ELSEVIER

International Biodeterioration & Biodegradation 52 (2003) 175–185

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

www.elsevier.com/locate/ibiod

Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance

Lisbeth R. Hilbert[a], Dorthe Bagge-Ravn[b], John Kold[c], Lone Gram[b,*]

[a] *Department of Manufacturing Engineering and Management, Technical University of Denmark, Bldg. 204, DK-2800 Kgs. Lyngby, Denmark*
[b] *Danish Institute for Fisheries Research, Department of Seafood Research, Søltofts Plads, c/o Technical University of Denmark, Bldg. 221, DK-2800 Kgs. Lyngby, Denmark*
[c] *Biotechnological Institute, Holbergsvej 10, DK-6000 Kolding, Denmark*

Abstract

The aim of this study was to evaluate whether hygienic characteristics of stainless steel used in the food industry could be improved by smoothing surface roughness (Ra) from Ra 0.9–0.01 μm. The adherence of *Pseudomonas* sp., *Listeria monocytogenes* and *Candida lipolytica*, to stainless steel was not affected by surface roughness ranging from grit 4000 polished stainless steel (Ra < 0.01) to ground stainless steel (Ra 0.9). Neither adhesion of *Ps. aeruginosa* nor its removal by an alkaline commercial cleaner in a flow system was affected by surface roughness. Pitting corrosion resistance was evaluated in a commercial disinfectant and in 1 M NaCl. Electropolished and grit 4000-polished steel proved more corrosion resistant as opposed to grit 80- and 120- polished surfaces. In conclusion, the surface finish did not influence bacterial attachment, colonziation or removal, but is an important parameter for the corrosion resistance of the surface.

Keywords: Microbial adhesion; Bacterial adhesion; Stainless steel; Roughness Ra; Corrosion resistance

1. Introduction

Microbial adhesion and biofilm formation are major concerns in the food processing industry (Carpentier and Cerf, 1993; Kumar and Anand, 1998; Zottola and Sasahara, 1994). Adhered microorganisms, microorganisms embedded in biofilms or microorganisms hiding in cracks or crevices may escape cleaning and disinfecting procedures and be a source of (re)-contamination of food products during processing. A large number of studies have unequivocally demonstrated that the processing equipment can be a source of contamination of food products. Hence, spoilage bacteria (*Pseudomonas* sp.) of ground turkey were harboured in a grinder and did not originate from the raw meat (Michiels et al., 1997). Similarly, spoilage yeasts of semi-preserved herring were hidden in a rubber lining in a processing tank (Hjelm, 2000). Contamination of ready-to-eat foods with the human pathogenic bacterium *Listeria monocytogenes* is commonly caused by (re)-contamination during processing (Autio et al., 1999; Fonnesbech Vogel et al., 2001; Miettinen et al., 2001; Senczek et al., 2000; Unnerstad et al., 1996). Adhered and biofilm-forming microorganisms may also have other adverse effects such as decreasing heat transfer (Criado et al., 1994; Lewin, 1984) or causing corrosion (Costerton and Lappin-Scott, 1989; Characklis and Cooksey, 1983; Dubey et al., 1995). A major part of the pre-requisite programme (Good Hygienic Practices programme) of a food manufacturing plant is therefore to ensure that microbial biofilms do not form or are efficiently removed.

The adhesion of bacteria to a surface depends on a number of microbiological, physical, chemical, and material-related parameters. Especially the surface topography has been widely discussed as a parameter influencing bacterial adhesion, and Flint et al. (1997a) concluded based on review of existing literature "It may be possible to modify surfaces physically or chemically to reduce attachment (e.g. electropolishing of stainless-steel surfaces) to limit the adhesion of microorganism". Several parameters or measures have been used to characterize the material surface. Surface characteristics have primarily been based on two-dimensional characteristics such as the Ra, Rt and Rz values (De Chiffre, 1999). Amongst the most widely used is the surface

* Tel.: +45-45-25-2586; fax: +45-45-88-4774.
E-mail address: gram@dfu.min.dk (L. Gram).

0964-8305/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0964-8305(03)00104-5

roughness Ra value (which is the arithmetical mean deviation of the profile) and an Ra value of 0.8 μm or less has been recommended for dairies (3-A, 1996) and, in general, for food contact surfaces (EHEDG, 1993). Although widely used, the Ra value will typically not characterize features of the surface such as soft or sharp topography or the presence of scratches or porosities. During recent years, scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been used to give a three-dimensional visualisation of the surface topography including AFM determination of three-dimensional topographical parameters in the nanometre range (De Chiffre, 1999; Stout and Blunt, 1995).

With the recommendation of a minimum Ra value of 0.8 μm, a number of studies have evaluated if further reductions in numbers of adhering bacteria can be obtained by using even smoother surfaces with lower Ra values. However, experiments in milk showed no significant difference between adhesion on surfaces with Ra 0.4 and 0.035 μm (Barnes et al., 1999). Similar tendencies were found in a study of weldments and base metal, where no significant differences were found in bacterial adhesion (Tide et al., 1999). Conversely, it may be asked if rougher surfaces result in higher numbers of adhering bacteria, but Flint et al. (2000) found similar levels on surfaces with Ra values in the range 0.5–3.3 μm.

Hygienic quality is linked to cleanability (Mettler and Carpenter, 1999) and roughness of stainless steel is believed to be the most important factor in cleaning biofilms from surfaces (Leclercq-Perlat and Lalande, 1994; Wirtanen et al., 1995). However, a number of studies have documented that it is necessary to discern between bacterial removal and soil removal. Recently it was shown that bacteria are removed independent of Ra or topography in general, whereas removal of starch was dependent on surface defects, but not on the Ra value (Boyd et al., 2001).

While the issue of cleanability is focused on removal of soil and microorganisms, other features may be as important for the hygienic quality (Boulangé-Peterman, 1996). The ability of a surface to resist corrosion is of major importance, as corrosion pits may decrease cleanability markedly (Flint et al., 2000). Cases of corrosion failures, e.g. due to deviations from the recommended disinfection procedure, are not uncommon in the food industry. Corrosion resistance depends on material composition, mode of manufacturing, geometry and on the surface finish. On passive stainless steel, rougher surfaces are more susceptible than smoother surfaces to localised forms of corrosion such as pitting and crevice corrosion. This effect can be related to the surface nucleation of metastable pitting preceding propagation of pitting. Although a higher number of nucleation events take place on a smoother surface as compared to a rough surface (Burstein and Vines, 2001), propagation of the pits and formation of micropits does not occur as readily (Burstein and Vines, 2001; Zuo et al., 2002). On a rougher surface, several of the nucleation events will lead to propagation of pits and thereby corrosion.

The improvement of corrosion resistance by surface polishing of stainless steel can be measured as increase in pitting potentials and reduction in numbers of metastable pits (Hong and Nagumo, 1997). Improved corrosion resistance obtained by passivation treatment of 316 l in HNO_3 has been attributed not only to changes in the composition of the oxide layer to a more corrosion resistant form, but also to changes in surface topography to smoother surfaces with fewer flaws and defects (Wang et al., 2001).

