., 56, Ternopil, Ukraine, 46001
**State Agrarian and Engineering University in Podilya
Schevchenka str., 13, Kamianets-Podilskyi, Ukraine, 32300

1. Introduction

The presence of bacteria at surfaces of technological equipment in the dairy industry is regarded to be an important factor that could lead to the contamination of dairy products; it is considered an important hygienic problem [1]. At the same time, micro-organisms survive at the surfaces of technological equipment owing to the capability to create biofilm forms [2, 3]. In this regard, certain hygienic requirements are put to the surface of technological equipment that is used in the dairy industry, especially regarding the mark of steel, relief, and roughness. According to the EU Directive 93/43 [4, 5], based on the criteria for evaluation of hygiene of equipment, large areas of the surface in contact with a product must possess roughness not exceeding 0.8 μm. This is predetermined by that the surface roughness may promote or inhibit the adhesion and proliferation of biofilm forms of bacteria [6, 7]. In addition, the development of a biofilm reduces the effectiveness of sanitizing dairy equipment and thereby increases the microbial contamination of dairy products and reduces their shelf life.

Thus, it is a relevant task in the dairy industry to study the process of biofilms formation by bacteria, of different shapes and sizes, depending on roughness of the surface of stainless steel. Research in this area will make it possible to scientifically substantiate parameters for the roughness of surface of milk equipment, which would maximally reduce the process of microbial adhesion. Such studies could become the basis for the development of a technique to evaluate stainless steel for the presence of anti-adhesive properties.

2. Literature review and problem statement

Successful development of microbial biofilms is not possible without the adhesion of a microorganism to the surface. Scientists distinguish five main stages in the formation and development of biofilms at any surface [8]. Fig. 1 shows a schematic model of the formation and growth of a biofilm.

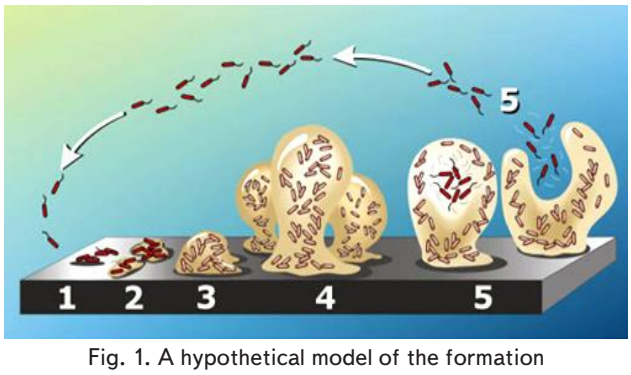


Fig. 1. A hypothetical model of the formation of a microbial biofilm

Stage 1 is the adhesion of bacteria to the surface; stages 2–4 is the growth of colonies and production of intercellular matrix, a biofilm formation; stage 5 is the dispersion (planktonic bacteria exit a biofilm). The microorganisms that are in the upper layer of the matrix of a biofilm release planktonic cells when breeding that colonize other surfaces [9]. However, the process of forming each film has certain features that are governed by the genetic, biochemical properties of a bacterial cell and by factors from the environment [10]. Thus, a biofilm is the microbial community, which is formed from cells attached to a surface, or to one another, while being in the matrix of synthesized extracellular substances [11, 12]. The main danger from biofilms at dairy equipment is that the extracellular matrix of a biofilm protects bacteria from the action of disinfectants. The microorganisms that survived colonize new surfaces and dairy products [1, 3].

It is believed that the adhesion of bacteria to the surface is a complicated physical-chemical process, which depends on: the properties of a surface (topography, roughness, hydrophobicity, chemical composition, surface energy) [13, 14]. It also depends on the initial number of microorganisms, their shape, size, temperature, and pH of the environment, etc. [15, 16]. That is why, while exploring the process of microbial adhesion and the formation of biofilms under laboratory conditions, it is not always possible to take into consideration the influence of production factors and physiological characteristics of microbial cells.

However, among many of those specified factors that affect the process of adhesion, researchers point to the role of the properties of a surface, which is considered to be the most essential [17, 18]. Consequently, three theories of microbial adhesion to the surface were proposed: thermodynamic, DLVO theory, and XDLVO [13]. The thermodynamic theory is based on that when microorganisms attach to the surface there is a change in the total Gibbs free energy, van der Waals forces. A DLVO theory is based on that the colloidal particles in a lyophobic disperse system can freely approach each other until there is a contact between their liquid diffuse shells. A XDLVO theory is based on the thermodynamic and DLVO theories [13]. However, researchers believe that all three theoretical models, which are aimed at revealing the essence of microbial adhesion to the surface, are designed for a perfect colloid system. Under industrial conditions, microbial adhesion is a much more complex process and the attachment of micro-organisms can occur in different ways [19, 20].

In the dairy industry, equipment is mostly made from the corrosion-resistant stainless steels AISI-304, AISI-316, AISI-321 [21]. These steels can have different surface roughness when delivered, 0.2–3.2 μm [22]. Studies into the influence of topography and roughness of surface on the microbial adhesion are rather ambiguous. Thus, according to research [23, 24], there is a correlation between a surface roughness and the bacterial adhesion, with the attachment of microorganisms to the surface increasing with an increase in roughness. It is reported [17, 25] that forming a biofilm occurred much slower at the surface with a roughness of up to 0.4 μm, compared with the surface roughness greater than 0.8 μm. By using an electron microscopy, researchers in [7, 26] found that the primary adhesion of microbial cells occurs along the hollows of surface roughness, since under such conditions there is an increase in the contact area between a microbial cell and the surface. However, other studies indicate [27, 28] that there is practically no correlation between the surface roughness of stainless steel and the microbial adhesion. In addition, there are different data reported on the effect of a wetted surface on the microbial adhesion. It was found that the number of bacteria in adhesion decreased with an increase in surface hydrophobicity, and the microorganisms that attached to hydrophobic surfaces were easier removed by increasing the force of the flow during circulation of fluid [29–31]. However, other researchers [32] note that there is no correlation between surface wettability and the microbial adhesion. In addition, it was discovered that the biofilms that formed at surfaces with a large roughness reduced the efficiency of heat transfer in heat exchangers-condensators, by about 15 % [17].

