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AN OVERVIEW OF THE INFLUENCE OF STAINLESS-STEEL SURFACE PROPERTIES ON BACTERIAL ADHESION

VPLIV LASTNOSTI POVRŠINE NERJAVNEGA JEKLA NA ADHEZIJO BAKTERIJ

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The adhesion and growth of bacteria on the surface of stainless steel promotes corrosion of the material, microbiological contamination, healthcare problems and results in economic losses. There are numerous factors influencing the adhesion of bacteria to stainless steel, and material properties are one of the most important ones. In particular, surface roughness, topography, chemistry and surface energy can promote or inhibit the adhesion and growth of bacteria. Surface roughness and topography are generally accepted as crucial parameters, especially when the surface features are comparable to the size of the bacteria. The roughening of the surface increases the area available for adhesion and protects the bacteria from environmental factors, like liquid shear stress, mechanical forces and disinfectants. The surface chemistry and surface energy of the material can also affect microbial attachment and survival. The surface chemistry of stainless steel is significantly affected by the formation of an ultra-thin passive chromium-rich oxide film on the surface in the presence of an oxidative environment. Surface energy is also an important factor in the initial adhesion and it is commonly known that the minimal relative adhesion to surfaces occurs at surface energies ranging between 20 mN/m and 30 mN/m (Baier curve). Materials with a high surface energy, such as stainless steel, are mainly hydrophilic, frequently negatively charged and susceptible to contamination, and thus are rarely clean. This paper presents an overview of the mechanism and theories of bacterial adhesion on surfaces in general, together with a comprehensive overview of stainless-steel surface properties that may influence the adhesion of bacteria. Here we give a literature review and discuss how to manage the stainless-steel surfaces in food processing, medicine and other industries in order to reduce the adhesion of bacteria.

Keywords: stainless steel, surface properties, adhesion, bacteria

Adhezija in rast bakterij na površini nerjavnega jekla pospešuje korozijo materiala, povzroča mikrobiološko kontaminacijo, zdravstvene težave in posledično gospodarsko škodo. Na adhezijo bakterij vplivajo številni dejavniki, med katerimi so lastnosti materiala med pomembnejšimi. Sem spadajo predvsem hrapavost, topografija, kemijska sestava in površinska energija. Hrapavost in topografija površine sta splošno sprejeta kot ključna dejavnika, ki vplivata na adhezijo bakterij na površino, predvsem kadar so topografske značilnosti na površini primerljive velikosti z bakterijo. Povečana hrapavost po eni strani pomeni večjo površino za pritrjevanje, po drugi strani pa ščiti bakterije pred okoljskimi dejavniki, kot so strižne napetosti v tekočih medijih, mehanske sile in razkužila. Kemijska sestava površine in površinska energija materiala lahko tudi vplivata na adhezijo in preživetje bakterij. Na kemijske lastnosti površine izrazito vpliva oksidna plast, ki se ustvari na površini nerjavnega jekla. Površinska energija je prav tako pomemben dejavnik pri začetni fazi adhezije. Splošno znano je razmerje med adhezijo in površinsko energijo, ki ga opisuje Baierjeva krivulja, kjer minimalno adhezijo na površini opazimo pri površinski energiji med 20 mN/m in 30 mN/m. Materiali z visoko površinsko energijo, kot je na primer nerjavno jeklo, so pogosto hidrofilni, negativno nabiti in dovzetni za kontaminacijo, tako da so te površine redko čiste. Članek daje splošen pregled mehanizmov in teorije adhezije bakterij na površine. Podrobno je podan pregled površinskih lastnosti nerjavnega jekla, ki lahko vplivajo na adhezijo bakterij. V članku razpravljamo o tem, kako zmanjšati adhezijo bakterij v živilskopredelovalni industriji ter pri medicinski uporabi. Za uspešno zmanjševanje neželene adhezije bakterij je potrebno poglobljeno znanje o dejavnikih, ki v največji meri vplivajo na adhezijo bakterij.

Ključne besede: nerjavno jeklo, lastnosti površine, adhezija, bakterije

1 INTRODUCTION

Bacteria generally exist freely or as a population attached to surfaces.[1] When available, bacteria prefer to grow on surfaces[2] forming aggregates known as biofilms. Bacterial adhesion and the subsequent biofilm formation is a complex physico-chemical process consisting of several stages, including the development of a surface-conditioning film, the approach of bacteria to the surface, adhesion (initial reversible and subsequent irreversible adhesion), the growth and division of organisms and finally detachment and dispersal of cells.[1,3–5]

The adhesion of bacteria to surfaces depends on the properties of the material (surface topography, roughness, surface chemistry and surface energy), the bacteria (surface charge, surface hydrophobicity and appendages) and the surrounding environment (type of medium, temperature, pH, period of exposure and bacterial concentration).[6,7] Among them, the material surface properties are one of the most important.

Stainless steels are commonly used in industrial, medical and food-processing applications,[8,9] and the adhesion of bacteria to stainless steel represents a chronic source of microbial contamination that leads to the deterioration of food, healthcare problems, the enhanced corrosion of stainless steel and reduces the performance of plants, pipelines, cooling towers and heat exchangers.[1,3,10–12]

Stainless steels can be produced in various grades and finishes, and additional surface treatments can affect surface physico-chemical properties.[13–15] The same type of stainless steel may have distinctly different surface properties, including topography, roughness, molecular composition, electrochemistry and physico-chemistry.[15] Additionally, an ultra-thin oxide film composed of chromium and iron oxides forms on the stainless-steel surface, which makes the steel resistant to corrosion.[14,15] The surface properties of stainless steel depend on the stainless-steel grade, the surface finish applied and the cleaning process used.[15] The passive oxide layer is also very susceptible to contamination from the environment (dissolve solutes and molecules from air)[16,17] and contamination can alter the surface properties and influence the adhesion.

The influence of stainless-steel surface properties on the adhesion and retention of bacteria has been extensively investigated. There have been numerous studies on different stainless steels, including AISI 302[18], AISI 304[8,13,19–32], AISI 316[13,23–26,32–37] and AISI 430[13,25] using different bacteria. However, it is still not clear as to which characteristics of stainless steels are favourable for bacterial adhesion as they are often interrelated.[15]

To reduce microbial adhesion and retention on stainless-steel surfaces it is necessary to understand the factors governing microbial adhesion through the systematic research of the various surface properties involved.

