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# Metal surface characteristics dictate bacterial adhesion capacity

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ABSTRACT

Bacterial adhesion can be dictated by different surface characteristics. In this study we concentrate on the surface roughness of a stainless steel material. We prepared the stainless steel surfaces by 3D polishing, brushing, grinding and electropolishing. Untreated stainless steel surfaces were also considered. The corresponding surface roughness was assessed by profilometry and atomic force microscopy. In experiments we have used different types of bacteria. The rate of adhered bacteria on metal surfaces was determined spectrophotometrically. The results showed that the rate of adhered bacteria increases with increasing surface roughness. Scanning electron microscopy was used to image surfaces in order to determine locations of adhered bacteria. The increased adhesion of bacteria on more rough surfaces results from an increased interplay between the increasing effective surface area and increasing numbers of cracks, voids and gaps.



## 1. Introduction

Microbial contamination of different contact surfaces which has implications for human health and the environment creates major problems in different technologies like food, pharmacy and various service industries. By understanding the relationship between surface conditions and microbial adhesion, strategies can be developed to inhibit the attachment of bacteria and spores [1–5].

Bacterial adhesion to a material surface is a complicated process that is affected by various physico-chemical properties of the bacteria cell and substratum surface [6]. The physicochemical properties of surfaces are governed by factors such as environment (temperature and pH), surface characteristics such as hydrophobicity and charge and, in the case of a micro-organism hydrophobicity, flagellation and motility [1]. The basic stages of bacterial adhesion are generally described by a two-stage kinetic binding model. In a first stage there is an initial, rapid and easily reversible interaction between the bacteria cell surface and the material surface. The adhesion of bacteria within an interface is mainly governed by electrostatic, van der Waals, and hydrophobic effects and contact interactions [7,8]. Generally, the interaction free energy of the adhesion process shows two minima. The first minimum appears at a separation of 10 nm and is few $kT$ (thermal energy, $T$ is the absolute temperature and $k$ is the Boltzmann constant). In this minimum the microorganism is weakly and reversibly bound. The second stage includes specific and non-specific interactions between so-called adhesion proteins expressed on bacterial surface structures (fimbriae or pili) and binding molecules on the material surfaces. The second minimum in the interaction free energy appears at a contact distances of 1 nm. Here the microorganism is strongly and irreversibly adhered. The microorganism has to surpass a large energy barrier of a few $kT$ to overcome from the first into the second minimum at the contact point. The characterization of the surface topography of the material on a submicrometer scale can be obtained with atomic force microscopy [9].

In general, bacterial adhesion is a complicated process influenced by many factors. Boulané-Petermann [10] represents two physico-chemical theories that can be applied to predict simple cases of bacterial adhesion. However, these models are limited in their applicability owing to the complexity of bacterial surfaces and the surrounding medium. Various factors that can affect the bacterial adhesion process have been listed, all directly linked to the solid substratum, the suspension liquid or the microorganism (e.g. for stainless steel surfaces, it is important to take into account

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the grade of steel, the type of finish, surface roughness, the cleaning procedures used and the age of the steel). Research on biofilms growing on stainless steel has confirmed results obtained with other materials, regarding resistance to disinfectants, the role of the extracellular matrix and the process by which the biofilm forms. However, it appears that the bactericidal activity of disinfectants on biofilms differs according to the type of surface on which they are growing.

Stainless steels are widely used in food and beverage manufacturing and processing industries for manufacture, bulk storage and transportation, preparation and presentation applications. Numerous studies have shown that *Listeria monocytogenes* is capable of adhering and forming biofilm on food contact surfaces [11] such as polystyrene, glass and stainless steel [12]. If the grade of stainless steel is correctly specified for the application, corrosion should not be encountered. Surface finish and condition is very important to the successful application of stainless steels. Smooth surfaces not only promote good cleanability but also reduce the risk of corrosion. The types of corrosion to which stainless steels can be susceptible can be useful in identifying problems due to wrong grade selection or inappropriate use of equipment [13]. Ismail et al. [14] described that the presence of microorganisms on a metal surface often leads to highly localized changes in the concentration of the electrolyte constituents, pH and oxygen levels. Dexter et al. [15] demonstrated that microorganisms often stimulate localized forms of corrosion, such as pitting, depending on the passive film forming and repairing capabilities of the metal or alloy.

Atomic force microscopy (AFM) [9] can be used to characterize the surface topography of a material on a submicrometer scale. A big advantage of AFM is the possibility of imaging surfaces with high resolution and the quantitative evaluation of selected surface features, including statistical analysis, which permits the roughness parameters to be determined. From contact angle measurements the surface hydrophobicity can be determined.