Thus, an analysis of the scientific literature revealed that the hygienic quality and cleanliness of technological equipment in the dairy industry after sanitizing might be closely associated with a relief and surface roughness. Therefore, it is a promising task to study the process of film formation on stainless steel with different surface roughness over a certain time and with different shapes and sizes of bacteria. Such a study will make it possible to deeper understand the process of the formation of microflora on equipment and, accordingly, the contamination of food products. In addition, elucidating the influence of a surface roughness on microbial adhesion would contribute to the improvement and modification of surfaces that prevent adhesion.

3. The aim and objectives of the study

The aim of this work was to determine the effect of varying roughness of stainless-steel surface on the process

of microbial adhesion and film formation, depending on the physiological and morphological characteristics of microorganisms that contaminate equipment. This will make it possible to substantiate the magnitude for a surface roughness of dairy equipment, which would maximally reduce the process of microbial adhesion and could be easily sanitized.

To achieve the set aim, the following tasks have been solved:

– to experimentally explore the process of film formation by rod-shaped and coccal forms of bacteria on stainless steel with a different surface roughness at a temperature of 25 °C;

– to model theoretically the process of degradation of biofilms on stainless steel with a different surface roughness during sanitation.

4. Materials and methods to study the process of film formation

Materials and equipment used in the experiment are described in more detail in [33].

Density of the formed microbial biofilms was determined as follows. We placed in sterile Petri dishes sterile plates made of stainless steel with a corresponding surface roughness and introduced to the dish a sterile meat peptone broth (MPB) and the test culture *E. coli* or *S. aureus* at such a concentration at which 1 to 10 thousand cells on average are contained per 1 cm^2 of the plate area. After 3, 6, 9, 12, 18, and 24 hours of incubation, at a temperature of 25 °C, the plates were removed from the Petri dishes, washed three times with phosphatic buffer from the planktonic (non-attached) micro-organisms and fixed the biofilms formed with a 96° ethyl alcohol. Upon fixing, the biofilms were stained in a 0.1-% solution of crystal violet and dried. Next, 7.0 cm^3 of 96° ethyl alcohol were poured onto each plate separately; it was left for 10 min. Following the exposure over 10 minutes, we took 5 cm^3 of washing solution from biofilms and determined the optical density spectrophotometrically at wavelength 570 nM.

At the optical density of the washing solution up to 0.5 units, the density of the formed biofilms was considered low and believed that steel exhibits excellent anti-adhesive properties; from 0.51 to 1.0 units – average, good anti-adhesive properties of the steel surface; at the density of solution 1.01–1.30 units, the density of the formed biofilms was considered high: satisfactory anti-adhesive properties of steel; larger than 1.31 – poor anti-adhesive properties of steel [1].

An electron-microscopic analysis of the biofilms formed on stainless steel was performed using an electron scanning microscope (REM 106I, Ukraine).

5. Results of studying the formation of biofilms and modeling the degradation at sanitizing

Earlier research has found that dairy equipment and finished products at dairy enterprises release bacteria of the genera *Bacillus, Lactobacillus, Staphylococcus, Enterococcus, Pseudomonas,* and the family *Enterobacteriaceae* [2]. Staphylococci refer to the optional-anaerobic fixed bacteria of coccoid forms the size of 0.5×1.5 μm. *E. coli* (*Escherichia coli*) is a moving stick that has the ability to move using flagella, optional anaerobe, the size of 1.1–1.5×2.0–6.0 μm [34].

At the first stage of research, we defined the process of formation of the biofilm *E. coli* and *S. aureus* on stainless steel with a roughness of 2.687±0.014 μm (Fig. 2).

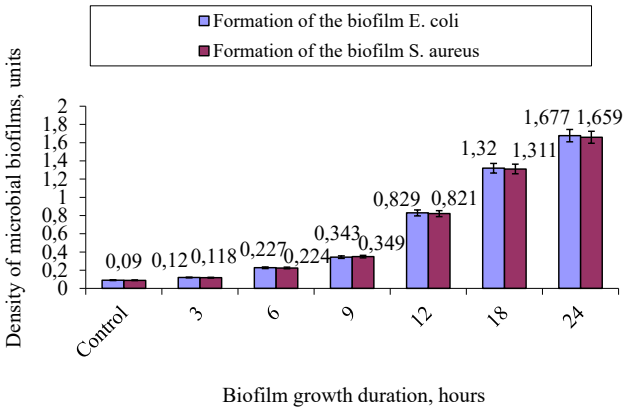


Fig. 2. Formation of the biofilms *E. coli* and *S. aureus* on stainless steel of grade AISI 321 with a roughness of 2.687±0.014 μm at a temperature of 25 °C

Data in Fig. 2 show that the optical density of microbial biofilms gradually grew during the time of incubation. However, the process of film formation was almost the same both for *E. coli* and *S. aureus* and did not depend on the size and shape of bacteria. Based on the acquired data, Fig. 3 shows a schematic model of the formation of a biofilm by these bacteria at a surface roughness of 3.00 μm.