This review summarizes the influence of the surface properties of stainless steel, especially surface roughness, topography, chemistry and energy on the adhesion and retention behaviour of bacteria. The aim of this review is to summarize the available literature data on the material surface characteristics that are responsible for bacterial adhesion. We will emphasize the stainless-steel-bacterial adhesion in order to provide information about how to produce and maintain the surfaces in order to reduce bacterial contamination.

2 THEORY AND MECHANISM OF BACTERIAL ADHESION TO A SURFACE

The adhesion of bacteria to a substrate surface is governed by the physico-chemical properties of both the substrate and the bacterium, and also the environmental conditions.[6,7,38] Bacteria may adhere to the surface either directly to the bare material (nonspecific adhesion) or indirectly to the conditioning film (specific adhesion) on the surface. Usually, nonspecific adhesion is investigated and these results are the closest to the predictions of theoretical models.[39] However, in natural environments the first step of the adhesion process is the formation of a conditioning layer[4,5] of organic and inorganic molecules that may alter the physico-chemical properties of the surface, provide a nutrient source for bacteria or inhibit the adhesion of certain bacteria.[5]

2.1 Theoretical background of bacterial adhesion

2.1.1 Physico-chemical Models of Bacterial Adhesion

Concepts developed in colloidal research are a common approach to predicting bacterial adhesion to surfaces.[2,10] If bacteria are treated as colloids in suspension, it is possible to model the bacterial adhesion to surfaces as the sum of the chemical and physical properties of bacteria and the material surface.[39] Three colloidal models are commonly applied when studying bacterial adhesion to surfaces: the thermodynamic theory, the Deryaguin–Landau–Verwey–Overbeek (DLVO) theory and the extended-DLVO (XDLVO) theory.[10,39,40]

2.1.1.1 Thermodynamic theory

The thermodynamic approach is based on the total change in the potential Gibbs free energy (energy available in a closed system) when a bacterium attaches to a surface and is calculated from the Lifshitz-van der Waals forces and Lewis acid-base interactions[39]:

$$\Delta G_{\mathrm{ADH}} = \Delta G_{\mathrm{LW}} + \Delta G_{\mathrm{AB}} \tag{1}$$

ΔG_{ADH} is the total change of the Gibbs free energy of adhesion, ΔG_{LW} is the Gibbs free energy change of the Lifshitz-van der Waals forces and ΔG_{AB} is the Gibbs free energy change of the Lewis acid-base forces. Thermodynamic theory assumes that adhesion is always reversible and distance independent. The theory does not include the effects of surface charge and the electrolyte concentration of the surrounding media. This theory is the most accurate with uncharged surfaces or in the presence of large quantities of ions.[39]

2.1.1.2 DLVO theory

The DLVO theory like the thermodynamic approach also assumes that adhesion is the sum of interfacial energies. However, the DLVO theory considers electrostatic forces instead of acid-base interactions[39]:

$$U^{\mathrm{DLVO}} = U^{\mathrm{LW}} + U^{\mathrm{EL}} \tag{2}$$

U^{DLVO} is the total interaction energy, U^{LW} is the Lifshitz-van der Waals interactions energy and U^{EL} is the electrostatic interaction energy. DLVO theory assumes that adhesion can be reversible and distance dependent. The theory is most accurate when electrostatic forces are predominant; however, it is limited due to the disregarded effects of the polar interactions.[39]

2.1.1.3 XDLVO theory

In an attempt to more accurately model bacterial adhesion the XDLVO theory combined the features of the thermodynamic approach and DLVO theory. The XDLVO model assumes that adhesion is the sum of the Lifshitz-van der Waals, electrostatic and Lewis acid-base interactions[39]:

$$U^{DLVO} = U^{LW} + U^{EL} + U^{AB} \quad (3)$$

U^{DLVO} is the total interaction energy, U^{LW} is the Lifshitz-van der Waals interaction energy, U^{EL} is the electrostatic interaction energy and U^{AB} is the Lewis acid-base interaction energy. Like with the DLVO, also XDLVO theory assumes that adhesion can be reversible and distance dependent.[39]

All three models favour bacterial adhesion when the product of the equations' theories is negative. An increase or decrease in bacterial adhesion for one set of parameters compared to a different set of parameters is calculated. These three theoretic models that predict the bacterial adhesion to surfaces were developed for ideal systems; however, the actual bacterial adhesion is complex and can behave completely differently from the prediction of the developed models.[39]

2.2 Mechanism of bacterial adhesion

Actual bacterial adhesion frequently deviates from the above-described adhesion models.[10] Solid materials exposed to various environments adsorb organic and inorganic material, thus forming a conditioning layer to which microorganisms attach.[10] The conditioning layer changes the physico-chemical properties of the surface and thus plays an important role in the bacterial attachment process.[5,10]

The adhesion of bacteria to solid surfaces is a two-phase process composed of an initial reversible (physical) followed by an irreversible (molecular and cellular) phase.[2,5,41] The adhesion of bacteria to the surface may be passive or active and this depends on the motility of the bacteria and the transportation of cells by gravity, the diffusion of bacteria and the fluid dynamic forces.[3,5] Initial adhesion also depends on the physico-chemical properties of bacteria cells, their growth phase and the availability of nutrients.[3]

The adhesion of bacteria to surfaces occurs rapidly, within seconds.[42,43] Planktonic microbial cells are transported from the suspension to the conditioned surface either by bacterial appendages or by physical forces, thus enabling an initial reversible adhesion.[1,3,41] The long-range physical forces, including van der Waals forces, steric and electrostatic interactions, influence the initial reversible adhesion. During this initial stage the bacteria still show Brownian motion and can be easily removed from the surface.[3,41]

After an initial reversible adhesion a number of cells adhere irreversibly. In this phase molecular reactions between bacterial surface structures and substrate surfaces become predominant. In contrast to reversible adhesion, various short-range forces such as dipole-dipole interactions, hydrogen, ionic and covalent bonding and hydrophobic interactions are involved.[1–3,41] Once the bacteria attach, irreversible strong physical or chemical forces are required to remove them from the surface.[3]

