

MODIFICATIONS IN HYGIENIC PROPERTIES OF STAINLESS STEEL SURFACES DUE TO REPEATED SOILING AND CLEANING

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ABSTRACT

Regular and effective cleaning of food contact surfaces is a basic requirement to guarantee product safety in food processing industry. Cleaning in place (CIP) investigations are often focused on the influence of surface properties. Many studies used new surfaces. In contrast it has been reported that new surfaces are rapidly conditioned by the process environment.

In this paper the influence of repeated soiling and cleaning on hygienic properties of surfaces has been investigated. For this purpose two conditioning procedures have been chosen to mimic industrial applications. The first conditioning procedure has been designed to study possible influences due to the cleaning fluids NaOH and HNO₃. The second conditioning procedure has been designed to study possible influences due to soiling and cleaning. Gelatinised starch has been chosen as cohesive test soil. It has been found that repeated soiling and cleaning may affect wetting properties, surface charges and surface chemical composition of stainless steel. The Fe/Cr ratio of the passive layer has been changed after repeated cleaning with NaOH and HNO₃. After repeated soiling/cleaning organic surface contaminations have been determined. However, constant values of the contact angle have been found after 3 soiling and cleaning runs. The cleaning rate has been slightly affected by the treatment.

INTRODUCTION

Food producers have the legal responsibility to ensure product safety (Regulation of European commission 178/2002). To guarantee high food quality regular and effective cleaning of food processing machines is essential. Extensive work has been done to understand the parameters dictating the cleaning processes (Jensen 2003; Fryer and Asteriadou 2009; Gordon, Brooker et al. 2010). Cleaning in place investigations are often focused on the influence of surface properties. Most of the studies have used new surfaces (Liu 2006; Bobe, Hofmann et. al 2007; Detry, Sindic et al. 2011). The procedures used for assessing the cleanability are based on a single soiling step followed by one cleaning step. Apart from the test soil this procedure takes into account possible surface contaminations which

may come from processing steps or may be absorbed from the surrounding atmosphere (Landoulsi, Genet et al. 2008)

In contrast it has been reported that new surfaces are rapidly conditioned by contact with aqueous media and food products (Mettler and Carpentier 1998; Verran 2006; Caillou, Gerin et al. 2008; Landoulsi, Genet et al. 2008). The aim of industrial cleaning processes is to restore the initial clean status. According to Graßhoff (1992) and Verran (2006) surfaces hardly regain their initial clean status. The accumulation of organic soil on surfaces may mask the actual surface and affect interfacial interactions between soil and machine surface (Marshall 1996; Storgards, Simola et al. 1999). This organic layer is usually called conditioning film. But also common cleaning fluids (i.e. NaOH and HNO₃) may have an influence on surface properties as well as detergents and disinfectants (Boulangé-Petermann 1993; Santos, Nylander et al. 2004; Landoulsi, Genet et al. 2008)

It is also reported that the surface conditioning affects the hygienic status of surfaces (Flint, Palmer et al. 2001; Parkar, Flint et al. 2001; Verran 2006; Herrera, Cabo et al. 2007). The impact of conditioning layers on microorganism attachment and resistance of microorganisms to cleaning procedures is not fully understood. Studies prior to this work have demonstrated that repeated fouling and cleaning can alter the cleanability of surfaces (Verran 2006). Leclercq-Perlat (1994) observed that surfaces cleaned with HNO₃ and NaOH were more difficult to clean than reference samples. Leclercq-Perlat (1994) also stated that cleaning procedures can change the chemical composition of the surfaces which directly influences cleanability. Jullien (2008) found an increased number of adherent spores after successive runs of soiling and cleaning.

Boulangé-Petermann (2004) and Graßhoff (1992) suggested that cleaning investigations should take account of the presence of conditioning layers. Therefore cleaning investigations should include tests after repeated fouling and cleaning.

The objective of this work is to study possible consequences of repeated soiling and cleaning on hygienic properties of stainless steel. For this purpose conditioning procedures are chosen to mimic industrial application conditions. In this work the key parameter that determines

the hygienic property of a surface is the main cleaning rate derived from the cleaning time necessary to remove all test soil. The possible influences on physico-chemical properties (surface topography, surface free energy, zeta-potential and surface chemical composition) are monitored.