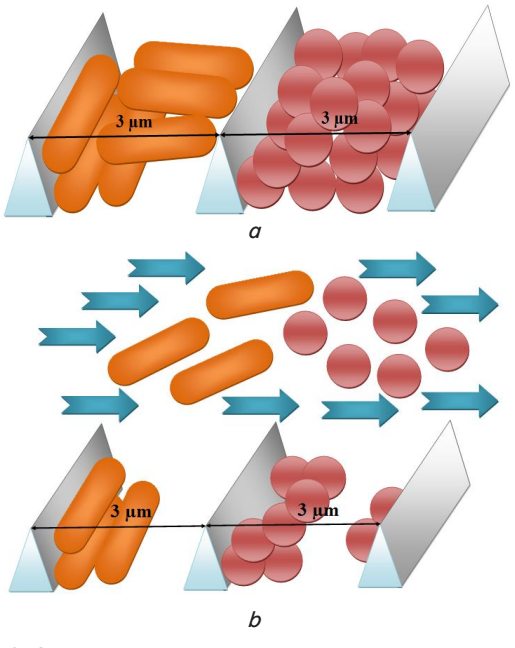
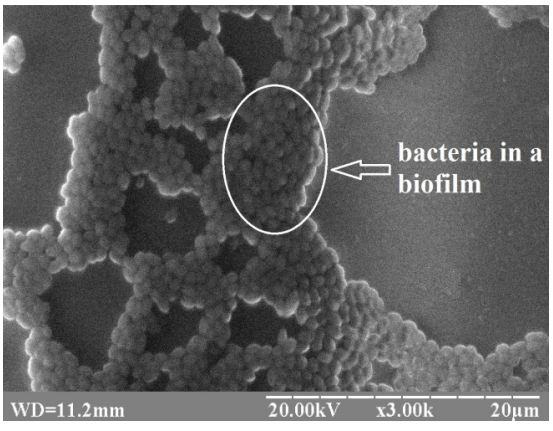


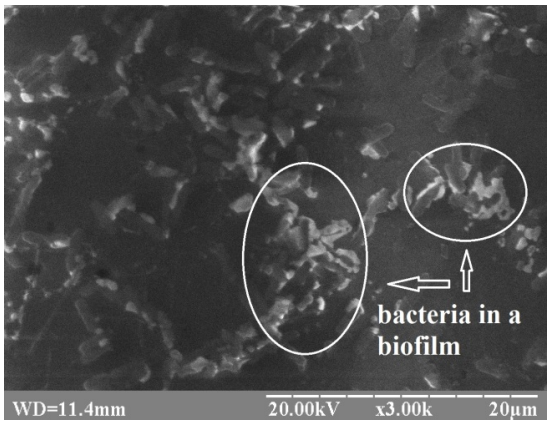
Fig. 3. Schematic model of the formation and destruction of biofilms by rod-shaped and coccal forms of bacteria on stainless steel of grade AISI 321 with a roughness of 3.00 μm: *a* – prior to sanitizing; *b* – during sanitizing

One can see (Fig. 3) that the rod-shaped and coccoid forms of bacteria freely attach in the hollows of roughness, so that under such conditions there is good adhesion to the surface and there starts the initial process of the first stage of a biofilm formation. During sanitizing, both coccoid and rod-shaped bacteria can remain in the hollows of roughness. In fact, cells in the formed biofilm in hollows are protected from the effect of detergents.

An electron-microscopic analysis of the process of forming a biofilm by the rod-shaped and coccal forms of bacteria after 6 h of incubation is shown in Fig. 4.



a



b

Fig. 4. The process of biofilm formation by microorganisms after 6 h of incubation: *a* – coccal forms; *b* – rod-shaped forms

Fig. 4 shows that the bacteria begin to produce the exopolysaccharide matrix, which surrounds cells and protects them from harmful environmental factors. In addition, following this period, one observes the separate, freely placed bacteria without a biofilm at the surface.

Therefore, the data obtained indicate that a surface roughness of stainless steel of 2.687±0.014 μm the process of forming the microbial biofilms by rod-shaped and coccal forms of bacteria proceeds smoothly and does not depend on the shape and size of the bacteria.

Fig. 5 shows results from studying the process of forming the biofilms *E. coli* and *S. aureus* on stainless steel with a roughness of 0.95±0.092 μm.

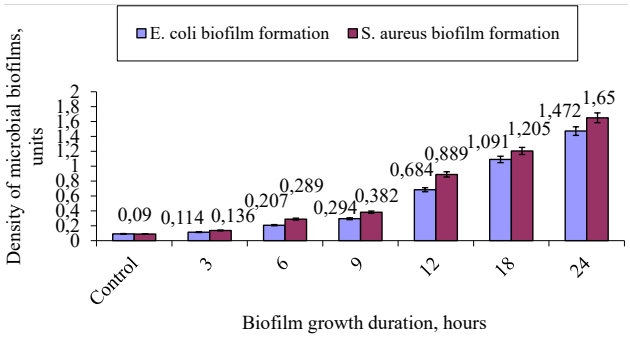
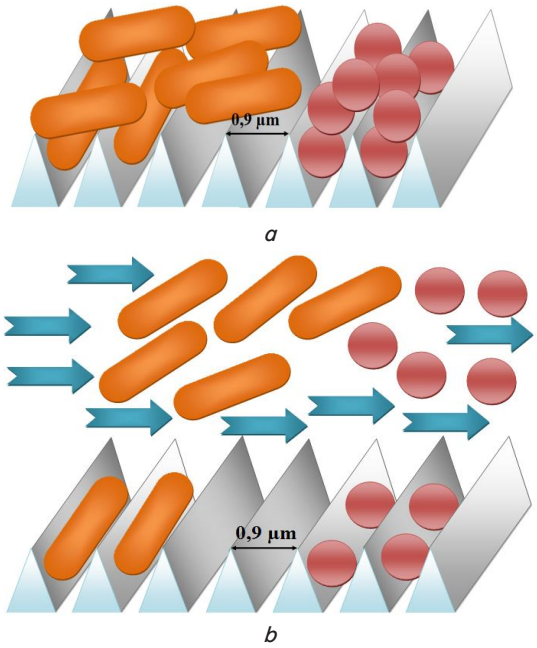