The irreversibly attached bacterial cells start to grow, divide and form microcolonies, the basic structural unit of the biofilm.[1,3,44] The production of additional extracellular polymeric substances (EPS) helps to strongly bind the cells to the surface and stabilize the microcolonies from the environmental fluctuations[1,3] and the presence of nutrients in the conditioning film and the surrounding environment determines the rapid growth and division of cells.[1] The biofilm not only enables the strong attachment of the cells to the surface, but also helps collect diffuse nutrients, acts as a protection against environmental stress, antibiotics and disinfectants and enables intercellular communications. As the biofilm ages the attached bacteria detach and disperse from the biofilm and colonize new niches.[1,3]

3 FACTORS AFFECTING BACTERIAL ADHESION TO SURFACES

Bacterial adhesion is a complex process affected by the characteristics of the bacteria, environmental properties and the physical and chemical properties of a material surface.[6,7,38] Environmental factors including the type of medium, temperature, pH, shear stress of the flowing medium, bacterial concentration, chemical treatment and the presence of antibiotics may influence bacterial adhesion by either changing the surface characteristics of the bacteria and material or influencing the interactions in a reversible phase of adhesion. Furthermore, different bacterial species and strains adhere differently for a given material. This is due to the differences in the physic-chemical characteristics of the bacteria, including surface hydrophobicity, surface charge, appendages and EPS production.[6,7] The physical and chemical properties of a material surface that can influence bacterial adhesion to the material surface include surface roughness, topography or physical configuration, chemical composition, surface energy and hydrophobicity.[6,7] However, the surface characteristics can be quickly altered by the adsorption of organic and inorganic compounds forming a conditioning layer.[3,5,10] This review will focus on the properties of stainless steel that can influence bacterial adhesion.

3.1 Stainless-steel surface properties affecting bacterial adhesion

Stainless steels are iron-based alloys containing at least 10.5 % Cr with numerous alloying elements that improve the mechanical and corrosion properties.[9] Stainless steel is the material of choice in the food-processing industry[14,15,45], mainly because it is inert, resistant to corrosion, stable at various temperatures and hygienic.[45,46]

Stainless steel can be produced in various grades (AISI 302, AISI 304, AISI 316, AISI 420) and finishes (2B, 2R, 2D, number 4 finish), thus having different surface properties (chemistry, topography, roughness, energy).[13,14] Furthermore, additional surface treatments such as mechanical or electro polishing can be applied to modify the surface topography and roughness and achieve functionally and aesthetically improved surfaces.[13,45] When the commonly used 2B surface finish is additionally grinded/polished with SiC papers and diamond paste, different surface patterns are obtained (**Figure 1**).[47] Stainless steel forms an ultra-thin oxide film on the surface composed of chromium and iron oxides that protects the steel from corrosion. The composition of the oxide film depends on the metal substrate, the surface finish and the surrounding environment.[15]

3.1.1 Effect of surface roughness and topography on bacterial adhesion

Stainless steel produced in different surface finishes is designated by a system of standardized numbers: No. 1, 2D, 2B, and 2BA for unpolished finishes; and No. 3, 4, 6, 7, and 8 for polished finishes.[9] The 2B pickling finish, the 2R bright annealed finish and finish 4 are the most often used.[14,15] During production, stainless steel goes through annealing and pickling processes where the stainless steel is softened and descaled. These processes clean the surface of the material prior to processing to a given finish.[14] After cold rolling, which reduces the thickness of the steel, final annealing (in oxidising atmosphere) and pickling follows and the surface finish obtained is designated as a 2D surface finish. When the 2D surface finish is finally light passed on polished rolls 2B or pickling finish is obtained.[9,15] To achieve a bright finish or a 2R finish, the stainless steel is annealed in a protective atmosphere and the final pickling process is avoided.[15] Finish 4 is achieved when 2D or 2R sheets are further polished with fine-grained polish belts.[9,15] The surface composition, topography and roughness for a given material may differ considerably according to the different surface finishes applied.[15]