MATERIALS AND METHODS

Material

From a range of surface modifications used in a previous study (Mauermann, Eschenhagen et al. 2009) stainless steel (SS) AISI 316 samples with a pickling (2B) finish were selected. After electropolishing the samples were provided in the dimensions of 20mm x 40mm x 1 mm.

Prior to the first use the samples were subjected to the following cleaning procedure: i) 5 min rinsing with distilled water, ii) 5 min cleaning by immersion in 0,5% NaOH, iii) 5 min cleaning by immersion in ethanol solution and 5 min rinsing with distilled water. Samples were then dried and protected from environment in a sample blister. Without a further treatment (conditioning) these samples are denoted as “new samples”.

Model food soil

The model food soil consists of gelatinised potato starch and the fluorescent tracer fluorescein. Dispersions of potato starch (17.5 g, Cargill Deutschland GmbH, Krefeld) in distilled water (250 ml, 10^{-3} mol/l KCl) were prepared in a glass container to yield a 7 % starch suspension. The initial temperature of the dispersion was 50 °C. By means of a thermostat the dispersion was heated up in order to gelatinise the starch. After cooking and stirring for 30 min at 90 °C the starch solution was cooled down for 30 min. For better visual evaluation of the starch during cleaning 0.012 % Fluorescein AP (C.I. 45350) were added. Exposed to UV-VIS light fluorescein emits a green fluorescence shine. Subsequently the starch solution was kept in a climate chamber at 60 °C. Microscopic analysis proved that the starch solution is fully gelatinised after this procedure.

Conditioning procedures

The aims of the conditioning procedures were to mimic a typical application in food processing machines at laboratory scale and to obtain surfaces similar to those found in food processing machines. The changes of surface properties with time should be monitored. The experiments were focused on the factors food contact, cleaning fluids and temperature. The first conditioning procedure was designed to study possible influences due to industrial cleaning runs with NaOH and HNO₃. A second conditioning procedure was designed to study possible influences due to soiling and cleaning runs.

Conditioning procedure by cleaning steps

Fig. 1 shows the design for the study with repeated cleaning runs. In order to get a reference prior to the first cleaning run the surface roughness, contact angle, zeta-potential and surface chemical composition were determined. Samples were first placed in glass containers with 2.5 % (w/w) NaOH for 45 min at 75°C followed by an immersion in 1% (w/w) HNO₃ for 45 min at 75°C. The

surface roughness and the contact angle were measured at the end of each cleaning run. Before each measurement the surfaces were rinsed carefully with reverse osmosis (RO) water. Both after the immersion in NaOH and after the immersion in HNO₃ the samples were rinsed with RO-water. After the last cleaning run the zeta-potential and the chemical composition of the surface were determined in addition.

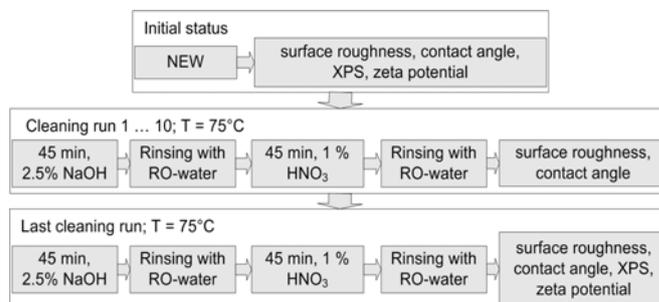


Fig. 1 Conditioning procedure - cleaning

Conditioning procedure by soiling and cleaning steps

Previous experiments had revealed (data not shown) that due to remainings of the test soil the physico-chemical properties of an optically clean stainless steel surface may change following repeated soiling and spray cleaning runs. In order to determine whether intensive industrial cleaning processes can remove the test soil from surface completely and whether the surface properties will get affected a two step cleaning process was used. A spray cleaning step was used to study the effect on cleaning rate and the second step was introduced to remove possible soil remainings.