Clearly, several aspects of surface topography influence the hygienic quality. Typically, studies have either focused on bacterial adhesion or on bacterial removal or on corrosion resistance. The purpose of the present study was to evaluate these different parameters for the same surfaces and to elucidate if changes in surface smoothness, measured as Ra value, had any effect on hygienic quality of stainless steel.

Materials and methods

Two systems were used when studying interaction between microorganisms and different steel surfaces. Microbial adhesion was studied on $10 \times 20 \times 1$ mm disks placed vertically in a glass beaker. Bacterial adhesion and the effect of cleaning were studied in a "test-rig" (described below) into which plugs with different surfaces were inserted.

Stainless-steel surfaces

Stainless-steel plates $10 \times 20 \times 1$ mm were made of sheet SS 2343 (AISI 316) with surface finish 2 B (cold rolled, annealed, pickled and lightly rolled). Composition was as follows: C 0.03, Si 0.49, Mn 1.7, Cr 16.7, Ni 10.7, Mo 2.5, P 0.032, S 0.001, Fe rest. Five different surface finishes were applied: (a) 2B finish as delivered, (b) wet polished with 80 grit or ground, (c) wet polished with 120 grit, (d) electropolished in a bath containing 65–67 vol% of 85% phosporic acid (H_3PO_4), 30 vol% of 96% sulfuric acid (H_2SO_4) and 5–3 vol% H_2O at 55°C at 20 A dm^{-2} for 15 min and (e) polished to grit 4000 leaving a mirror-like surface. Plates were cathodically degreased in a cyanide bath at 10 A dm^{-2} for 2 min, rinsed in deionised water and dried in hot air before use.

For experiments in the test-rig (Kolding model), cylindrical studs with exposed surface area of 3 cm^2 were prepared from a rod of SS 2343 (AISI 316L) with composition: C 0.011, Si 0.70, Mn 1.50, Cr 16.65, Ni 10.52, Mo 2.51, Cu 0.51, Ti 0.039, P 0.029, S 0.027, Cb 0.006, N 0.072, Fe rest, peeled and annealed. The surface treatments (b)–(e) described above were applied. Surfaces were ground instead of polished with 80 grit paper in treatment (b).

In addition, cylindrical studs with a diameter of 10 mm were prepared of AISI 316L (stainless-steel specimen ø10 × 10 mm of SS 2343 (AISI 316L) with composition C 0.021, Si 0.65, Mn 1.46, Cr 16.9, Ni 10.16, Mo 2.02, P 0.027, S 0.025, N 0.07, Fe rest, drawn and annealed) for corrosion

testing. The following surface treatments were applied: glass bead blasting, polishing with grit 80, polishing with grit 800, pickling and electropolishing.

The surface finish was characterised by SEM with a JEOL 5900 at 15 kV to characterise the visual appearance at magnifications ranging from 50 to 3000 times. Roughness measurements were obtained on a Taylor–Hobson Surtronic 3P instrument. A tracing length of 5.6 mm, 2 μm stylus, and cut-off 0.8 mm were used. Roughness was measured perpendicular to the grinding scratches, and each measurement is a mean of five measurements on each specimen. The results are presented as means of data from 2 to 4 individual specimens.

Microorganisms

The adhesion of microorganisms to stainless-steel disks submerged in a suspension of microorganisms in a beaker was tested with a Gram-negative (*Pseudomonas* sp.), a Gram-positive (*Listeria monocytogenes*) bacteria and a yeast (*Candida lipolytica*). These isolates originated from food processing equipment sampled after cleaning and disinfection of the equipment. The bacteria were isolated from a factory producing cold-smoked salmon (Bagge-Ravn et al., 2003; Fonnesbech Vogel et al., 2001) and the yeast from a factory producing semi-preserved herring (Bagge-Ravn et al., 2003). Bacterial adhesion and the effect of cleaning were tested using *Pseudomonas aeruginosa* ATCC 15442.

Microbial adhesion

Microbial adhesion to stainless-steel surfaces with the four different surface finishes described above, (a), (b), (c) and (e), was studied in the model system described by Bagge et al. (2001). The four different stainless-steel disks were cleaned (soaking overnight in a 10% Deconex solution, rinsed and de-greased with acetone) and sterilised by autoclaving. The bacteria were precultured in Tryptone Soya Broth (TSB, Oxoid CM129) with agitation at 25°C and harvested after 24 h ($3000 \times g$ for 10 min), resuspended and diluted in phosphate-buffered saline (PBS; 0.8% NaCl, 0.02% KCl, 0.144% Na_2HPO_4, 0.0024% NaH_2PO_4). The yeast was precultured with agitation at 25°C for 48 h in MYGP (0.3% yeast extract, 0.3 malt extract, 1% glucose, 0.5% peptone) and harvested as described above.

A surface layer (conditioning film) was formed on the sterile disks by submersion in dilute (1:7) TSB for 30 min with agitation. Thereafter, the holders were transferred to a new sterile beaker containing the microorganism in PBS at approximately 10^6 colony forming units per mililitre (cfu ml^{-1}). Adhesion was allowed to take place on both sides of the disk under slow stirring at room temperature.

Disks were sampled at different time points, and the adhered microorganisms were quantified by indirect conductometry (Bagge et al., 2001; Johansen et al., 1997) using the Malthus® instrument. The disks were transferred to a test tube containing a growth medium (TSB for the *Pseudomonas* sp., Brain Heart Infusion (BHI, Oxoid CM225) for *L. monocytogenes* or MYGP for *C. lipolytica*). The growth of the adherent microorganisms developed CO_2, which diffused to an inner tube containing sterile NaOH. Electrodes measure the change of conductance in the NaOH as CO_2 dissolved in the alkali. The detection time, i.e. the time point at which a significant change in conductance occurs, is inversely related to the initial number of microorganisms. The initial number of microorganisms on the surfaces was calculated by use of a calibration curve constructed comparing detection times and colony counts of a 10-fold dilution series of each of the microorganisms. For these experiments, linear regression was used to construct the calibration curve.

Bacterial adhesion in a flow system and effect of cleaning

The effect of different surface roughness on bacterial adhesion was studied using the "Kolding test-rig". This is a macro version of the Modified Robbin's Device. It consists of a stainless-steel squared tube (29 mm × 29 mm × 140 cm) in which 36 positions are available for insertion of surfaces (Fig. 1) with a diameter of 19.4 mm (area of 3 cm^2). Suspensions may be pumped through or circulated at flow rates varying from 0.5 to 2.5 m s^{-1}. Surfaces (completely at level with the inner side of the tube wall) were inserted into the squared tube and sterilised by autoclaving. Alternatively, the inserts were autoclaved separately and aseptically inserted in the rig. The pump and remaining circulation system was disinfected by steam (121°C for 30 min). The surfaces were conditioned by circulating Nutrient Broth (NB, Oxoid CM1) at a flow rate of 1.0 m s^{-1} for 30 min.