Fig. 5. Formation of the biofilms *E. coli* and *S. aureus* on stainless steel of grade AISI 321 with a roughness of 0.95±0.092 μm at a temperature of 25 °C

Data in Fig. 5 show that the process of biofilm formation at the surface of stainless steel with a roughness of 0.95±0.092 μm proceeds in a more intense fashion for *S. aureus* than for *E. coli*. Thus, over 3 h of incubation, the optical density of the formed biofilms *S. aureus* was 1.2 times greater than the density of the biofilms formed by *E. coli*. After 6 h of incubation at such roughness, the density of the biofilms formed by *S. aureus* amounted to 0.289 units and was 1.4 times ($p<0.05$) higher than that of *E. coli*. Over the subsequent hours of incubation, the density of biofilms gradually increased, however, from hour 6 to hour 12 the biofilms *S. aureus* were found to be on average 1.3 times ($p<0.05$) denser than the biofilms formed by *E. coli*. At hour 18 and hour 24 of incubation, the difference between the density of the biofilms formed by *E. coli* and *S. aureus* did not exceed 1.1 times. This indicates that, up to hour 18 of incubation, *E. coli* forms biofilms on stainless steel with a roughness of 0.955 μm at the protrusions in roughness.

A schematic model of biofilm formation on stainless steel with a roughness of 0.95 μm is shown in Fig. 6.



a

b

Fig. 6. Schematic model of the formation and destruction of biofilms by rod-shaped and coccal forms of bacteria on stainless steel of grade AISI 321 with a roughness of 0.90 μm: *a* – prior to sanitizing; *b* – during sanitizing

Data in Fig. 6 show that at such roughness of the stainless-steel surface the coccoid forms of bacteria, due to smaller sizes, stay put in the hollows of roughness, they are faster at adhesion and they form a biofilm. At the same time, the rod-shaped forms of bacteria, particularly *E. coli*, can attach to the hollows of roughness the size of 1 μm lengthwise only, because of their larger size. As a result, the adhesion of *e. coli* is slower while the density of biofilms is lower. Sanitization would destroy the biofilms formed at the protrusions of roughness while the hollows would host the biofilms formed by the survived coccal bacteria.

Fig. 7 shows results from studying the formation of the biofilms *E. coli* and *S. aureus* on stainless steel with a roughness of 0.63±0.087 μm.

Data in Fig. 7 show that the optical density of the biofilms formed by *S. aureus* after 3 h of incubation was 1.4 times ($p<0.05$) greater than that of the biofilm formed

by *E. coli*. At hour 6 of incubation, the intensity of film formation for *S. aureus* at such roughness of steel was 1.5 times ($p<0.05$) faster than that for *E. coli*. From hour 9 to hour 12 of incubation of the test cultures, we found a 1.4-time denser ($p<0.05$) biofilm, on average, for *S. aureus*. At hour 18 of incubation, the difference between the density of the biofilms formed by *S. aureus* and *E. coli* decreased to 1.2 times, at hour 24 – to 1.1 times. This indicates that during this time *e. coli* was very good at adhesion at the protrusions of roughness and began an intensive process of film formation.

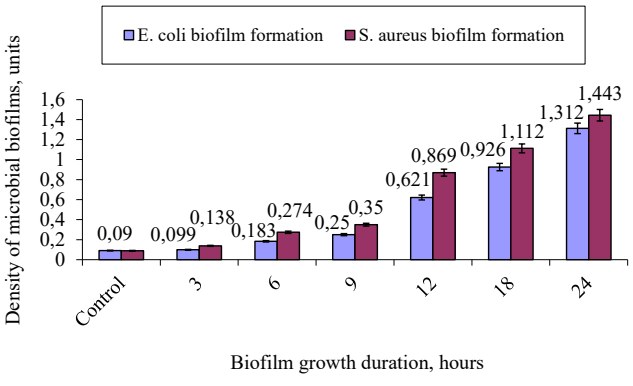


Fig. 7. Formation of the biofilms *E. coli* and *S. aureus* on stainless steel of grade AISI 321 with a roughness of 0.63±0.087 μm at a temperature of 25 °C

A schematic model of the process of film formation by *E. coli* and *S. aureus* on stainless steel with a roughness of 0.63 μm is shown in Fig. 8.

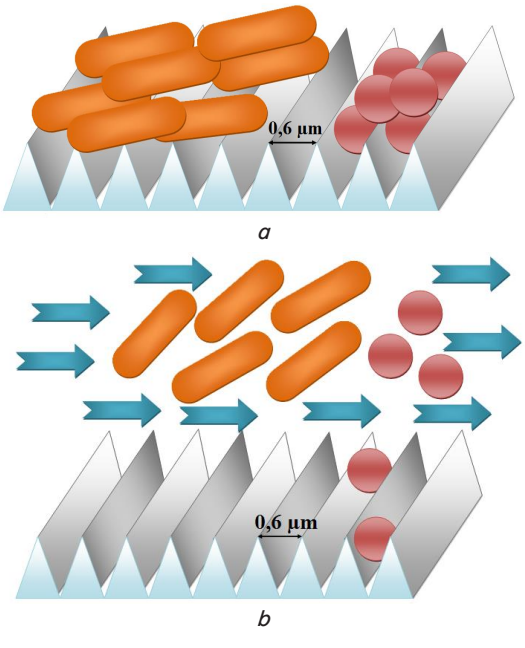


Fig. 8. Schematic model of the formation and destruction of biofilms by rod-shaped and coccal forms of bacteria on stainless steel of grade AISI 321 with a roughness of 0.63 μm: *a* – prior to sanitizing; *b* – during sanitizing

Data in Fig. 8 make it possible to assume that at a surface roughness of stainless steel of 0.63 μm the rod-shaped bacteria, specifically *E. coli*, are capable of adhesion and starting the process of forming a biofilm only at protrusions, not penetrating the hollows. At the same time, at such a surface roughness, the coccoid bacteria the size of up to 0.63 μm are capable of adhesion in hollows. The biofilms formed in the hollows of roughness are denser and are better protected from environmental factors, such as sanitization by washing and disinfecting agents in the food industry.