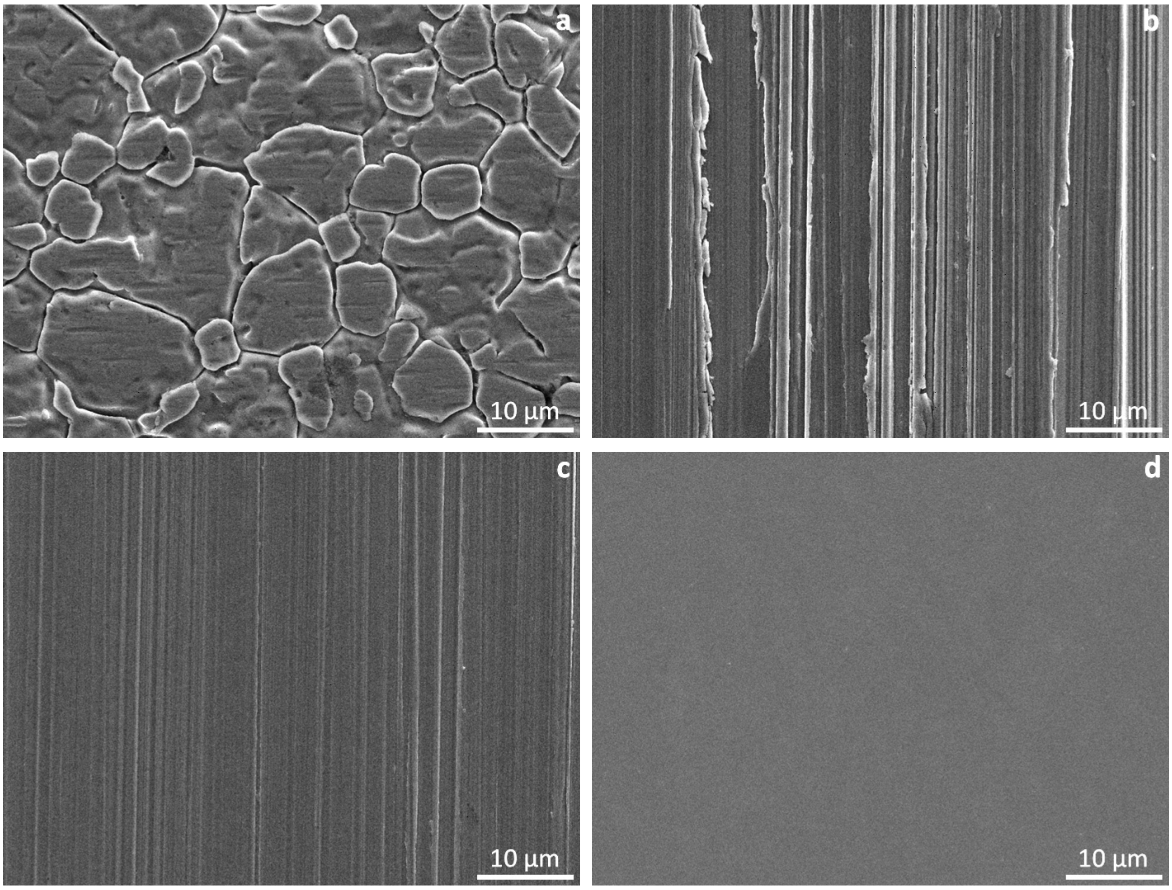

Figure 1: Secondary-electron (SE) images taken on scanning electron microscope illustrate the surface features of different surface finishes of AISI 316L stainless steel: a) 2B surface finish, b) 2B surface finish grinded with 100 SiC grit paper, c) 2B surface finish grinded with 800 SiC grit paper and d) 2B surface finish polished with diamond paste 3 µm and 1 µm to mirror finish. The 2B surface finish (a) has a network of subsurface crevices between the oxide grain boundaries; mechanically grinded surface finishes (b, c) exhibit scratch patterns with long linear alternating grooves and ridges; and the mirror surface finish (d) is the smoothest without pronounced topography features.[47]

Slika 1: Slike sekundarnih elektronov (SE), posnete z vrstičnim elektronskim mikroskopom, prikazujejo topografske karakteristike različno obdelanih površin nerjavnega jekla AISI 316L: a) 2B površina, b) 2B površina, brušena z granulacijo papirja 100 SiC, c) 2B površina, brušena z granulacijo papirja 800 SiC in d) 2B površina, polirana z diamantno pasto 3 µm in 1 µm. 2B površina (a) ima mrežo razpok med oksidnimi zrni na površini; na mehansko poliranih površinah (b, c) je opazen vzorec prask z dolgimi izmeničnimi dolinami; polirana površina (d) na drugi strani nima izrazite topografije.[47]

When studying bacterial adhesion to surfaces, a comprehensive characterization of the surface-roughness parameters and visualisation of the surface topography is very important.[38,48] Surface roughness is a two-dimensional parameter of a material surface and is usually described as the arithmetic average roughness (R_a) and the root mean square roughness (R_q)[6,38], whereas the topography is a-three dimensional parameter and describes the shape of the surface features.[6] The R_a and R_q, are commonly reported surface-roughness parameters when investigating bacterial adhesion; however, they are measures of the height variation without information about the topography (surface features).[38,48] Therefore, it is important to measure the spatial or amplitude parameters that give information about the spatial variation and to visualize and describe the morphological features of the surface.[38]

Stainless-steel grades AISI 302, AISI 304 and AISI 316 are most often used in adhesion studies due to their application in the food industry and medicine.[14,15,33,49] In the literature regarding adhesion and retention, bacteria genus Escherichia, Staphylococcus, Listeria, Pseudomonas, Streptococcus and Salmonella are the most often studied.[14] Although a number of studies have investigated the influence of the surface topography and roughness of different stainless steels on the adhesion of different bacteria the conclusions from these studies are not consistent.[38,48]

Several researchers including Jullien et al.[13], Ortega et al.[19], Whitehead and Verran[23], Flint et al.[26], Peterman et al.[27], Hilbert et al.[50] and observed no direct correlation between the surface roughness of the AISI 304/316 stainless R_a ranging between 0.01 μm and 3.3 μm and the adhesion of bacteria or spores. Arnold et al.[8,30] and Ortega et al.,[29] on the other hand, reported a positive correlation between the adhesion of bacteria and the surface roughness of the AISI 304 stainless steel. However, Goulter-Thorsen et al.[20] reported that *E. coli* attached in greater numbers to significantly smoother AISI 304 stainless steels. Also interesting are the findings of Medilanski et al.[51] who reported that minimal adhesion occurs at R_a = 0.16 μm and attachment to both rougher and smother surfaces was significantly higher. The increased adhesion of bacteria on rougher surfaces may be explained due to the increase in the surface area available for adhesion[6,7] and the roughening of the surface might also facilitate a firmer attachment by providing more contact points.[52] The opposing observations reported between the different studies are probably due to the various experimental conditions, different bacterial species tested, the material studied and methods used for bacteria detection.[26,42]

Besides surface roughness, also the topography of the surface or surface features such as pits, crevices, scratches, grooves and ridges, play an important part in the adhesion process.[15,38,43,45] Many researchers concluded that if the surface features are comparable to the size of the bacteria they can promote bacterial attachment and increase the subsequent microbial retention.[6,7,43,48]

The bacteria attach differently to surfaces with different surface topographies or special surface features and often the pattern of adhesion reflects the surface topography (**Figure 2**).[47] Medilanski et al.[51] studied the influence of an AISI 304 stainless-steel surface topography (three surface finishes with scratches and two without observable scratches) on the adhesion of four bacteria strains (*Desulfovibrio desulfuricans*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Rhodococcus* sp.) and found that bacterial cells attach into scratches in the longitudinal orientation when the width of the scratches corresponds to the width of the bacterial cells. Rougher surfaces with wider scratches exhibit a higher fraction of bacteria adhered in other orientations and the smoothest surfaces exhibit a random cell orientation.[51] Flint et al.[26] observed that surface flaws (scrapes, scratches and pitting) on AISI 304 stainless steel did not always affect the number of adhered bacteria; however, bacteria often aligned with the lines created by the surface flaws. Similar observations were made by Whitehead and Verran[23] on AISI 304 and AISI 316 stainless steel. Barnes et al.[21] compared the 2B and 8 mirror finish of AISI 304 stainless steel and reported that *Staphylococcus aureus* attach in greater numbers to a rougher 2B surface finish, whereas little differences between the 2B and number 8 mirror finish were observed for *Listeria monocytogenes.* Furthermore, scanning electron microscopy revealed that bacteria cells did not orient exclusively along polishing lines.[21] Using microbial retention assays with a range of differently sized, unrelated microorganisms on engineered surfaces (silicon wafers) with controlled topographical features Whitehead et al.[53,54] and Verran et al.[55] demonstrated that the size of the surface features is important with respect to the size of the bacteria, and its subsequent retention.