Ps. aeruginosa was pre-cultured at 25°C in TSB (Oxoid CM129) for 18–24 h reaching approx. 10^8 cfu ml^{-1}. Colony counting by surface plating was used to verify the bacterial density. A volume of 15 l NB was inoculated with 1.5 ml outgrown culture and after 1/2–1 h, the level of bacteria in all sections of the test-rig was approx. 10^4 cfu ml^{-1}. The inoculated medium was circulated from 24 to 48 h during which counts increased to 10^8 cfu ml^{-1} in the liquid. At the end of the cycle, cold tap water was circulated to remove loosely attached bacteria. Half of the inserted surfaces were removed aseptically and the number of adhering *Ps. aeruginosa* quantified using direct conductometric measurements in the BacTrac® instrument. BacTrac® cells were filled with 50 ml each of sterile BiMedia 001A (Sy-Lab, Austria) and the electrodes (positioned at the bottom) were covered by a plastic lid. Free diffusion of medium and bacteria is allowed and the test-rig surfaces are prevented from physical contact with the electrodes.

A standard curve comparing cfu ml^{-1} of several dilution series to detection times was constructed by comparing DT of 10-fold dilution steps of *Ps. aeruginosa* to cfu ml^{-1}. Repeated measurements demonstrated that the relationship was not linear at very low bacterial densities, and the

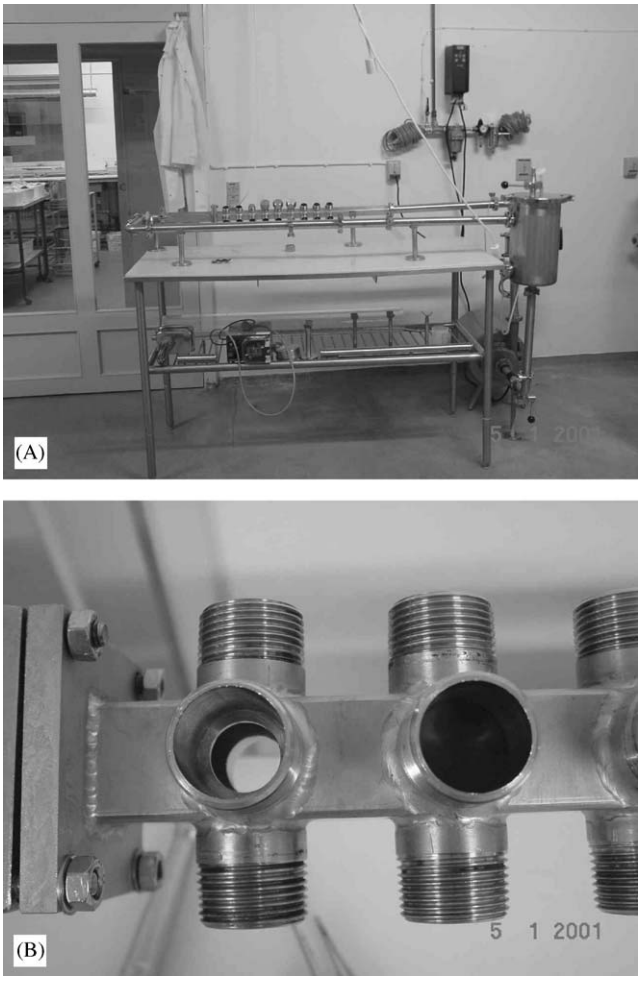

Fig. 1. (A) The Kolding test-rig for bacterial adhesion formation and (B) magnification of insert holders in the Kolding test-rig.

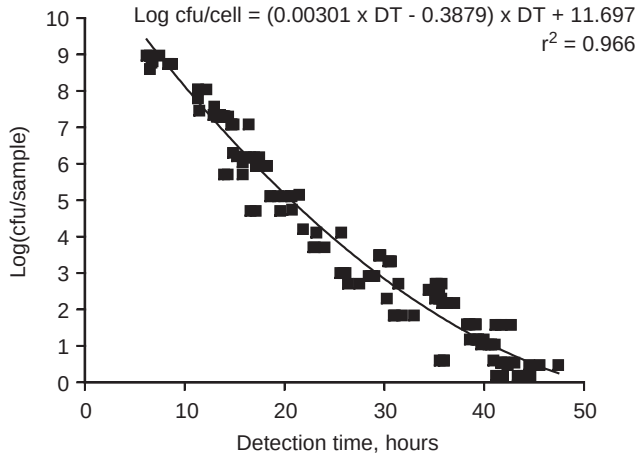

Fig. 2. Standard curve comparing cfu ml^{-1} (initial counts) of *Pseudomonas aeruginosa* with detection times in the BacTrac® system.

cfu ml^{-1}–DT curve was fitted using a Gaussian equation (Fig. 2). Subsequently, this curve was used to estimate bacterial numbers on the test-rig-insert surfaces. A clean-in-place (CIP) procedure was done using MC103 (Novadan, Denmark), which is an alkaline (pH 10.5) tenside containing cleaning agent. CIP was done at either 0.5 or at 2.5 m s^{-1}. After CIP, the system was rinsed with cold tap water, and the test surfaces removed for estimation of bacterial numbers as described above.

Between trials, surfaces were cleaned using 2% MC103 followed by soaking in 2% Extran (MA 02 neutral, Merck) for 2 h. The surfaces were gently scrubbed and rinsed in tap water and de-mineralised water.

A total of five independent experiments were conducted with three types of stainless-steel surfaces. In each experiment, three (or two) surfaces were sampled per surface per treatment. The number of replicates for each experiment is shown in Table 1. Individual measurements were log-transformed and statistical comparisons of means were based on *t*-test.

Corrosion testing

Electrochemical polarisation techniques were used to characterise the corrosion resistance of stainless steel with different surface finish. For active–passive materials, like stainless-steel, measurement of pitting potentials are used for ranking the aggressiveness of different media or the corrosion resistance of different alloys in a specific media. We used pitting potentials to rank the corrosion properties of stainless-steel AISI 316L with different surface treatments when exposed to a commercial oxidising disinfectant or a 1 M NaCl solution. Experiments were all run at 25°C. The commercial disinfectant that was used in a 0.2 vol% solution was based on hydrogen peroxide, acetic acid and peracetic acid in deionised water resulting in a pH of 3.9. 250 mg/l Cl^- was added as NaCl to accelerate pitting initiation and the media was under oxygen gas purging. The experiments in 1 M NaCl (solubilised in deionised water) were run under nitrogen gas purging and the pH was 6.5.

AISI 316 plates similar to those in the static microbial adhesion studies were exposed to the commercial disinfectant. Cylindrical specimens made from AISI 316L rod material corresponding to the material used for the studies of bacterial adhesion in a flow system were exposed to the NaCl solution. The specimens were rinsed in distilled water and ethanol and dried in hot air before mounting in the electrode holder in a 400 ml volume temperature-controlled glass cell. Platinum was used as counter electrode and standard calomel electrode (SCE) as reference. Pitting potential measurements were conducted on a PGP 201 Potentiostat/Galvanostat from Radiometer operated by Voltamaster 1 with a scan rate of 10 mV min^{-1}. The curve in commercial disinfectant was run from −300 mV to the open circuit potential (225–300 mV vs. SCE) to 100 μA current. When exposed to 1 M NaCl, the curve was run from −50 mV vs. SCE to +1000 mV vs. SCE. Pitting potentials were defined as the potential at which current density exceeded 10 μAcm^{-2}.