Fig. 4 shows the design of the study with repeated soiling and cleaning. The conditioning procedure consisted of three steps i) cleaning at a mean flow velocity of 1.4 m/s with cleaning solution of 2.5 % (w/w) NaOH for 45 min and 1 % (w/w) HNO₃ for 45 min at 75 °C, ii) soiling with food model soil starch and iii) spray cleaning with 0.5 % (w/w) NaOH. Fig. 2 shows the schematic drawing of the test rig for the first cleaning step.

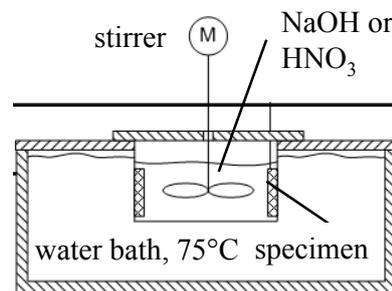


Fig. 2 Schematic drawing of the cleaning test rig

The soiling of the samples was realised by means of an apparatus that applies a defined and homogeneous starch layer (Fig. 3). Before soiling, the samples are cleaned and aligned on a precision base plate. Ductile and sticky leveling compounds are arranged on the back side of the samples. Thereafter a plate is pressed against the base plate, turned and again placed on the base plate. Limiters on the plate are assuring that the pressing is done under

reproducible conditions. By means of ductile leveling compounds the top of every coupon is in the same plane. After this procedure the samples are ready for the soiling step. For coating 2 ml of gelatinised starch is evenly spread over the samples. Subsequently the wiper is moved over by means of a linear drive and removes the excess soil. As recommended in Lang (1991) the velocity of the linear drive as adjusted to 3 cm/s. The coating gap defines the thickness of the soiling layer and can be adjusted by spacers of different height. Thus different mass of starch can be applied. Following the samples are dried for 24 h at 23 °C. Rheological measurements with the gelatinised starch have shown that the shear- and time-dependent properties of the starch do not change significantly within 3h. Within this period the coating behaviour should not be effected.

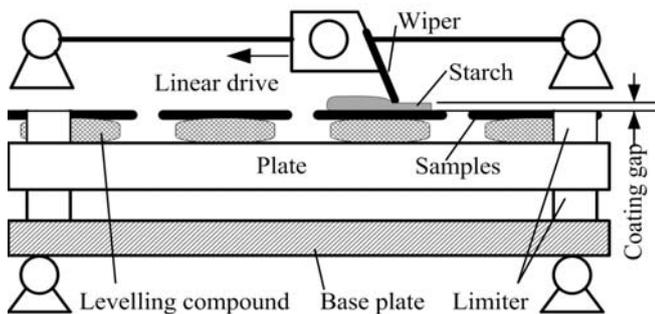


Fig. 3 Apparatus for application of starch layers

For spray cleaning a test rig was used upon which cleaning was realised by impingement of a cleaning spray. The cleaning process was optically monitored based on the fluorescence properties of the test soil. The result of this cleaning step is an optically clean surface. From the cleaning profil the main cleaning rate \bar{R} was derived (Equation 1).

$$\bar{R} = \frac{0.95 \cdot m}{x_{95}} \quad (1)$$

Here m represents the initial soil mass on the sample surface and x_{95} the cleaning time necessary to remove 95% of the initial soil load. Further information on the spray cleaning process and the determination of the cleaning characteristic is given in Mauermann, Eschenhagen et al. (2010).

After each cleaning run the surface roughness and the contact angle were measured. Before each measurement the surfaces were rinsed carefully with distilled water. After the last cleaning run the zeta-potential and the chemical composition of the surface were determined in addition.