The wear of the surfaces may change the adhesion and retention of bacteria[14,45] with the introduction of new random features (i.e., scratches) different dimensions[45], especially on smooth polished surfaces. Studies of simulated worn surfaces demonstrated that the hygienic status of stainless steel was not affected in terms of microbial retention; however, the cleanability was affected in terms of the reduced removal of organic soil.[45] Holah and Thorpe[46] observed the increased retention of bacterial cells on abraded sinks compared to unused ones, this is due to the fact that rougher surfaces have an increase in the number of attachment sites, a larger surface contact area and topographical features that reduce the cleaning shear forces. Verran et al.[32] simulated wear on AISI 304 and AISI 316 stainless steel and studied the retention of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results showed that wear corresponding to R_a < 0.8 μm did not significantly affect the retention of microorganisms, but the pattern of

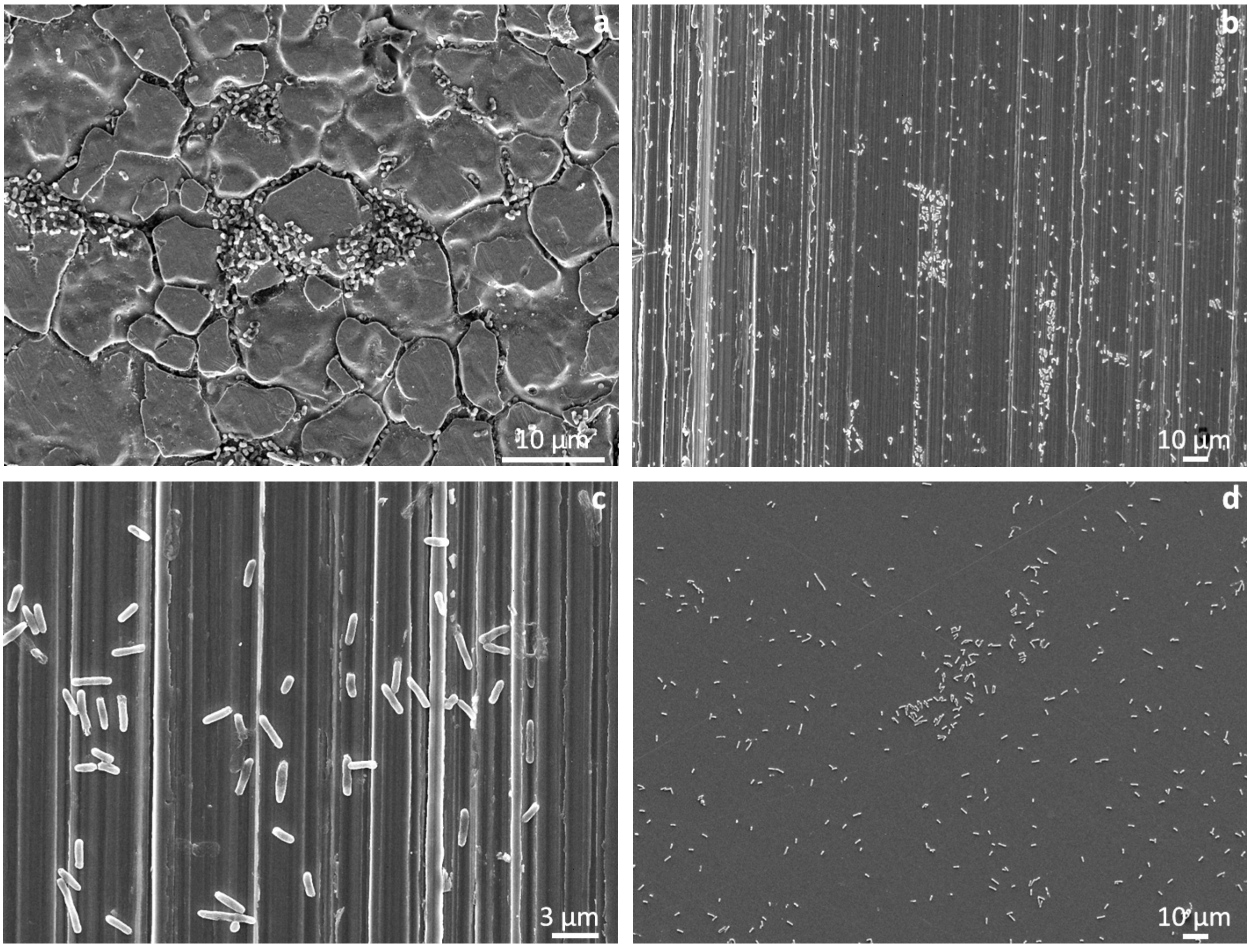

Figure 2: SE images of attachment patterns of *Escherichia coli* cells to surfaces with different surface finishes of AISI 316L stainless steel: a) 2B surface finish, b) 2B surface finish grinded with 100 SiC grit paper, c) 2B surface finish grinded with 800 SiC grit paper and d) 2B surface finish polished with diamond paste 3 µm and 1 µm to mirror finish. On 2B surface finish (a) microorganisms attach to the crevices between oxide grain boundaries, whereas on mechanically polished surface finishes (b, c) bacteria align often along longitudinal scratches (when comparable to the size of the bacteria). On the other hand, mirror finish (d) exhibited a less pronounced topography and microorganisms were observed to be distributed across the surfaces more randomly.[47]

Slika 2: SE-slike razporeditve celic bakterije *Escherichia coli* na različno obdelanih površinah nerjavnega AISI jekla 316L: a) 2B površina, b) 2B površina, brušena z granulacijo papirja 100 SiC, c) 2B površina, brušena z granulacijo papirja 800 SiC in d) 2B površina, polirana z diamantno pasto 3 µm in 1 µm. Na površini 2B (a) se bakterije pritrjujejo v razpoke med oksidnimi zrni na površini, medtem ko se na mehansko brušenih vzorcih (b, c) bakterije pogosto orientirajo vzdolž prask (kadar so primerljivih velikosti z bakterijami). Po drugi strani poliran vzorec nima izrazite topografije in razporeditev bakterij na površini je naključna.[47]

the attachment was highly affected by the surface topography.[32] Linear surface features will be more easily cleaned along rather than across the features and presumably also more easily than surfaces with random linear features across the surface. Furthermore, an increase in the surface roughness may cause the entrapment of microorganisms within the surface features and reduce the cleanability; however, if the surface features are significantly larger than the microbial cells, then they are relatively easily removed from the surface.[14] Therefore, it is important to visualise the surface features as well as measure the roughness parameters as the wear of food contact surfaces can affect the topography without any observable change in roughness.[45]