Table 1
Overview of stainless-steel surfaces and replicates tested in the Kolding test-rig model

Experiment	Material	Before cleaning		After cleaning		
		No. of replicates	Log(cfu cm^{-2})	Flow	No. of replicates	Log(cfu cm^{-2})
B	Ground	3	6.19	High	3	0.38
	Polished 4000	3	6.32		2	0.30
	Polished 120	3	6.32		2	0.52
C	Ground	3	6.33	High	3	1.66
	Polished 4000	3	6.33		2	0.74
	Polished 120	3	6.49		3	1.33
D	Ground	3	6.80	Low	3	4.30
	Polished 4000	1	6.34		4	4.69
	Polished 120	3	6.89		3	2.91
E	Ground	3	6.39	Low	3	1.94
	Polished 4000	3	6.19		3	2.70
	Polished 120	3	6.36		2	1.44
F	Ground	2	6.97	High	3	2.27
	Polished 4000	3	6.77		3	3.20
	Polished 120	3	7.17		3	1.98

Results

Topography of surfaces with different finish

Ra values obtained by different surface treatments ranged from 0.9 to 0.01, indicating differences in the two-dimensional topography of the surfaces (Table 2). Rz is the horizontal distance from the highest peak to the lowest valley and Rz values followed like all measured topographical parameters the variation in Ra (Fig. 3). RSm, which is a measure of the average profile element width, distance from one top to the next valley, increased with increasing roughness Ra in all but one case: the electropolished surface (Fig. 3).

Rod and plate material responded differently to the surface treatments. For instance, electropolishing of the rod

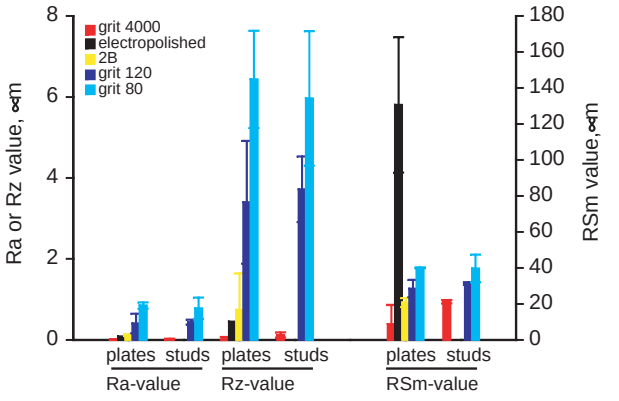

Fig. 3. Roughness data for plate and stud specimen. Ra, Rz and RSm values are all measured in micrometer. Error bars are standard deviations.

Table 2
Roughness data for AISI 316

Base material	Treatment	Ra (μm)	
		Avg.	Avg ± standard variation
Sheet	As delivered—pickled	0.1	0.12 ± 0.02
Sheet	Polished grit 120	0.4	0.37 ± 0.20
Sheet	Polished grit 80 ~ grinding	0.9	0.86 ± 0.1
Sheet	Electropolished	0.08	0.078 ± 0.03
Sheet	Polished grit 4000	<0.01	(0.009 ± 0.003) (below detection limit)
Rod	Ground	0.8	0.78 ± 0.26
Rod	Polished grit 120	0.5	0.45 ± 0.16
Rod	Polished grit 4000	0.02	0.02 ± 0.01
Rod 2	Pickled	0.4	0.396
Rod 2	Electropolished	0.3	0.270
Rod 2	Polished grit 80	0.9	0.854
Rod 2	Polished grit 800	0.2	0.233
Rod 2	Glass bead blasted	2.0	1.950

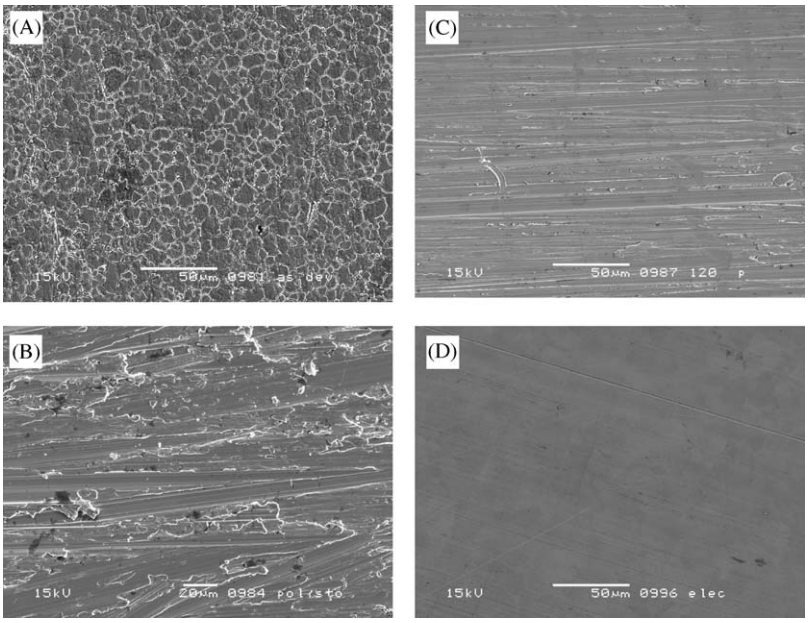

Fig. 4. SEM micrographs of different surface finishes (A) As delivered—2B pickled, (B) polished grit 80 ~ ground, (C) polished grit 120, (D) electropolished.

material was not successful and only caused pitting corrosion of the surface. Several tests of variation of material and electropolishing procedure lead to the conclusion that material inhomogenity due to alloy elements added to obtain machinable material caused a poorer corrosion resistance of rod material as compared to plate. The mirror-like surface polished with grit 4000 was therefore used to obtain a very low Ra value comparable to the electropolished, but the surface properties are not identical to an electropolished surface, where the top layer is chemically removed. For corrosion studies, a rod with a smaller diameter was, however, successfully electropolished.

SEM micrographs of different surface finishes on AISI 316 clearly demonstrated that the surfaces were visually different (Fig. 4). The 2B pickled surface was characterised by visible grain boundaries due to the pickling, whereas the two surfaces ground at 120 and 80 grit were characterised by scratches. The electropolished surface was very smooth but a single scratch from previous polishing was still evident. The mirror-like surface from 4000 grit polishing of rod material could not be visualised by SEM due to the resolution of SEM.

Adhesion of microorganisms to stainless-steel surfaces

Pseudomonas sp., *Listeria monocytogenes* and *Candida lipolytica* adhered readily to the surfaces. The number of adhered microorganisms increased until a stationary state was reached. (Fig. 5). The number of adhered bacteria was higher than the number of adhered yeast. The bacterial number increased from 10^3 cfu cm^{-2} immediately after immersion to 10^6 or 10^7 cfu cm^{-2} after 8 h of incubation. The yeast increased from approximately 10^5–10^6 cfu cm^{-2} after incubation for 8 h.

The cell number in the suspension varied for the three experiments, from 5.4×10^4 cfu ml^{-1} for the yeast, 3.8×10^6 cfu ml^{-1} for the *Pseudomonas* sp. and to 7.1×10^6 cfu ml^{-1} for *L. monocytogenes*.