In addition, the research results reported here indicate that the process of film formation on stainless steel with a roughness of 0.63 μm was slower for rod-shaped and coccal bacteria, compared with a roughness of surface of 2.687 and 0.95 μm.

Fig. 9 shows results of forming the biofilms *E. coli* and *S. aureus* on stainless steel with a roughness of 0.018±0.16 μm.

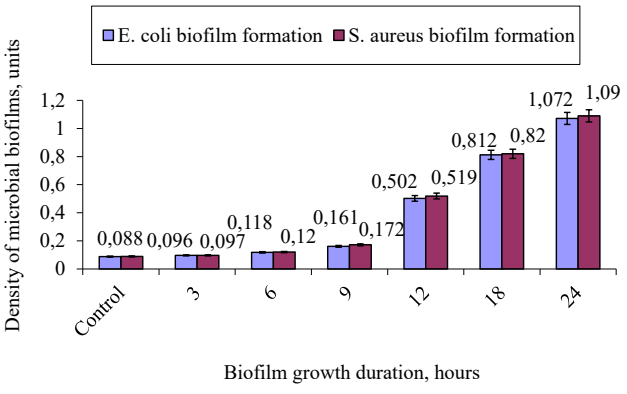


Fig. 9. Formation of the biofilms *E. coli* and *S. aureus* on stainless steel of grade AISI 321 with a roughness of 0.16±0.018 μm

Data in Fig. 9 show that at a steel surface roughness of 0.16±0.018 μm we found no probable difference in the density of the biofilms formed by *E. coli* and *S. aureus* over the period of incubation of 24 h. This indicates that the process of adhesion and film formation occurs at the protrusions of roughness, both for *E. coli* and *S. aureus*.

A schematic model of the *S. aureus* and *E. coli* film formation on stainless steel with a roughness of 0.2 μm is shown in Fig. 10.

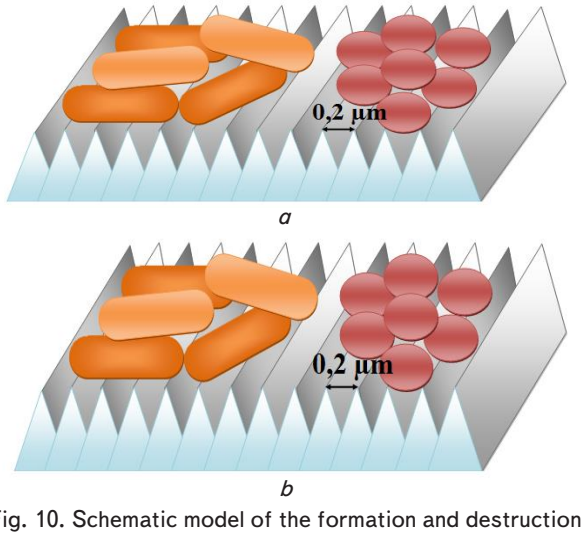


Fig. 10. Schematic model of the formation and destruction of biofilms by rod-shaped and coccal forms of bacteria on stainless steel of grade AISI 321 with a roughness of 0.2 μm: *a* – prior to sanitizing; *b* – during sanitizing

The schematic model of biofilm formation at a roughness of 0.2 μm has revealed (Fig. 10, *a*) that at such size of bacteria the adhesion is possible only on the protrusions of rough-

ness. At the same time, during sanitizing by washing and disinfecting agents (Fig. 10, *b*), at such roughness, there occurs a slight degradation of the biofilm of rod-shaped and coccal forms of bacteria, since the protrusions at surface roughness do not protect biofilms thereby destroying adhesive forces.

6. Discussion of results of studying the formation and degradation of biofilms at the surfaces of steel with a different roughness