3.1.2 Effect of surface chemistry, hydrophobicity and energy

Stainless steels, produced in various grades and finishes, also vary in surface properties like chemistry, topography, roughness and surface energy.[14,15] Stainless steel forms an invisible oxide film (passivation) on the surface composed of chromium and iron oxides that protects the steel from corrosion.[14,15,45] The composition of the oxide film depends on the metal substrate, the surface finish and the surrounding medium.[15] When scratched from surface the oxide layer forms within seconds and due to the speed of re-passivation it is difficult to determine the exact chemical composition of the surface.[45] The passive film on a stainless steel is not static but it changes (grows, dissolves and may adsorb or incorporate anions) according to the environment.[56]

From a physico-chemical standpoint, the energy characteristics of stainless steel depend on the surface finish and on the cleaning process used[15] and a high- or low-energy surface can be obtained depending on the cleaning treatment.[15,28]

Surface energy is inversely proportional to the thickness of the contaminating carbon layer that is not eliminated by cleaning. The cleaning also affects the surface charge of the steel. For a given surface finish, and with a pH above the isoelectric point, a more or less negatively charged surface can be obtained.[15] In the food industry, the electrostatic interactions are repulsive because stainless-steel surfaces are generally negatively charged at neutral or alkaline pH and microorganisms are also negatively charged at these pH values in low-concentration aqueous solutions. In weakly charged liquids such as water, repulsive electrostatic interactions are significant, whereas in high electrolyte concentrations (milk, wine) the effect of surface charge is obscured.[15] Metals compared to polymers have a high surface energy, they are mainly hydrophilic, frequently negatively charged[6,15,17] and when exposed adsorb dissolved solutes or atmospheric contaminants, thus being rarely clean.[17] On the other hand, metal oxides provide positively-charged surfaces that can significantly increase the adhesion of negatively-charged bacteria to surfaces, primarily due to their positive charge and hydrophobicity.[40]

It is thought that hydrophobic materials are more susceptible to bacterial adhesion in contrast to hydrophilic.[6,7] The adhesion of vegetative cells, bacterial spores and freshwater bacteria has been shown to increase with increasing surface hydrophobicity. The cell attachment to hydrophobic plastic occurs very quickly compared to hydrophilic surfaces (metals oxides, metal and glass) where longer exposure times are needed.[43] Marine *Pseudomonas* sp. attach in large numbers to hydrophobic plastics with little or no surface charge, moderate to hydrophilic metals with a positive or neutral surface charge and few to hydrophilic, negatively charged materials such as glass and oxidized plastics.[6] Teixeira et al.[57] reported that hydrophobic and hydrophilic bacteria attach in greater numbers to relatively hydrophobic surfaces with a low surface energy like AISI 316 and AISI 304 stainless steel compared to polymethylmethacrylate (PMMA) and glass which are more hydrophilic. Sinde and Carballo[58] studied the adhesion of *Salmonella* spp. and *Listeria monocytogens* strains to AISI 304 stainless steel, rubber and polytetrafluorethylene (PTFE). The attachment results showed that in general *Salmonella* and *Listeria monocytogens* strains adhered in greater numbers to more hydrophobic material (rubber and PTFE), with stainless steel being the least hydrophobic.[58] Boulangé-Petermann et al.[28] studied the wettability of AISI 304 stainless steel with 2B and 2RB surface finishes with respect to the cleaning process. The cleaning process affected the wettability of a solid stainless steel surface; however, the results obtained regarding bacterial adhesion showed no direct correlation between the wettability or surface energy and the adhesion of *Streptococcus thermophiles*.[28] Flint et al.[26] studied the adhesion of thermoresistant streptococci (*Streptococcus thermophilus* and *Streptococcus waiu*) to different substrates (stainless steel, aluminium, zinc, cooper and glass) and different grades of stainless steel (AISI 304L and AISI 316L). The influence of substrate hydrophobicity, charge and a thin oxide film on stainless steel surfaces was also investigated with respect to the adhesion of thermo-resistant streptococci.[26] The results showed that bacteria preferentially attach to stainless steel and zinc compared to copper, aluminium and glass. Bacteria adhere in higher numbers to AISI 316L stainless steel with a 2B surface finish compared to AISI 304L stainless steel with the same surface finish, indicating the role of the chemical composition on the adhesion.[26] On the other hand, Percival et al.[59,60] reported a greater number of adhering viable cells on AISI 304 stainless steel compared to grade AISI 316 in a water piping system over a period of a few months. Flint et al.[26] also observed that negatively charged surfaces attracted more bacteria than positively charged surfaces and the unpassivated stainless-steel surface (without oxide layer) reduced the adhesion of thermo-resistant streptococci, thus suggesting that a stainless-steel surface oxide film enhances adhesion. However, when stainless steel is exposed to air repassivation occurs and the ability to attract bacteria is restored.[26]

The influence of surface energy on adhesion has been studied extensively and the surface energy of the material is an important factor influencing adhesion. The Baier curve demonstrates the relationship between the relative adhesion of organisms and the energy of the surface where minimal adhesion occurs between 20–30 mN/m.[61] The surface energy of the substrate also depends on the conditioning layer (defined by the surrounding environment) and surface structure with surface irregularities. In an aqueous environment a conditioning film forms immediately after exposure of the surface and changes the substrate properties and affects microbial retention.[43]

More comprehensive investigations on different stainless steels with well-known properties (roughness, topography and surface chemistry) are necessary in order to determine the effect of surface chemistry on bacterial adhesion. However, surface physico-chemical properties are interrelated, therefore, it is difficult to draw the conclusions of the effects on adhesion due solely to one of them.[15]

4 CONCLUSIONS

Bacterial adhesion is governed by properties of the material surface, bacterial surface characteristics and the surrounding environment, therefore a comprehensive and multidisciplinary approach is necessary in order to improve the understanding of factors contributing to the adhesion and retention of bacteria to surfaces.