There was no significant difference in numbers of microorganisms adhering to the four different surfaces of stainless steel. The surface with lowest Ra value was the mechanically polished (grit 4000, Ra 0.01). In previous experiments, an electropolished steel surface (Ra 0.1) was also tested and the same number of microorganisms adhered to this surface as to a grit 400 polished surface (data not shown).

Bacterial adhesion in test-rig and effect of cleaning

Counts of *Ps. aeruginosa* increased in the nutrient broth from 10^4 to 10^8 cfu ml^{-1} in 24 h. Five different experiments were conducted with the three types of surfaces (Table 1). Counts on surfaces reached 5×10^6–5×10^7 cfu $surface^{-1}$ in all experiments (Fig. 6). No difference was seen in number of adhering bacteria on the three different surfaces.

The CIP procedure resulted in removal of bacteria. Counts decreased to 10–10^4 cfu $surface^{-1}$. Again, no systematic difference was seen in cleanability between the three stainless-steel surfaces. The CIP procedure was conducted using low flow 0.5 m s^{-1} (two experiments) and high flow 2.5 m s^{-1} (three experiments). The highest level of bacteria remaining after cleaning was detected after a low-flow cleaning cycle. Conversely, the lowest level of bacteria remaining after cleaning was seen after a high-flow cleaning cycle (Fig. 6F). However, one CIP cleaning at low

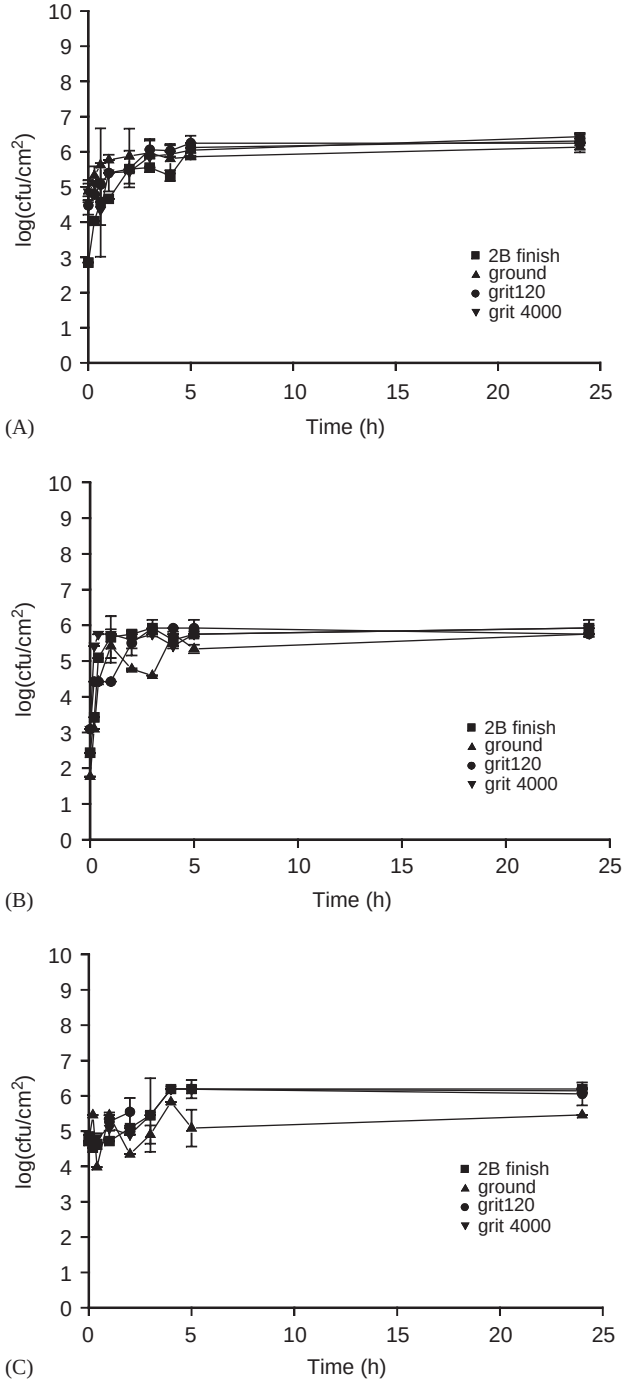

Fig. 5. Adhesion of microorganisms to steel surfaces. (A) *Pseudomonas* sp., (B) *Listeria monocytogenes* and (C) *Candida lipolytica*. Error bars are standard deviations of triplicate measurements.

flow resulted in a lower count than one of the high-flow cleaning procedures.

Corrosion resistance

Polarisation curves for electropolished and grit 4000-polished plates in disinfectant revealed that the test surfaces were characterised by a high degree of passivity (Fig. 7A). High initial corrosion potentials indicated a noble passive surface on the electropolished stainless steel. The currents were low but increased steadily except in a distinct passive region at higher potentials. Final breakdown and pitting occurred at very high potentials. The other surfaces (Fig. 7B) were less passive and large current fluctuations indicating onset and termination of small pits were seen. The fluctuations were largest and appeared at low potentials for grit 80. Grit 120 displayed fewer fluctuations than grit 80, but the actual breakdown of grit 80 occurred at a very high potential actually giving this surface a high pitting potential. The 2B finish, pickled and lightly rolled, had a low pitting potential but showed few current fluctuations.

Experiments in 1 M NaCl gave three different patterns of pitting potentials (Fig. 8). Grit 800 and electropolished displayed a high degree of passivity and low currents until breakdown. Large current fluctuations occurred on grit 80 and glass bead blasted surfaces even at low potentials. Pitting potentials of grit 80 and glass bead blasted surfaces were low. The pickled surface displayed high currents at low potentials, but the actual pitting potential which indicated total breakdown was high. Pitting potentials determined in 1 M NaCl clearly correlated with the Ra value (Fig. 9).

Discussion

It is recommended that surfaces to be used in the food industry do not have an Ra value above 0.8 μm. Although we have not been able to find scientific data substantiating this value, we assume that surfaces with higher Ra Values are believed to be of poorer hygienic quality. Hence, it could be anticipated that lower Ra values, resulting in smoother surfaces, would have improved hygienic characteristics. In the present study, we have evaluated three parameters of importance for the hygienic quality of stainless steel: (i) the number of bacteria adhering to a surface, (ii) the cleanability of the surface and (iii) the corrosion resistance.