The development of a biofilm on technological equipment creates serious problems at dairy enterprises related to the contamination of dairy products and the reduction in their shelf life. In addition, metallic surfaces are increasingly corroded, the efficiency of heat transfer decreases, the resistance against the friction of fluid during sanitizing increases, which generally lead to economic losses [17, 35, 36]. Part of researchers believe that stainless steel with a low surface roughness demonstrates the best anti-adhesive properties, bringing the number of bacteria attached bacteria to it considerably lower, which means sanitization could be more efficient [6, 17, 25]. Our research has found that at the surface of stainless steel of grade AISI 321 with a roughness of 2.687±0.014 μm the process of film formation for *E. coli* and *S. aureus* proceeded equally over the period from hour 3 to hour 24 of incubation, and did not depend on the size and shape of the bacteria. At the surface of stainless steel with a roughness of 0.95±0.092 μm the process of film formation for *S. aureus* was more intense than that for *E. coli*. Over the first 3 h of incubation the optical density of the formed biofilms *S. aureus* was 1.2 times greater than the density of biofilms formed by *E. coli*. Over the next 15 hours of incubation, the formed biofilms *S. aureus* were on average 1.3 times denser. From hour 18 to hour 24 of incubation, no reliable difference between the density of the biofilms formed by *S. aureus* and *E. coli* was detected. That allows us to assume that *S. aureus*, due to a ball-like shape, can stay put in the hollows of roughness 0.95±0.092 μm and are better at adhesion to the surface. At the same time, *E. coli*, owing to a rod-like shape, at such a surface roughness, can stay put in a hollow only lengthwise and form biofilms. However, it was found that the intensity of the process of film formation by bacteria at the surface with a roughness of 0.95±0.092 mm depends on the shape and size of the bacteria only until hour 18 of incubation. When we used in the course of research the stainless steel with a roughness of less than 0.8 μm, as is recommended in the food industry according to hygienic norms [4, 5], it was established that at a surface roughness of 0.63±0.087 μm the intensity of film formation by *S. aureus* was, on average, 1.4 times faster than that for *E. coli*, up to hour 18 of incubation. At the same time, at a roughness of 0.16±0.018 μm, the process of film formation proceeded equally for *S. aureus* and *E. coli*, but the formed biofilms were of lower density compared with those that formed at a roughness of 0.63±0.087 μm. The data obtained are consistent with research [6], where it is indicates that the adhesion of *L. monocytogenes* on stainless steel with roughness below 0.8 μm was slower than at the surface with a roughness of 30 μm. However, it was established that in addition to the surface roughness, the process of biofilm formation is affected by the shape and size of the cells of microorganisms. That relates to that at roughness 0.95 and 0.63 μm the coccoid forms of microorganisms formed biofilms in a more intense fashion than the rod-shaped ones. We support scientists [7, 14] in whose opinion this phenomenon is associated with an increase in the area of contact between bacteria and a surface.

Therefore, we believe that the efficient sanitizing of dairy equipment should involve the maximum surface roughness of 0.5 μm. Such a treatment is the optimal solution for the prevention of film formation by both coccal and rod-shaped forms of bacteria.

In addition, we support the idea that the process of microbial adhesion to the surface, in addition to the physical-chemical properties of a surface, is affected by such cellular structures of microorganisms as flagella, fimbria, pili, as well as such environmental factors as temperature, pH, the initial number of microorganisms, duration of incubation. Typically, upon sanitizing, a surface and the environmental conditions are practically stable, so the survival of bacteria is associated with the resistance to available disinfectants and the ability to form spores.

Thus, by summarizing, one can note that the roughness of surface and the size of bacteria play a key role at the early stages of biofilm formation, that is when there is a process of adhesion of cells of microorganisms to the surface. At a temperature of 25 °C of the *E. coli* and *S. aureus* incubation at stainless steel with a roughness of 0.95 and 0.63 μm the process of intensive film formation depended on the size and shape of bacteria up to hour 18. Over the subsequent period of incubation, the size of bacteria did not exert any influence on the intensity of the process of biofilm formation.

From a practical point of view, the identified patterns of the more intense process of film formation by coccal bacteria at a surface with a smaller roughness than that by the rod-shaped ones makes it possible to determine the conditions for machining the surface of steel to the specified roughness, at which the process of adhesion is slower. This will allow the application of a substantiated approach to the use of equipment with the defined roughness of surface in order to achieve certain effects under industrial implementation. Specifically, by reducing the attachment of microorganisms to a surface and by bringing down dairy products contamination.

However, under production conditions, when installing equipment, the importance of surface roughness is not always a priority, neither the duration of its operation nor the impact of sanitizing agents that could eventually destroy the surface relief. Thus, promising areas in the further research in this field include determining the impact of a service time of stainless steel with a different surface roughness on microbial adhesion and the formation of biofilms, depending on the applied alkaline and acidic detergents and disinfectants at sanitizing.

Therefore, in order to plan measures to decrease the adhesion and biofilm formation at the surfaces of stainless steel in dairy industry, it is necessary to consider the grade of steel, surface roughness, the operation time of equipment, the physiological features of microflora that contaminates its, and the technology of sanitizing. In addition, using technological equipment with a roughness less than 0.5 μm would make it possible to lower the intensity of film formation process and to improve hygienic cleanliness of equipment.

7. Conclusions

1. Our research has found that at the surface of stainless steel with a roughness of 2.687±0.014 μm the process of film formation for *E. coli* and *S. aureus* proceeded equally from hour 3 to hour 24 and did not depend on the size of bacteria. This allows us to argue that the rod-shaped and coccoid bacteria freely attach to the hollows of roughness and form biofilms. The patterns in the formation of biofilms at the surface of steel with a roughness of 0.95±0.092 μm imply that the process of film formation for *S. aureus* was more intense than that for *E. coli*. Over 3 h of incubation, the density of the formed biofilms *S. aureus* was 1.2 times higher compared with the biofilms *E. coli*. In the next 15 hours of incubation, the formed biofilms *S. aureus* were, on average, 1.3 times denser. This gives grounds to assume that *S. aureus*, due to a ball-like shape, can stay put in the hollows of roughness at 0.95±0.092 μm and become better at adhesion to the surface. At the same time, *E. coli*, owing to a rod-like shape, can, at such a surface roughness, adhere in a hollow only lengthwise. It has been proven that at a surface roughness of 0.63±0.087 μm the intensity of film formation by *S. aureus* was on average 1.4 times faster than that for *E. coli*. At the same time, at a roughness of 0.16–0.018 μm, the process of film formation proceeded equally for *S. aureus* and *E. coli*, but the biofilms were of lower density compared with those that formed at a roughness of 0.63±0.087 μm.