A fundamental aspect of the study of bacterial adhesion and attachment to surfaces is the need for reliable quantification of the microbiological population that attaches to the material surface. In the past, few methods for bacteria counting have been introduced including direct counting methods, such as scanning electron microscopy (SEM), and indirect counting methods such as colony forming units (CFU) plate count and staining methods [44,45].

In this study we consider bacterial adhesion on five different stainless steel surfaces. Besides the untreated surface (3C) we use 3D polished, brushed, ground and electropolished surfaces. The objective of this study is to investigate the influence of surface roughness on the degree of bacterial adhesion. Regarding the finishing process of the surface, roughness of the surface varies. The roughness lies in the region of few tens of nanometers to submicrometer. The hydrophobicity is kept as constant as possible. The roughness of surfaces is determined by profilometery and AFM. The bacterial adhesion rate is measured using crystal violet staining method.

## 2. Methods

### 2.1. Bacteria and growth conditions

Strains used in this study were selected from culture collection of Laboratory for food microbiology at Dept. of Food Science, Biotechnical Faculty (designation ŽM and ŽMJ), namely *Bacillus cereus* ŽMJ 3 (apple vinegar), *L. monocytogenes* ŽM 520 (Danish Meat Research Institute, Roskilde, Denmark DMRICC 3633), *Staphylococcus aureus* ŽMJ 72 (type strain ATCC2 5923), *Staph. aureus* ŽM 518 (ATTC 24213) as Gram-positive bacteria, and *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ŽMJ 87 (laboratory strain), *P. aeruginosa* ŽM 517 (ATTC 15442), *Salmonella enterica* Typhimurium ŽM 375 (ATTC 14028), as Gram-negative bacteria.

The bacteria were preserved as frozen stock at −80 °C in Tryptone Soya Broth, Oxoid CM0129, Hampshire, England (TSB) with 15% glycerol. Prior to all experiments, cultures were transferred into TSB (4 mL) and incubated overnight at 37 °C with shaking (75 $min^{-1}$). Cultures were then transferred to Tryptone Soya Agar, Oxoid CM0131 (TSA) plates, and incubated for 24 h at 37 °C. These plates were kept for further experiments at 4 °C. Prior to each experiment, 1 colony from TSA was transferred to TSB (4 mL) and incubated overnight at 37 °C with shaking (75 $min^{-1}$), except for *L. monocytogenes* and *B. cereus* strains that were incubated at 25 °C.

### 2.2. Metal surfaces

In this study stainless steel AISI 304 was used. This stainless steel material contains 18% chromium, 8% nickel, 2% manganese and the remainder is iron. The untreated surface of stainless steel is marked by C. Different surface treatments were used. 3D polishing of stainless steel (here denoted by 3D polished material) is a process based on laser polishing by remelting. Grinding is an abrasive machining process in which a spinning wheel covered in rough particles cuts chips of material from metallic substances. Electropolishing is an electrolytic polishing technique in which the metal (anode) on the surface is oxidized and dissolved in the electrolyte.

Brushing is polishing with a grit belt and then softening with a grit greaseless compound. A pattern of very fine lines parallel to the brushing direction is obtained. In this way we achieved five different surfaces with different levels of roughness. 1 mm thick pieces having dimensions of 1 cm × 1 cm were cut from larger pieces of stainless steel material for surface and microstructural investigations. For bacterial adhesion measurements, disks with a diameter of 3 mm as well as 2 cm × 2 cm stainless steel pieces were cut and the edges of the pieces were polished in order to reduce the error of the measurements by minimizing the adhesion of microbes on the side surfaces.

### 2.3. Surface roughness (AFM and profilometery)

Atomic force microscopy was used for the characterization of the surface topography of materials on a submicrometer scale. A big advantage of this method is the possibility of imaging surfaces with high resolution and the quantitative evaluation of selected surface features, including statistical analysis, which permits the roughness parameters to be determined. In the present study VEECO Dimension 3100 AFM system was used and measurements were made in contact mode. With high roughness levels AFM was not able to efficiently follow the surface, so a mechanical profilometer Form Talysurf Series 2 from Taylor-Hobson Ltd., Leicester, Great Britain was used.

### 2.4. Contact angle

Surface hydrophobicity was determined by contact angle measurement. This angle depends on surface tensions between three components of the system: $\gamma_{sl}$(solid – liquid), $\gamma_{lg}$(liquid – gas) and $\gamma_{sg}$(solid – gas). At equilibrium conditions we can calculate the contact angle ($\theta_c$) from Young's equation

$$\gamma_{sg} - \gamma_{sl} - \gamma_{lg} \cos \theta_c = 0. \quad (1)$$

Young's equation assumes a perfectly flat and rigid surface. Wenzel's model takes into account the roughness of the surface and describes the apparent contact angle on a rough surface