We have demonstrated that when stainless-steel surfaces with lower Ra values (range of < 0.01–0.9 μm) were exposed to bacteria, both under static and under flow conditions, the surface smoothness did not affect the number of attaching bacteria. Although, it has been suggested that, for instance, electropolishing of stainless steel would reduce attachment (Flint et al., 1997a), our data are similar to recent studies (Barnes et al., 1999; Tide et al., 1999) finding no repelling of bacteria on surfaces of lower Ra values as compared to surfaces with higher Ra values. It should be mentioned that a few studies have actually concluded that attachment of colloidal particles occurs more readily on smoother surfaces with lower Ra values (Bowen et al., 2001; Kerr et al., 1999). It could be argued that the adhesion force of colloidal particles is not a relevant model for living bacteria; however, if a smoother surface attract organic material

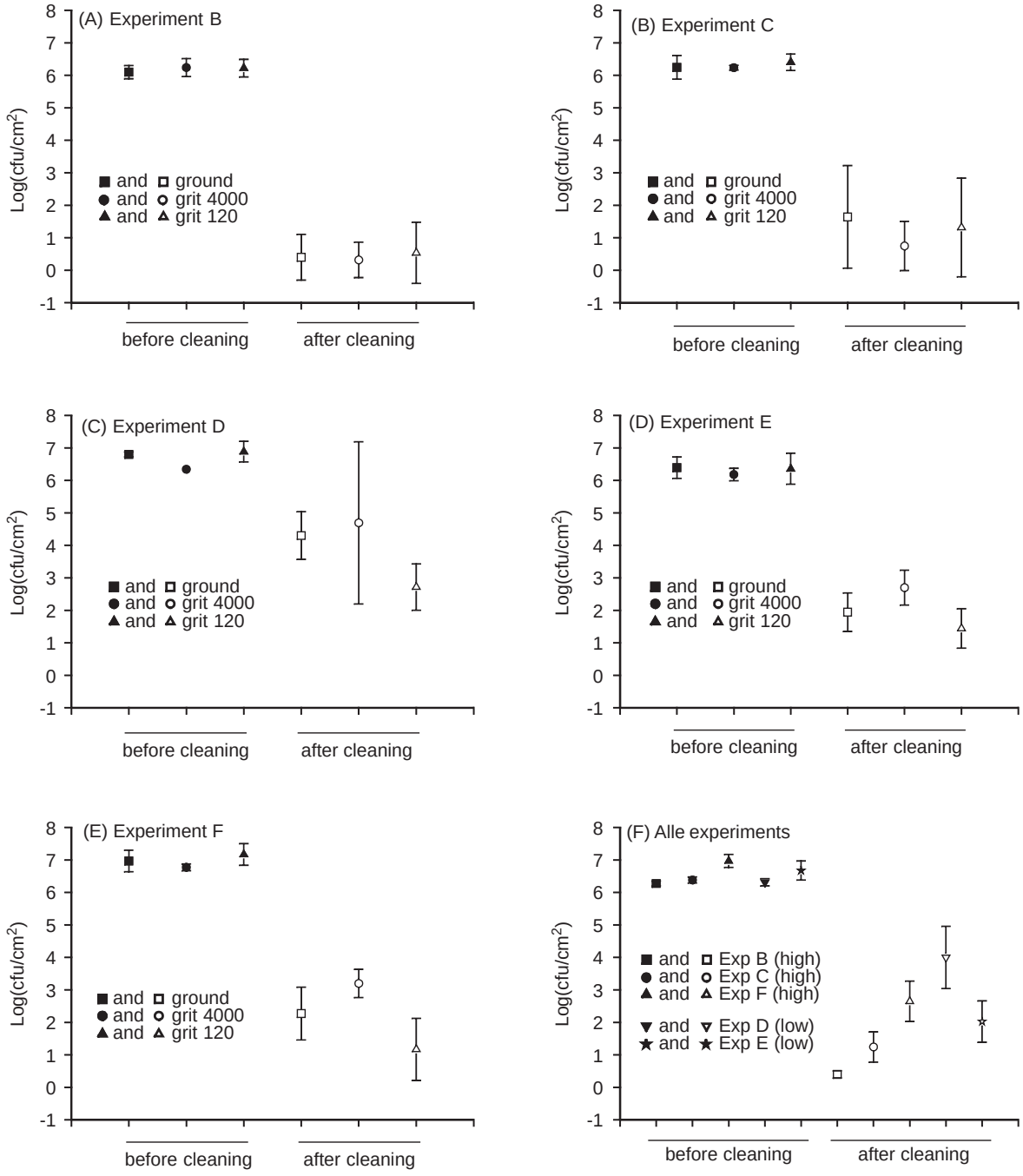

Fig. 6. Level of *Pseudomonas aeruginosa* on three different steel surfaces inserted in a test-rig. cfu/surface before and after cleaning. (A–E): five different experiments. Figure F: compilation of all surfaces before cleaning and after cleaning for the five experiments. Solid symbols: before cleaning. Closed symbols: after cleaning. Error bars are standard deviations.

easier than a rougher surface, this could explain why the level of bacterial attachment to the different surfaces is similar.

Surface Ra values below 0.5 have been found to improve cleanability of edible oils and food emulsions (Michalski et al., 1998); however, in our study, the Ra value did not change the number of bacteria removed during cleaning and disinfection. In agreement with this, Mettler and Carpenter (1999) could not correlate mean roughness to cleanability.

When examining SEM micrographs of the different surfaces (Fig. 4) it is evident that the topography of the surfaces (smoothness) was very different and appeared to correlate with the Ra value. However, it could be argued that the Ra value does not characterise the surface finish in a sufficient manner, and clearly, the RSm value did not change in the same manner as the Ra value (Fig. 3). The RSm value of the electropolished plate surface was extremely high compared to the other surfaces. This is probably explained

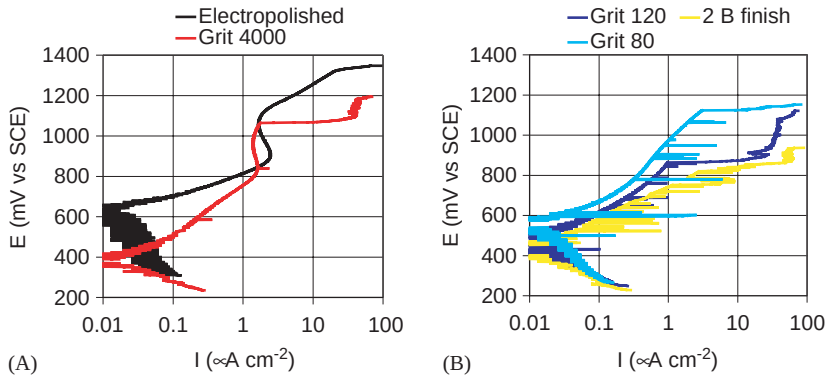

Fig. 7. Pitting potential curve of stainless steel in 0.2% commercial disinfectant + 250 ppm Cl^-. E_{pit} is taken at 10 μA cm^{-2}, E_{cor} at the transition from cathodic to anodic current = minimum current in the plot.

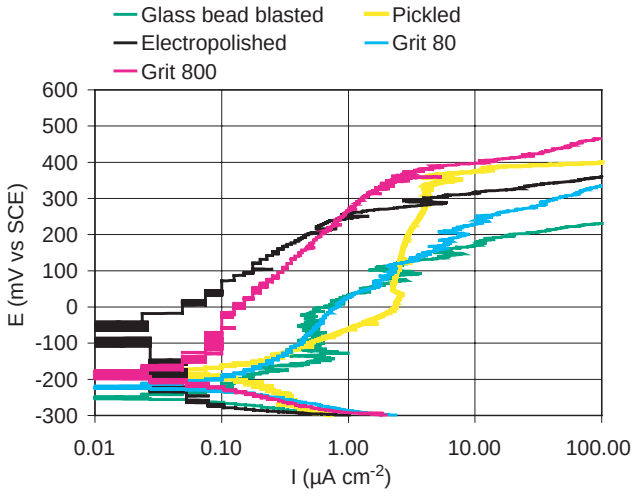

Fig. 8. Pitting potential curves for AISI 316 in 1 M NaCl.