2. It has been theoretically modeled that the process of degradation of the biofilms made from stainless steel would proceed, at sanitizing, in the most difficult manner at a surface roughness of 2.687–0.95 μm. This relates to that the hollows in roughness could host both coccoid and rod-shaped bacteria that formed biofilms. At the same time, at a steel surface roughness of 0.63–0.16 μm, the formed biofilms are less protected by hollows in roughness and thus the degradation process would occur faster.

Thus, given the mechanisms that were established based on theoretical and experimental data, one could argue that the roughness of a surface and the size of bacteria play a key role at the early stage of biofilm formation. Therefore, the use of equipment with a roughness not exceeding 0.5 μm in dairy industry would make it possible to reduce the attachment of microorganisms to the surface and to bring down the contamination of dairy products.

References

1. Formation of biofilms on dairy equipment and the influence of disinfectants on them / Kukhtyn M., Berhilevych O., Kravcheniuk K., Shynkaruk O., Horyuk Y., Semaniuk N. // Eastern-European Journal of Enterprise Technologies. 2017. Vol. 5, Issue 11 (89). P. 26–33. doi: https://doi.org/10.15587/1729-4061.2017.110488
2. The influence of disinfectants on microbial biofilms of dairy equipment / Kukhtyn M., Berhilevych O., Kravcheniuk K., Shynkaruk O., Horyuk Y., Semaniuk N. // EUREKA: Life Sciences. 2017. Issue 5. P. 11–17. doi: https://doi.org/10.21303/2504-5695.2017.00423
3. Persistence strategies of Bacillus cereus spores isolated from dairy silo tanks / Shaheen R., Svensson B., Andersson M. A., Christiansson A., Salkinoja-Salonen M. // Food Microbiology. 2010. Vol. 27, Issue 3. P. 347–355. doi: https://doi.org/10.1016/j.fm.2009.11.004
4. Council directive 93/43/EEC on the hygiene of foodstuffs // Official Journal of the European Communities. 1993. No L 175/1.
5. Whitehead K. A., Verran J. The effect of surface properties and application method on the retention of Pseudomonas aeruginosa on uncoated and titanium-coated stainless steel // International Biodeterioration & Biodegradation. 2007. Vol. 60, Issue 2. P. 74–80. doi: https://doi.org/10.1016/j.ibiod.2006.11.009
6. The retention of bacteria on hygienic surfaces presenting scratches of microbial dimensions / Verran J., Packer A., Kelly P., Whitehead K. A. // Letters in Applied Microbiology. 2010. Vol. 50, Issue 3. P. 258–263. doi: https://doi.org/10.1111/j.1472-765x.2009.02784.x
7. Picosecond laser treatment production of hierarchical structured stainless steel to reduce bacterial fouling / Rajab F. H., Liauw C. M., Benson P. S., Li L., Whitehead K. A. // Food and Bioproducts Processing. 2018. Vol. 109. P. 29–40. doi: https://doi.org/10.1016/j.fbp.2018.02.009
8. Finkel J. S., Mitchell A. P. Genetic control of Candida albicans biofilm development // Nature Reviews Microbiology. 2011. Vol. 9, Issue 2. P. 109–118. doi: https://doi.org/10.1038/nrmicro2475
9. Monds R. D., O'Toole G. A. The developmental model of microbial biofilms: ten years of a paradigm up for review // Trends in Microbiology. 2009. Vol. 17, Issue 2. P. 73–87. doi: https://doi.org/10.1016/j.tim.2008.11.001
10. Psl trails guide exploration and microcolony formation in Pseudomonas aeruginosa biofilms / Zhao K., Tseng B. S., Beckerman B., Jin F., Gibiansky M. L., Harrison J. J. et. al. // Nature. 2013. Vol. 497, Issue 7449. P. 388–391. doi: https://doi.org/10.1038/nature12155
11. Correction: Biofilm Formation As a Response to Ecological Competition / Oliveira N. M., Martinez-Garcia E., Xavier J., Durham W. M., Kolter R., Kim W., Foster K. R. // PLOS Biology. 2015. Vol. 13, Issue 8. P. e1002232. doi: https://doi.org/10.1371/journal.pbio.1002232
12. Monds R. D., O'Toole G. A. The developmental model of microbial biofilms: ten years of a paradigm up for review // Trends in Microbiology. 2009. Vol. 17, Issue 2. P. 73–87. doi: https://doi.org/10.1016/j.tim.2008.11.001
13. Bacterial Adhesion and Biomaterial Surfaces / Moriarty T. F., Poulsson A. H. C., Rochford E. T. J., Richards R. G. // Comprehensive Biomaterials. 2011. P. 75–100. doi: https://doi.org/10.1016/b978-0-08-055294-1.00007-6
14. An overviev of the influence of stainless-steel surface properties on bacterial adhesion / Hoevar M., Jenko M., Godec M., Drobne D. // Materials and technology. 2014. Vol. 48, Issue 5. P. 609–617.
15. Surface topographical factors influencing bacterial attachment / Crawford R. J., Webb H. K., Truong V. K., Hasan J., Ivanova E. P. // Advances in Colloid and Interface Science. 2012. Vol. 179-182. P. 142–149. doi: https://doi.org/10.1016/j.cis.2012.06.015
16. Merritt K., An Y. H. Factors Influencing Bacterial Adhesion // Handbook of Bacterial Adhesion. 2000. P. 53–72. doi: https://doi.org/10.1385/1-59259-224-4:53