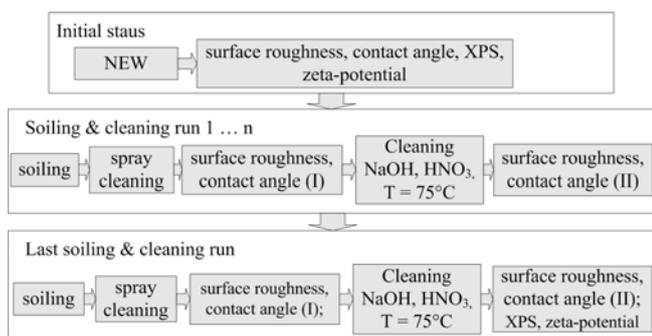


Fig. 4 Conditioning procedure soiling and cleaning

Physico-chemical surface properties

Surface roughness

For assessment of surface topography in this study non-contact high-resolution scandisk confocal microscopy (μ Surf, Nanofocus Germany) and chromatic confocal imaging (MicroGlider, FRT, Germany) were used. Further information about the surface characterisation of stainless steel is provided in Calvimontes, Bellmann et al. (2011). To consider possible anisotropic properties of the sample the 3D arithmetic means surface roughness S_a , the 3D mean roughness depth S_z , the 3D reduced peak height S_{pk} and the 3D reduced valley depth S_{vk} were assessed.

Surface charges

To determine the electrokinetic potential of the test surfaces streaming potential measurements were conducted using the electrokinetic analyser EKA by Anton Paar/Graz/A. A streaming channel was built between two samples and the measuring fluid was pumped through the channel. The streaming potential U was determined versus the loss of pressure p and the zeta-potential ζ was calculated by the modified Smoluchowski equation:

$$\zeta = \frac{dU}{dp} \frac{\eta \kappa}{\epsilon \epsilon_0} \quad (2)$$

with conductivity κ and viscosity η of the fluid.

Wetting properties

To determine the static contact angle the measuring device OCA 40 Micro (Data Physics, Germany) was used. 20 μ L deionized water drops were placed on the surface with the help of a microliter syringe and an automatic moving table. Thereafter the needle of the syringe was withdrawn from the drops and the contact angle was determined by means of the software SCA 20th. Five water droplets were applied to each sample to determine the static contact angles. In order to avoid edge effects the sessile droplet had diameters not more than 4.5 mm and distances of leastwise 5 mm to the edges. The small error bars in Fig. 5 correspond with the assumption that edge effects do not disturb the contact angle measurements.

Surface chemical composition

All surface analysis were carried out by means of an AXIS ULTRA (Kratos Analytical, Manchester, UK) X-ray photoelectron spectrometer (XPS). The spectrometer was equipped with a monochromatic Al K α ($h\nu = 1486.6$ eV) X-ray source of 300 W at 15 kV. The kinetic energy of photoelectrons was determined with a hemispherical analyser set to pass energy of 160 eV for wide-scan spectra and 20 eV for high-resolution spectra. During all measurements, electrostatic charging of the sample was avoided by means of a low-energy electron source working in combination with a magnetic immersion lens. Later, all recorded peaks were shifted by the same value that was necessary to set the C 1s peak to 285.00 eV.

Quantitative elemental compositions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function.

Spectrum background was subtracted according to Shirley (1972). The high-resolution spectra were dissected by means of the Kratos spectra deconvolution software. Free parameters of component peaks were their binding energy (BE), height, full width at half maximum and the Gaussian-Lorentzian ratio.

RESULTS

Conditioning procedure by cleaning steps

Wetting properties

After various cleaning treatments with NaOH and HNO₃ at 75 °C for 45 min stainless steel samples exhibited a lower contact angle than the reference (Fig. 5). The samples are more hydrophilic.

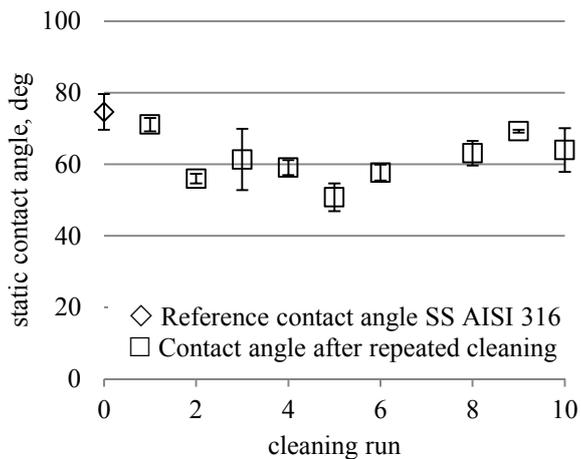


Fig. 5 Static contact angle of stainless steel conditioned by repeated cleaning with 2.5% NaOH and 1 % HNO₃

Surface chemical composition

The relative atomic concentration Fe/Cr of stainless steel was determined by XPS on new surfaces and conditioned surfaces (NaOH-HNO₃). After the cleaning runs the Fe/Cr ratio was reduced from 1.44 to 0.62. Obviously, the cleaning procedure affected the chemical composition of the stainless steel.