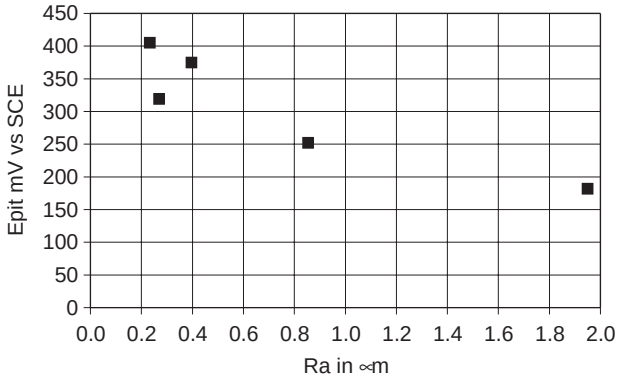

Fig. 9. Pitting potentials for AISI 316 in 1 M NaCl as a function of Ra.

by the levelling effect of electropolishing as compared to polishing which only reduces the size of the scratches. The Ra value is widely used but features of surface structure, such as soft or sharp topography or the presence of scratches or porosities, are not revealed by this parameter. Such features are probably important from a hygienic point of view.

There is a great need to determine if one (or several) surface characterising parameters can reflect the degree to which bacteria adhere to or are removed from a surface. Unfortunately, since we did not find any differences in adhesion or cleanability, we are unable to progress this topic further. Several studies have suggested that surface defects such a cracks and crevices are more likely to reflect the degree of soiling and microbial attachment on a surface. Thus, Verran et al. (2000) concluded that surface defects as characterised by Atomic Force Microscopy (AFM) could be correlated to starch removal. Similarly Mettler and Carpenter (1999) found that grooves extending below the core profile trapped dirt and decreased cleanability. In a long-term study of biofilm formation in a potable water system, Ra values did not correlate with biofilm formation whereas the presence of scratches, grooves, and deformation as revealed by AFM caused a higher degree of biofilm formation (Percival et al., 1998).

It has been suggested that AFM which provides both a 3-dimensional view of the surface and enables the determination of two-dimensional parameters is the best technique to evaluate the topographic properties of a surface (Arnold et al., 2001; Verran et al., 2000). AFM allowed Arnold et al. (2001) to give a detailed characterisation of and to compare stainless steel with different surface finish. It was concluded that bacterial attachment was affected by surface characteristics. However, bacterial numbers were estimated using SEM and differences in attachment of only a factor of 2–4 were seen. As bacterial numbers are normally log-transformed before comparison, such differences are very small and may not be significant.

SEM and other microscopic techniques are useful to provide a visual impression of bacteria on a surface and may be used as a (semi)quantitative technique. However, quantification is very difficult when the bacteria are clumped or positioned in layers. Also, the detection limit

(magnification and field of view) of the microscope does not enable detection and quantification at low cell densities, for instance following cleaning and disinfection. As our goal was to compare the levels or numbers of bacteria attaching (or being removed) from very high to very low levels, a different technique had to be used. We therefore used an indirect growth-dependent method to quantify microorganisms on the surfaces. This technique has been used in several studies to quantify microbial biofilms (Bagge et al., 2001; Flint et al., 1997b; Johansen et al., 1997, 1999) and has the advantage of being able to quantify very low levels of organisms. Even a single cell remaining on the 2 cm^2 surface will eventually multiply and be detected by the instrument. Several studies have demonstrated that the relationship between detection times and initial bacterial levels is not strictly linear at very low and very high bacterial densities. In this study, we show that this can be overcome by constructing a standard curve using other analysis than linear regression (Fig. 2).

The Ra value had little effect on the microbial parameters, however, the corrosion resistance was indeed improved by smoothing the surface since grit 4000-polished steel resisted a commercial disinfectant to a higher degree than grit 120-polished steel. This is in agreement with other studies (Zuo et al., 2002; Hong and Nagumo, 1997), where it has been demonstrated that surface treatments like mechanical polishing, electropolishing or pickling increase corrosion resistance, while rough surfaces are less resistant. Plotting pitting potentials vs. Ra values (Fig. 9) illustrated a direct effect of roughness on pitting resistance when surfaces were exposed to 1 M NaCl. Pitting potentials were fairly high for surfaces with Ra below 0.8 μm, but pitting resistance was lowered for more rough surfaces.

The simple pitting potentials alone cannot, however, characterise the corrosion properties and the stability of the current must also be evaluated. In the case of the commercial disinfectant, ranking corrosion resistance based only on pitting potentials would lead to the following ranking: electropolished > grit 80 > grit 4000 > grit 120 > 2B finish. However, due to the fluctuating current indicating onset and termination of small pits, especially on the grit 80 surface, this surface actually has a poor corrosion resistance in spite of an apparent high pitting potential.

The grit 4000-polished surface with fine corrosion properties is not an industrially applicable surface, whereas an electropolished or pickled surface can be obtained in many types of equipment. Pickling improved corrosion properties slightly in 1 M NaCl as compared to the very rough surfaces, but the 2B finish did not enhance corrosion resistance in the commercial disinfectant even though this finish also includes a pickling treatment.

In conclusion, we have demonstrated that the attachment to and removal of microorganisms from stainless-steel surfaces did not depend on surface roughness when varied between Ra values of 0.01 and 0.9 μm. In contrast, the corrosion resistance against disinfecting agents and NaCl was higher on the surfaces smoothed by electropolishing and grit 4000 polishing than on the rougher surfaces. Our study clearly demonstrates that one must consider a range of aspects when addressing the issue of hygienic quality of surfaces. Therefore, hygiene must be improved by interdisciplinary work focused on extending hygienic lifetime by use of corrosion-resistant materials and appropriate design allowing effective cleaning and disinfection.

Acknowledgements

The study was supported by a grant from The Danish Agency for Trading and Industry of the Danish Ministry of Commerce. We thank H.C. Dam and Michael Albæk for assistance with preparation of stainless-steel surfaces, and Janne Nielsen, DIFRES and Kersti Haugan, formerly BI, for work on bacterial adhesion.

References

3-A, 1996. Accepted practices for farm milk cooling and storage systems. Number 611-00.

Arnold, J.W., Boothe, D.H., Bailey, G.W., 2001. Parameters of treated stainless steel surfaces important for resistance to bacterial contamination. Transactions of the ASAE 44, 347–356.

Autio, T., Hielm, S., Miettinen, M., Sjöberg, A.-M., Aarnisalo, K., Björkroth, J., Mattila-Sandholm, T., Korkeala, H., 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Applied and Environmental Microbiology 65, 150–155.

Bagge, D., Johansen, C., Huber, I., Hjelm, M., Gram, L., 2001. *Shewanella putrefaciens* adhesion and biofilm formation on food processing surfaces. Applied and Environmental Microbiology 67, 2319–2325.

Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J.N., Johansen, C., Gram, L., 2003. The microbial ecology of processing equipment in different fish industries—during production and after cleaning and sanitizing. International Journal of Food Microbiology, in press.

Barnes, L.-M., Lo, M.F., Adams, M.R., Chamberlain, A.H.L., 1999. Effect of milk proteins on adhesion of bacteria to stainless steel surfaces. Applied and Environmental Microbiology 65, 4543–4548.