The adhesion of bacteria to stainless steel and retention on surfaces can enhance the corrosion of steel, present the source of contamination in the food-processing industry, cause healthcare problems in medicine and decreases the performance of equipment in other industries, thus causing economic losses. Therefore, it is important to control and reduce the adhesion process.

Stainless-steel surface properties including roughness, topography, chemistry, surface energy and hydrophobicity affect the adhesion of bacteria. These factors are interdependent.

The surface topography and roughness play a crucial role, especially when they are comparable to the size of the bacteria and can promote the adhesion and retention while reducing the cleanability of the surface. On the other hand, hydrophobicity and surface energy also play an important role in the adhesion process as hydrophobic surfaces are more susceptible to adhesion in comparison to hydrophilic ones and a low surface energy is better than a high surface energy.

The physico-chemical properties of the substrate are important in initial cell adhesion; however, once a biofilm is formed the effect of surface properties on adhesion diminishes, but the effect on retention and cleanability is still observable.

In order to reduce or manage the adhesion to stainless-steel surfaces in food processing, medical application and other industries, knowledge of factors that govern bacterial adhesion is necessary for each material being used. It is important to take into account the grade of the steel, the surface finish applied, the surface roughness, the cleaning procedures used and the age of the steel.

A surface-modification approach should concentrate on a reduction of the initial bacterial adhesion process and, on the other hand, cleaning protocols used should be improved, to increase the removal of bacteria. With wear these protocols should be adjusted (intensified).

Stainless steel is hard, inert, hygienic and has good wear resistance compared to plastic and ceramics, and when using smooth surfaces with effective cleaning and disinfection procedures this is the best approach to reducing adhesion in food processing, medicine and industry.

However, we also have to take into account the properties of different bacteria and surrounding environment where stainless steel is exposed. Therefore, the adhesion of a particular group of bacteria that are expected to contaminate the surfaces should be tested.

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5 REFERENCES

[1] T. R. Garrett, M. Bhakoo, Z. B. Zhang, Progress in Natural Science, 18 (**2008**), 1049–1056

[2] Y. H. An, R. B. Dickinson, R. J. Doyle, Mechanisms of Bacterial Adhesion and Pathogenesis of Implant and Tissue Infections, In: Y. H. An, R. J. Friedman (Eds.), Handbook of Bacterial Adhesion, Humana Press, New Jersey 2000, 1–27

[3] C. G. Kumar, S. K. Anand, International Journal of Food Microbiology, 42 (**1998**), 9–27

[4] B. Gottenbos, H. C. van der Mei, H. J. Busscher, Models for studying initial adhesion and surface growth in biofilm formation on surfaces, In: J. D. Ron (Ed.), Methods in Enzymology, Academic Press, Massachusetts, 1999, 523–534

[5] S. L. Percival, S. Malic, H. Cruz, D. W. Williams, Introduction to Biofilms, In: S. Percival, D. Knottenbelt, C. Cochrane (Eds.), Biofilms and Veterinary Medicine, Springer-Verlag, Berlin Heidelberg 2011, 41–68

[6] K. Merritt, Y. H. An, Factors Influencing Bacterial Adhesion, In: Y. H. An, R. Friedman (Eds.), Handbook of Bacterial Adhesion, Humana Press, New Jersey 2000, 53–72

[7] Y. H. An, R. J. Friedman, Journal of Biomedical Materials Research, 43 (**1998**), 338–348

[8] J. W. Arnold, G. W. Bailey, Poultry Science, 79 (**2000**), 1839–1845

[9] S. D. Washko, G. Aggen, Wrought stainless steel, In: ASM International Handbook Committee (Eds.), Properties and selection: Irons, Steels, and High-Performance Alloys, ASM International, Ohio 1990, 2004–2195

[10] K. Hori, S. Matsumoto, Biochemical Engineering Journal, 48 (**2010**), 424–434

[11] Z. Lewandowski, H. Beyenal, Mechanisms of Microbially Influenced Corrosion, In: H. C. Flemming, P. S. Murthy, R. Venkatesan, K. Cooksey (Eds.), Marine and Industrial Biofouling, Springer-Verlag, Berlin Heidelberg, 2009, 35–64

[12] J. P. Marechal, C. Hellio, International Journal of Molecular Sciences, 10 (**2009**), 4623–4637

[13] C. Jullien, T. Benezech, B. Carpentier, V. Lebret, C. Faille, Journal of Food Engineering, 56 (**2003**), 77–87

[14] J. Verran, Testing surface cleanability in food processing, In: H. L. M. Lelieveld, A. Mostert, J. T. Holah (Eds.), Handbook of Hygiene Control in the Food Industry, CRC press, New York, 2005, 556–571

[15] L. Boulangé-Petermann, Biofouling, 10 (**1996**), 275–300

[16] O. Santos, T. Nylander, R. Rosmaninho, G. Rizzo, S. Yiantsios, N. Andritsos, A. Karabelas, H. Müller-Steinhagen, L. Melo, L. Boulangé-Petermann, C. Gabet, A. Braem, C. Tragardh, M. Paulsson, Journal of Food Engineering, 64 (**2004**), 63–79

[17] M. Fletcher, Methods for Studying Adhesion and Attachment to Surfaces, In: R. Grigorova, J. R. Norris (Eds.), Methods in Microbiology, Academic Press, Massachusetts 1990, 251–283

[18] L. Rivas, N. Fegan, G. A. Dykes, International Journal of Food Microbiology, 115 (**2007**), 89–94

[19] M. P. Ortega, T. Hagiwara, H. Watanabe, T. Sakiyama, Food Control, 21 (**2010**), 573–578

[20] R. M. Goulter-Thorsen, E. Taran, I. R. Gentle, K. S. Gobius, G. A. Dykes, Journal of Food Protection, 74 (**2011**), 1359–1363

[21] L. M. Barnes, M. F. Lo, M. R. Adams, A. H. L. Chamberlain, Applied and Environmental Microbiology, 65 (**1999**), 4543–4548

[22] J. H. Ryu, L. R. Beuchat, Applied and Environmental Microbiology, 71 (**2005**), 247–254

[23] K. A. Whitehead, J. Verran, International Biodeterioration & Biodegradation, 60 (**2007**), 74–80

[24] E. Vanhaecke, J. P. Remon, M. Moors, F. Raes, D. Derudder, A. Vanpeteghem, Applied and Environmental Microbiology, 56 (**1990**), 788–795