Surface roughness

For stainless steel no significant changes in arithmetic means surface roughness S_a were observed. Over eight cleaning runs the coefficient of variation was below 4 %. The same trend was found for the mean roughness depth S_z, reduced peak height S_{pk} and reduced valley depth S_{vk}.

Surface charges

After the cleaning procedures the isoelectric point (IEP = pH|_{ζ=0}) for stainless steel shifted by ca. one pH unit. The zeta-potential values in the region of low pH-values increased significantly (Fig. 6).

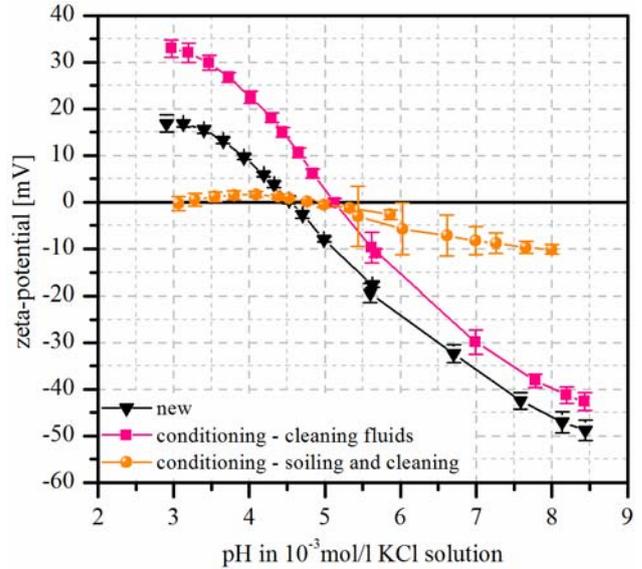


Fig. 6 Streaming potential measurements of new stainless steel, stainless steel after conditioning procedure-cleaning fluids and after conditioning procedure-soiling/cleaning

Conditioning procedure by soiling and cleaning steps

Wetting properties

The static contact angles over the number of soiling and cleaning are depicted in Fig. 7. The values indicated with “I” are results from the measurements after soiling with starch and the spray cleaning step. The values indicated with “II” are results from the measurements after soiling, spray cleaning and intensive cleaning with NaOH - HNO₃. A decrease in contact angle of stainless steel arose from the repeated treatment. The values decreased until they reach almost constant values after 3 - 4 soiling and cleaning runs. The values after spray cleaning were higher compared to the values after the cleaning step with NaOH and HNO₃. After 3-4 treatments there are almost no differences between the two values I and II.

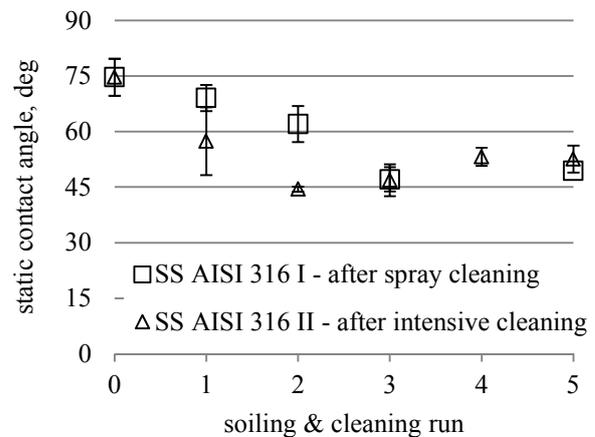


Fig. 7 Static contact angle of stainless steel conditioned by repeated soiling and cleaning. I – after soiling and spray cleaning until optical cleanliness; II – after cleaning with NaOH - HNO₃ at 75°C for 45 min

Surface roughness

A small effect of the combined treatment on the arithmetic means surface roughness S_a was observed. Over nine soiling and cleaning runs the coefficient of variation was 6.4 %. The same trend was found for the mean roughness depth S_z , the reduced peak height S_{pk} and reduced valley depth S_{vk} .