17. Impact of the surface roughness of AISI 316L stainless steel on biofilm adhesion in a seawater-cooled tubular heat exchanger-condenser / García S., Trueba A., Vega L. M., Madariaga E. // Biofouling. 2016. Vol. 32, Issue 10. P. 1185–1193. doi: https://doi.org/10.1080/08927014.2016.1241875
18. Effect of long-term operation on steels of main gas pipeline: Structural and mechanical degradation / Maruschak P., Panin S., Chausov M., Bishchak R., Polyvana U. // Journal of King Saud University – Engineering Sciences. 2018. Vol. 30, Issue 4. P. 363–367. doi: https://doi.org/10.1016/j.jksues.2016.09.002
19. Whitehead K. A., Verran J. The Effect of Substratum Properties on the Survival of Attached Microorganisms on Inert Surfaces // Marine and Industrial Biofouling. 2009. P. 13–33. doi: https://doi.org/10.1007/978-3-540-69796-1_2
20. An Y. H., Dickinson R. B., Doyle R. J. Mechanisms of Bacterial Adhesion and Pathogenesis of Implant and Tissue Infections // Handbook of Bacterial Adhesion. 2000. P. 1–27. doi: https://doi.org/10.1385/1-59259-224-4:1
21. Partington E. Stainless Steel in the Food & Beverage Industry // Materials and Applications. 1-st ed. 2006. 24 p.
22. Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry / Jullien C., Bénézech T., Carpentier B., Lebret V., Faille C. // Journal of Food Engineering. 2003. Vol. 56, Issue 1. P. 77–87. doi: https://doi.org/10.1016/s0260-8774(02)00150-4
23. Bacterial Adhesion and Surface Roughness for Different Clinical Techniques for Acrylic Polymethyl Methacrylate / Dantas L. C. de M., Silva-Neto J. P. da, Dantas T. S., Naves L. Z., das Neves F. D., da Mota A. S. // International Journal of Dentistry. 2016. Vol. 2016. P. 1–6. doi: https://doi.org/10.1155/2016/8685796
24. Whitehead K. A., Benson P. S., Verran J. Developing application and detection methods for Listeria monocytogenes and fish extract on open surfaces in order to optimize cleaning protocols // Food and Bioproducts Processing. 2015. Vol. 93. P. 224–233. doi: https://doi.org/10.1016/j.fbp.2014.07.007
25. Whitehead K. A., Verran J. The Effect of Surface Topography on the Retention of Microorganisms // Food and Bioproducts Processing. 2006. Vol. 84, Issue 4. P. 253–259. doi: https://doi.org/10.1205/fbp06035
26. Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance / Hilbert L. R., Bagge-Ravn D., Kold J., Gram L. // International Biodeterioration & Biodegradation. 2003. Vol. 52, Issue 3. P. 175–185. doi: https://doi.org/10.1016/s0964-8305(03)00104-5
27. Milledge J. J. The cleanability of stainless steel used as a food contact surface: an updated short review // Food Sci. Technol. 2010. Vol. 24, Issue 3. P. 18–20.
28. Retention of bacteria on a substratum surface with micro-patterned hydrophobicity / Bos R., Mei H. C., Gold J., Busscher H. J. // FEMS Microbiology Letters. 2000. Vol. 189, Issue 2. P. 311–315. doi: https://doi.org/10.1111/j.1574-6968.2000.tb09249.x
29. Bacterial Retention on Superhydrophobic Titanium Surfaces Fabricated by Femtosecond Laser Ablation / Fadeeva E., Truong V. K., Stiesch M., Chichkov B. N., Crawford R. J., Wang J., Ivanova E. P. // Langmuir. 2011. Vol. 27, Issue 6. P. 3012–3019. doi: https://doi.org/10.1021/la104607g
30. Antibacterial Fluorinated Silica Colloid Superhydrophobic Surfaces / Privett B. J., Youn J., Hong S. A., Lee J., Han J., Shin J. H., Schoenfisch M. H. // Langmuir. 2011. Vol. 27, Issue 15. P. 9597–9601. doi: https://doi.org/10.1021/la201801e
31. Bioinspired Hierarchical Surface Structures with Tunable Wettability for Regulating Bacteria Adhesion / Dou X.-Q., Zhang D., Feng C., Jiang L. // ACS Nano. 2015. Vol. 9, Issue 11. P. 10664–10672. doi: https://doi.org/10.1021/acsnano.5b04231
32. Femtosecond laser surface texturing of titanium as a method to reduce the adhesion of Staphylococcus aureus and biofilm formation / Cunha A., Elie A.-M., Plawinski L., Serro A. P., Botelho do Rego A. M., Almeida A. et. al. // Applied Surface Science. 2016. Vol. 360. P. 485–493. doi: https://doi.org/10.1016/j.apsusc.2015.10.102
33. Study of the influence of Savinase®Evity 16L enzyme on biofilms formation of staphylococcus aureus on stainless steel with different roughness / Kukhtyn M., Kravcheniuk K., Beyko L., Horiuk Y., Skliar O., Kernychnyi S. // EUREKA: Life Sciences. 2019. Issue 2. P. 26–32. doi: http://dx.doi.org/10.21303/2504-5695.2019.00858
34. Bergey's manual of systematic bacteriology / P. Vos, G. Garrity, D. Jones, N. R. Krieg et. al. (Eds.) // The Firmicutes. Springer, 2009. doi: https://doi.org/10.1007/978-0-387-68489-5
35. Biofilm Formation in Milk Production and Processing Environments; Influence on Milk Quality and Safety / Marchand S., De Block J., De Jonghe V., Coorevits A., Heyndrickx M., Herman L. // Comprehensive Reviews in Food Science and Food Safety. 2012. Vol. 11, Issue 2. P. 133–147. doi: https://doi.org/10.1111/j.1541-4337.2011.00183.x
36. Main microbiological and biological properties of microbial associations of "Lactomyces tibeticus" / Kukhtyn M., Vichko O., Berhilevych O., Horyuk Y., Horyuk V. // Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016. Vol. 7, Issue 6. P. 1266–1272.