Boulangé-Peterman, L., 1996. Processes of bioadhesion on stainless steel surfaces and cleanability: a review with special reference to the food industry. Biofouling 10, 275–300.

Bowen, W.R., Lovitt, R.W., Wright, C.J., 2001. Atomic force microscope studies of stainless steel: surface morphology and colloidal particle adhesion. Journal of Materials Science 36, 623–629.

Boyd, R.D., Cole, D., Rowe, D., Verran, J., Paul, A.J., West, R.H., 2001. Cleanability of soiled stainless steel as studied by atomic force microscopy and time of flight secondary ion mass spectrometry. Journal of Food Protection 64, 87–93.

Burstein, G.T., Vines, S.P., 2001. Repetitive nucleation of corrosion pits on stainless steel and the effects of surface roughness. Journal of the Electrochemical Society 148, B504–B516.

Carpentier, B., Cerf, O., 1993. Biofilms and their consequences, with particular reference to hygiene in the food industry—a review. Journal of Applied Bacteriology 75, 499–511.

Characklis, W.G., Cooksey, K.C., 1983. Biofilms and microbial fouling. Advances in Applied Microbiology 29, 93–137.

Costerton, J.W., Lappin-Scott, H.M., 1989. Behavior of bacteria in biofilms. ASM News 55, 650–654.

Criado, M.-T., Suárez, B., Ferreirós, C.M., 1994. The importance of bacterial adhesion in the dairy industry. Food Technology 48, 123–126.

De Chiffre, L., 1999. Industrial Survey on ISO surface texture parameters. Annals of the CIRP 48/3, 74–77.

Dubey, R.S., Namboodhiri, T.K.G., Upadhyay, S.N., 1995. Microbiological influenced corrosion of mild steel in cultures of sulphate reducing bacteria. Indian Journal of Chemical Technology 2, 327–329.

EHEDG, 1993. Hygienic design of closed equipment for the processing of liquid food. Trends in Food Science and Technology 11, 375–379.

Flint, S.H., Bremer, P.J., Brooks, J.D., 1997a. Biofilms in dairy manufacturing plant—description, current concerns and methods of control. Biofouling 11, 81–97.

Flint, S.H., Brooks, J.D., Bremer, P.J., 1997b. Use of the Malthus conductance growth analyser to determine numbers of thermophilic streptococci on stainless steel. Journal of Applied Microbiology 83, 335–339.

Flint, S.H., Brooks, J.D., Bremer, P.J., 2000. Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci. Journal of Food Engineering 43, 235–242.

Fonnesbech Vogel, B., Huss, H.H., Ojeniyi, B., Ahrens, P., Gram, L., 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Applied and Environmental Microbiology 67, 2586–2595.

Hjelm, M., 2000. Microflora from a processing environment producing semi-preserved herring. Identification, tolerance to preservation parameters and adhesion [in Danish]. Danish Institute for Fisheries Research, Department of Seafood Research. B.Sc. Thesis.

Hong, T., Nagumo, M., 1997. Effect of surface roughness on early stages of pitting corrosion of type 301 stainless steel. Corrosion Science 39, 1665–1672.

Johansen, C., Falholt, P., Gram, L., 1997. Enzymatic removal and disinfection of bacterial biofilms. Applied and Environmental Microbiology 63, 3724–3728.

Johansen, C., Bredtved, B.K., Møller, S., 1999. Use of conductance measurements for determination of enzymatic degradation of microbial biofilms. Methods in Enzymology 310, 353–361.

Kerr, A., Beveridge, C.M., Cowling, M.J., Hodgkiess, T., Parr, A.C.S., Smith, M.J., 1999. Some physical factors affecting the accumulation of biofouling. Journal of the Marine Biological Association of the United Kingdom 79, 357–359.

Kumar, C.G., Anand, S.K., 1998. Significance of microbial biofilms in food industry: a review. International Journal of Food Microbiology 42, 9–27.

Leclercq-Perlat, M.-N., Lalande, M., 1994. Cleanability in relation to surface chemical composition and surface finishing of some materials commonly used in food Industries. Journal of Food Engineering 23, 501–518.

Lewin, R., 1984. Microbial adhesion is a sticky problem. Science 224, 375–377.

Mettler, E., Carpenter, B., 1999. Hygienic quality of floors in relation to surface texture. Trans IChemE 77, Part C.

Michalski, M.C., Desobry, S., Hardy, J., 1998. Adhesion of edible oils and food emulsions to rough surfaces. Lebensmittel- Wissenschaft und Technologie 31, 495–502.

Michiels, C.W., Schellekens, M., Soontjens, C.C.F., Hauben, K.J.A., 1997. Molecular and metabolic typing of resident and transient fluorescent pseudomonad flora from a meat mincer. Journal of Food Protection 60, 1515–1519.

Miettinen, M.K., Palmu, L., Björkroth, K.J., Korkeala, H., 2001. Prevalence of *Listeria monocytogenes* in broilers at the abbatoir, processing plant, and retail level. Journal of Food Protection 64 (7), 994–999.

Percival, S.L., Knapp, J.S., Wales, D.S., Edyvean, R., 1998. Physical factors influencing bacterial fouling of type 304 and 316 stainless steels. British Corrosion Journal 33, 121–129.

Senczek, D., Stephan, R., Untermann, F., 2000. Pulsed-field gel electrophoresis (PFGE) typing of *Listeria* strains isolated from a meat processing plant over a 2-year period. International Journal of Food Microbiology 62, 155–159.

Stout, K.J., Blunt, L., 1995. Nanometres to micrometres: three-dimensional surface measurement in bio-engineering. Surface and Coatings Technology 71, 69–81.

Tide, C., Harkin, S.R., Geesey, G.G., Bremer, P.J., Scholz, W., 1999. The influence of welding procedures on bacterial colonization of stainless steel weldments. Journal of Food Engineering 42, 85–96.

Unnerstad, H., Bannerma, E., Bille, J., Danielson-Tham, M.-L., Waak, E., Tham, W., 1996. Prolonged contamination of a dairy with *Listeria monocytogenes*. Netherlands Milk and Dairy Journal 50, 493–499.

Verran, J., Rowe, D.L., Cole, D., Boyd, R.D., 2000. The use of atomic force microscope to visualise and measure wear of food contact surfaces. International Biodeterioration & Biodegradation 46, 99–105.

Wang, X.Y., Wu, Y.S., Zhang, L., Yu, Z.Y., 2001. Atomic force microscopy and X-ray photoelectron spectroscopy study on the passive film for type 316L stainless steel. Corrosion 57, 540–546.

Wirtanen, G., Aloha, H., Mattila-Sandholm, T., 1995. Evaluation of cleaning procedures in elimination of biofilm from stainless steel surfaces in open process equipment. Food and Bioproducts Processing 73, 9–16.

Zottola, E.A., Sasahara, K.C., 1994. Microbial biofilms in the food processing industry—should they be a concern? International Journal of Food Microbiology 23, 125–148.

Zuo, Y., Wang, H., Xiong, J., 2002. The aspect ratio of surface grooves and metastable pitting of stainless steel. Corrosion Science 44, 25–35.