Surface charges

For stainless steel a large change of electrokinetic properties after soiling and cleaning steps was found. The zeta-potential vs. pH curve shows a very flat characteristic. If the liquid phase remains constant the electrokinetic potential of the surface will be determined by the chemical constitution and surface polarity, topography (porosity and specific surface), and swelling respectively water adsorption processes. The influence of swelling processes on the electrokinetic properties has been discussed in Bellmann, Caspari et al. (2005). Swelling processes are assumed to expand the inner surface of the solid surface layer. It is to expect that swelling layer and electrochemical double layer overlap each other. (The double layer migrates in the swelling layer.) Hence, the slipping plane of the electrochemical double layer shifted to lower electrokinetic potentials (Fig. 6). Since the steel surface itself cannot swell, remaining starch residues may cause such behaviour (Bird 1995). To prove whether starch is still present on the surface, XPS measurements were carried out.

Surface chemical composition

Fig. 8 shows the high-resolution C 1s XPS spectrum of a stainless steel sample, which was contaminated with starch and subsequently cleaned. The shape of a C 1s spectrum recorded from starch sample is very different to C 1s spectra usually observed for organic substances. Hence, the surface soiling with starch allows separating randomly adsorbed surface contaminations, such as fats or hydrocarbons, from the applied starch soil. In order to analyse the binding states of carbon the recorded C 1s spectrum (thick line) was deconvoluted into six component peaks (thin lines). The binding energies of the component peaks give information on the chemical environment of the carbon atoms. Component peak A (285.00 eV) shows saturated hydrocarbons (C_xH_y). Hydrocarbons are very typical substances contaminating high-energy surfaces, such as metals and metal oxides. Their adsorption is driven by the fundamental tendency of all surfaces to minimize their surface free energy. The second intensive component peak C (286.53 eV) arose from $C-OH$ and/or $C-O-C$ groups. Such groups are consistent of surface-contaminating fats but also of saccharide molecules, such as starch. The assumption of the presence of residual starch molecules is supported by component peak D having a binding energy of 287.43 eV. This binding energy is typical for hemiacetal groups ($O-C-O$), a further consistent of saccharide molecules. Component peak E (288 eV) shows carbonic ester groups ($O=C-O-C$). The alcohol-sided carbon atoms of the carbonic ester groups ($O=C-O-C$) contribute to component peak C. If the intensity of component peak E is subtracted from the intensity of component peak C the

residual area of component peak C is five times higher than the intensity of component peak D. This ratio corresponds to the stoichiometry of saccharide ring molecules. Hence, it can be concluded that starch molecules remained on the stainless steel surface. Component peak B appeared from carbon atoms in α -position to highly electronegative functional groups, such as carbonic esters ($C-COOC$). Agreeing with the stoichiometry of fatty acids the intensity of B equals the intensity of component peak E. The chemical shift of component peak F is about 4.23 eV. This component peak indicates the presence of inorganic carbonates (CO_3^{2-}) and/or hydrogen carbonates (HCO_3^-).

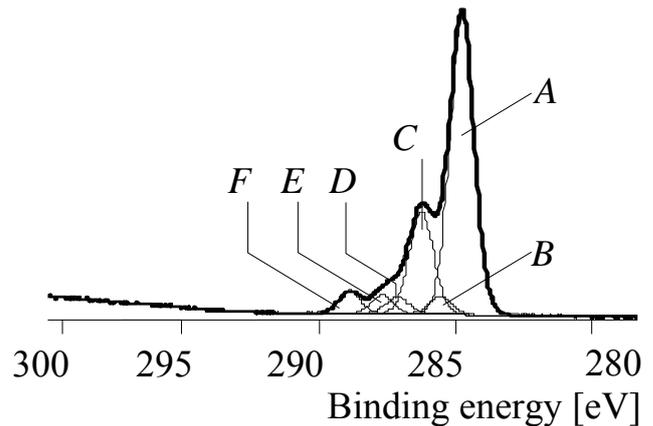


Fig. 8 High-resolution C 1s XPS spectrum recorded from the stainless steel sample after its soiling and cleaning. The assignment of the component peaks (A-F) to the different carbon bonds is discussed in the text.