[25] C. Jullien, T. Benezech, C. Le Gentil, L. Boulangé-Petermann, P. E. Dubois, J. P. Tissier, M. Traisnel, C. Faille, Biofouling, 24 (**2008**), 163–172

[26] S. H. Flint, J. D. Brooks, P. J. Bremer, Journal of Food Engineering, 43 (**2000**), 235–242

[27] L. Boulangé-Petermann, J. Rault, M. N. Bellon-Fontaine, Biofouling, 11 (**1997**), 201–216

[28] L. Boulangé-Petermann, B. Baroux, M. N. Bellon-Fontaine, Journal of Adhesion Science and Technology, 7 (**1993**), 221–230

[29] M. P. Ortega, T. Hagiwara, H. Watanabe, T. Sakiyama, Japan Journal of Food Engineering, 9 (**2008**), 251–259

[30] J. W. Arnold, D. H. Boothe, G. W. Bailey, Transactions of the Asae, 44 (**2001**), 347–356

[31] A. Allion, J. P. Baron, L. Boulangé-Petermann, Biofouling, 22 (**2006**), 269–278

[32] J. Verran, D. L. Rowe, R. D. Boyd, Journal of Food Protection, 64 (**2001**), 1183–1187

[33] W. C. Chiang, I. S. Tseng, P. Moller, L. R. Hilbert, T. Tolker-Nielsen, J. K. Wu, Materials Chemistry and Physics, 119 (**2010**), 123–130

[34] J. A. Barish, J. M. Goddard, Food and Bioproducts Processing, 91 (**2013**), 352–361

[35] M. Hjelm, L. R. Hilbert, P. Møller, L. Gram, Journal of Applied Microbiology, 92 (**2002**), 903–911

[36] G. G. Geesey, R. J. Gillis, R. Avci, D. Daly, M. Hamilton, P. Shope, G. Harkin, Corrosion Science, 38 (**1996**), 73–95

[37] C. Faille, J. M. Membre, J. P. Ussier, M. N. Bellon-Fontaine, B. Carpentier, M. A. Laroche, T. Benezech, Biofouling, 15 (**2000**), 261–274

[38] R. J. Crawford, H. K. Webb, V. K. Truong, J. Hasan, E. P. Ivanova, Advances in Colloid and Interface Science, 179 (**2012**), 142–149

[39] T. F. Moriarty, A. H. C. Poulsson, E. T. J. Rochford, R. G. Richards, Bacterial Adhesion and Biomaterial Surfaces, In: P. Ducheyne (Ed.), Comprehensive Biomaterials, Elsevier Ltd., Oxford 2011, 75–100

[40] F. M. Al-Abbas, J. R. Spear, A. Kakpovbia, N. M. Balhareth, D. L. Olson, B. Mishra, The Journal of Pipeline Engineering, 11 (**2012**), 63–72

[41] J. Palmer, S. Flint, J. Brooks, Journal of Industrial Microbiology & Biotechnology, 34 (**2007**), 577–588

[42] F. Riedewald, PDA Journal of Pharmaceutical Science and Technology, 60 (**2006**), 164–171

[43] K. A. Whitehead, J. Verran, The Effect of Substratum Properties on the Survival of Attached Microorganisms on Inert Surfaces, In: H. C. Flemming, P. S. Murthy, R. Venkatesan, K. Cooksey (Eds.), Marine and Industrial Biofouling, Springer-Verlag, Berlin Heidelberg 2009, 13–33

[44] J. W. Costerton, International Journal of Antimicrobial Agents, 11 (**1999**), 217–221

[45] J. Verran, P. Airey, A. Packer, K. A. Whitehead, Microbial Retention on Open Food Contact Surfaces and Implications for Food Contamination, In: A. I. Laskin, S. Sariaslani, G. M. Gadd (Eds.), Advances in Applied Microbiology, Academic Press, Massachusetts 2008, 223–246

[46] J. T. Holah, R. H. Thorpe, Journal of Applied Bacteriology, 69 (**1990**), 599–608

[47] M. Hočevar, M. Godec, M. Jenko, D. Drobne, N. Gunde-Cimerman, S. Novak, The adhesion of bacteria to austenitic stainless steel with different surface roughness, Proc. of the MC 2013: Microscopy conference, Regensburg, Germany, 2013, 35–36

[48] K. A. Whitehead, J. Verran, Food and Bioproducts Processing, 84 (**2006**), 253–259

[49] J. A. Disegi, L. Eschbach, Injury, 31 (**2000**), S2–S6

[50] L. R. Hilbert, D. Bagge-Ravn, J. Kold, L. Gram, International Biodeterioration & Biodegradation, 52 (**2003**), 175–185

[51] E. Medilanski, K. Kaufmann, L. Y. Wick, O. Wanner, H. Harms, Biofouling, 18 (**2002**), 193–203

[52] J. Verran, R. D. Boyd, Biofouling, 17 (**2001**), 59–71

[53] K. A. Whitehead, J. Colligon, J. Verran, Colloids and Surfaces B-Biointerfaces, 41 (**2005**), 129–138

[54] K. A. Whitehead, D. Rogers, J. Colligon, C. Wright, J. Verran, Colloids and Surfaces B-Biointerfaces, 51 (**2006**), 44–53

[55] J. Verran, A. Packer, P. Kelly, K. A. Whitehead, Letters in Applied Microbiology, 50 (**2010**), 258–263

[56] C. O. A. Olsson, D. Landolt, Electrochimica Acta, 48 (**2003**), 1093–1104

[57] P. Teixeira, Z. Lopes, J. Azeredo, R. Oliveira, M. J. Vieira, Food Microbiology, 22 (**2005**), 247–251

[58] E. Sinde, J. Carballo, Food Microbiology, 17 (**2000**), 439–447

[59] S. L. Percival, J. S. Knapp, R. G. J. Edyvean, D. S. Wales, Water Research, 32 (**1998**), 2187–2201

[60] S. L. Percival, J. S. Knapp, R. G. J. Edyvean, D. S. Wales, Water Research, 32 (**1998**), 243–253

[61] T. Vladkova, Surface Modification Approach to Control Biofouling, In: H. C. Flemming, P. S. Murthy, R. Venkatesan, K. Cooksey (Eds.), Marine and Industrial Biofouling, Springer-Verlag, Berlin Heidelberg 2009, 135–163