Analysing the elements, which are constituents of stainless steel, such as Fe and Cr indicated that the applied starch layer is thinner than the maximum information depth of the XPS method (ca. 8 nm), or the starch layer is not fully closed.

Cleaning rate

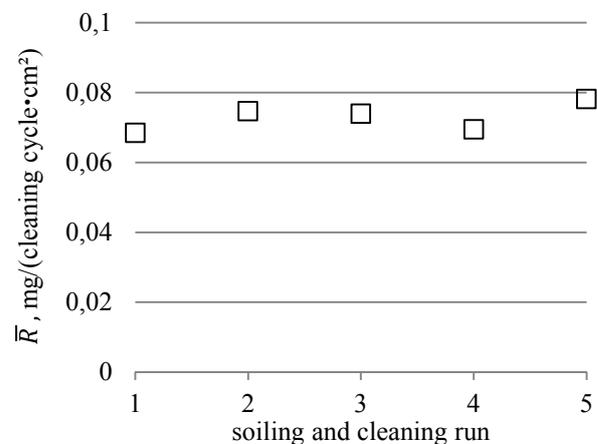


Fig. 9 Main cleaning rate of stainless steel as function of the number of soiling and cleaning runs. The higher the cleaning rate the better the cleanability.

Stainless steel was tested for its cleanability after repeated soiling and cleaning runs. The cleanability was estimated by the main cleaning rate. The main cleaning rate was calculated according to Equation 1 and the results are summarized in Fig. 9. Only a slight tendency to increasing cleaning rates was observed.

DISCUSSION

Metal and metal oxides are solids with high surface free energies (Kinloch 1990). The high surface free energy is a driving force leading to adsorption and the coverage of these surfaces with organic contaminants. Therefore XPS analysis often shows carbon as major element on the surface (Genet, Dupont-Gillain et al. 2008). In this paper in case of a new stainless steel surface the XPS data revealed a C/Fe atomic ratio of 46:6. The high carbon content could be attributed to a contamination layer that may come from material processing, pre-cleaning or even from the spectrometer itself (Landoulsi, Genet et al. 2008). The layer of organic contaminants is reducing the surface free energy (Mantel, Rabinovich et al. 1995). Hence, the water contact angle should increase compared to pristine surfaces (Caillou, Gerin et al. 2008). In the first conditioning procedure the cleaning with NaOH and HNO₃ may remove some of the organic contamination. Due to this the decreasing contact angle in Fig. 5 might be an effect of the repeated cleaning with NaOH and HNO₃.

On the other hand the chemical composition of the passive film of stainless steel surfaces is influenced by the pH of the environment (Landoulsi, Genet et al. 2008). According to Boulange-Petermann (1993) the changes in contact angle may partly be explained by the effect of HNO₃ on the passive film of stainless steel. Macdonald, Biaggio et al. (1992) have suggested a bilayer structure model for the passive film, with a Cr-rich inner layer. The outer layer consists mainly of Fe-oxides, which can be subject to dissolution by HNO₃. After 10 cleaning steps the Fe/Cr ratio of the passive layer shows a significant decrease and confirms this assumption. On the contrary Santos, Nylander et al. (2004) and Jullien (2008) attributed the change to the effects of NaOH. This may lead to chromium enrichment or the dissolving of iron present in the passive layer. Taking both effects into consideration it might be hypothesized that the water contact angle decrease in Fig. 5 is partly induced by the effect of cleaning solution on the passive layer and the removal of initial organic surface contaminants.

Immersion of new surfaces into an aqueous environment can result in a rapid adsorption of organic molecules as well (Marshall 1996; Verran 2006; Landoulsi, Genet et al. 2008). In food processing the daily use of the machines may lead to an accumulation of molecules from food products and / or cleaning fluids on the surface. The adsorption results in an alternation to the physic-chemical properties of the surface. The amount of molecules adsorbed are governed by the properties of the surface and the molecules present in the aqueous solution (Marshall 1996). In order to study possible consequences of repeated soiling and cleaning steps on hygienic properties of surfaces a second conditioning procedure was established.

After repeated soiling and cleaning the zeta-potential measurements revealed a flat curve (Fig. 6). This characteristic might be attributed to swellable surface contaminants, just as gelatinised starch that was used for soiling. Bird (1995) emphasises the swelling properties of starch by contact with fluids of different pH. During repeated soiling and cleaning a decrease of the water contact angle (Fig. 7) was found. This could be the result of two contrary processes: (i) the evolution of a starch layer on the surface and (ii) the removal of the surface contaminants. After 3-4 soiling and cleaning steps this leads to the formation of a contact angle plateau (Fig. 7) and is probably reflecting the accumulation of starch residuals on the surface. This assumption is supported by the results from the XPS measurements after the last soiling/cleaning step. The analyses are suggesting that starch molecules remained on the surface. However, from the given XPS analyses it cannot be clearly derived whether the contamination layer is completely masking the underlying substrate or just partially covering. Obviously this fact has to be considered when thinking about the impact of conditioning on the interactions between soil and underlying surface.

It was expected that the various modifications in surface properties must lead to changes in cleanability. In this study only a small effect on cleaning rate was observed. Indeed, after the conditioning procedure with repeated soiling and cleaning stainless steel showed a tendency to increasing cleaning rates. On the contrary Verran (2006), Leclercq-Perlat (1994) and Jullien (2008) reported a decreasing cleanability after repeated soiling and cleaning. But there are also reports with similar results (Parkar, Flint et al. 2001). However, it is difficult to compare the present results with those from published studies, since these studies used microorganisms for their cleaning tests. According to Liu (2006) cleaning results mainly from adhesive and / or cohesive failure of the present soil. It might be hypothesised that in alkaline environment gelatinised starch has a lower cohesive strength than adhesive strength. Corresponding results are presented in Schöler (2011). Therefore the removal of the starch layer will start after swelling from the top of the layer. At the given concentration NaOH a removal in chunks seems to be unlikely. It might be possible that with a soil removal predominated by bulk cohesive failure the effect of modifications in surface properties on cleanability is not easy to quantify. If bulk cohesive failure predominates and repeated soiling/cleaning results in an accumulation of starch on the surface, more starch-starch and less starch-substrate interaction should be considered at the interface. However, no clear conclusion can be drawn at this stage concerning the effect of surface conditioning.

CONCLUSIONS

1. Repeated soiling and / or cleaning of food contact material under conditions common in food processing, may significantly affect surface properties.
2. Surface conditioning should be taken into account for cleanability tests on materials employed in food processing.

3. Cleaning investigations should be conducted after a certain number of conditioning steps, if the interactions between soil and surface are the objective of the investigations.
4. The effect of surface conditioning on cleaning efficiency varies with the used soil matrix and the apparent surface conditions.
5. Further experimental work using adhesive test soils and surface modifications will be necessary to draw clearer conclusions concerning the effect of surface conditioning.

NOMENCLATURE

m	initial soil mass, mg/cm ²
p	loss of pressure, Pa
\bar{R}	main cleaning rate, mg/(cleaning cycle·cm ²)
S _a	3D arithmetic mean roughness (ISO 4287), μm
S _{pk}	3D reduced peak height, μm
S _{vk}	3D reduced valley depth, μm
S _z	3D mean roughness depth, μm
U	streaming potential, V
x ₉₅	cleaning time to remove 95% of initial soil mass on sample
ε	dielectric constant of the liquid
ε ₀	electric constant, F/m
ζ	Zeta potential, V
η	viscosity of the liquid, Pa s
κ	conductivity, S/m

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of Anja Caspari, Leibniz Institute of Polymer Research Dresden, for providing surface charge measurements and Roman Murcek and Peggy Roßmann, Fraunhofer AVV Dresden for supporting cleaning measurements. We also wish to acknowledge funding from the German AiF.

Das IGF-Vorhaben 16052 BR der Forschungsvereinigung Industrievereinigung für Lebensmitteltechnologie und Verpackung e. V. – IVLV, Schragenhofstr. 35, 80992 München, wurde über die AiF im Rahmen des Programms zur Förderung der industriellen Gemeinschaftsforschung und -entwicklung (IGF) vom Bundesministerium für Wirtschaft und Technologie aufgrund eines Beschlusses des Deutschen Bundestag gefördert.

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