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Influence of Surface Features on Microbial Colonization and Susceptibility to Corrosion of Stainless Steels Used in the Food Processing Industry

Geesey G.G., Gillis R.J., Zhang H -J, and Bremer, P.J

ABSTRACT

Surfaces of equipment used in the food processing industry are sources of microbial contamination of foods. Surface smoothness influences cleansability and disinfection of the equipment that comes in contact with the food. Stainless steels (ss) are commonly used as a material of equipment construction due to their hygienic properties. Proper treatment of stainless steel prior to commissioning for service can maximize surface smoothness and minimize initiation of localized corrosion, particularly at welded areas. "As received" 316L ss, which has been pickled but not polished, possesses a surface oxide film characterized by oxide grains and grain boundaries. The latter represent depressions in the film that are selectively colonized by bacteria found in corrosion deposits. Facultatively anaerobic bacteria and obligately anaerobic sulfate-reducing bacteria grow in intimate relationship to each other in a biofilm, selectively depleting the chromium and iron relative to nickel in subsurface regions of the oxide film at the oxide grain boundaries. Selective microbially-induced depletion of Cr in the surface oxide film may render the grain boundaries susceptible to localized attack and possibly pitting corrosion in the presence of Cl^- ions. The results reveal the need to treat stainless steels to achieve a homogeneous, passivating surface oxide film in order to minimize selective microbial colonization and microbially-induced chemical changes in the film that compromize film integrity and function.

Keywords

Microorganisms, Biofilms, Stainless Steels, Welds

Author Details

Dr. G. Geesey is professor of Microbiology. R. Gillis and H.-J Zhang are researchers at the Center for Biofilm Engineering at Montana State University, Bozeman, Montana, U.S.A.

Dr. P. Bremer is a microbiologist at the Seafood Research Unit, Crop & Food Research, Port Nelson, New Zealand.

Stainless steel (ss) is the most widely used material for food service equipme where food contact occurs.. This is due to the fact that it meets all of the requirements established by the food industry, including: corrosion resistance, durability, cleansability, economy, eye appeal and food flavor protection [1].

1 SURFACE PROPERTIES OF FOOD PROCESSING EQUIPMENT AND REQUIREMENTS OF THE FOOD PROCESSING INDUSTRY

Surfaces have been viewed as the most likely route of microbial contamination of food products [2]. The construction of dairy processing plant surfaces must be of such design to ensure products remain free from contamination of pathogenic and spoilage microorganisms. One of the factors that may influence biofilm accumulation on surfaces is surface roughness, R_a. The food processing industry specifies that surfaces in contact with products must be as smooth as possible. Rough surfaces are more difficult to clean than smooth surfaces because they offe greater surface area for microbial colonization. Welded regions of metal surfaces are prone to increased roughness compared to adjacent unwelded areas unless special care is taken to grind and polish the weld area. Ease of surface cleaning is directly related to ease of surface disinfection. Stainless steel exhibits excellent disinfection properties when sufficient surface smoothness is achieved.

A No. 4 surface finish is specified for stainless steel surfaces that are likely to com in contact with food during preparation. Such a surface finish will achieve a su ac roughness (R_a) value of 1 μm or less. At welded areas, this usually requires grinding using silicon carbide 150 grit to achieve minimal surface roughness. These treatments yield a stainless steel surface that is characterized by smooth shallow grooves [2]. Excessive polishing has been responsible for the loss of structural strength of welds and ultimately to failures of ss in dairy vessels. Depending on specific application, a variety of surface treatments have been described that, whe applied to stainless steels, permit a wide range of performance standards to be me [3].

2 ATTACHMENT OF BIOFILM-FORMING, FOOD-BORNE BACTERIA TO STAINLESS STEEL SURFACES

2.1 Biofilms

Microorganisms that have attached to and replicated on surfaces form what is popularly refered to as a biofilm [4]. Many food-borne pathogens such as *Salmonella spp., Yersinia spp.* and *Listeria monocytogenes* form biofilms on food preparation surfaces such as stainless steel. Food spoilage microorganisms also can exist in biofilms [5]. A typical biofilm may contain 10^7 cells/cm^2 bacterial cells, but cells can pile up on each other to produce thick, slimy deposits that retain as many as 10^{10} cells/cm^2 . Significant cell densities can accumulate within hour after surface cleaning and disinfection [5,6].

Microbial biofilms need not be visible to the naked eye to be a problem in the food industry. Low numbers of colony forming units (cfu) recovered on selective culture medium from swabs passed across the surface may not reflect the total densities of microorganisms adhering to the surface that could be transfered to food which comes in contact with the surface. Direct microscopic examination of the surface is necessary to appreciate the true extent of microbial surface contamination.

Numerous studies have described the attachment of food-borne pathogens to stainless steel surfaces. Adherence of the potential human pathogen *Yersinia enterocolitica* to 304 ss submerged in nutrient-rich broth was observed over a temperature range of 10-35C [7]. Although a No.4 surface finish was applied to the 304 ss test surface, electron microscopic examination revealed the presence of grooves and crevices where bacteria attached and multiplied. Whereas flagellar structures did not appear to be required for attachment in this study, fibrils were often seen extending from attached cell to the ss substratum.

Attachment of *L. monocytogenes* to four milk contact surfaces at ambient and cold storage temperatures was the subject of another study [8]. Again, 304 ss with No. 4 finish was used as the test substratum. The test surfaces were submerged in nutrient-rich broth for periods of 20-60 min and then examined by electron microscopy. Grooves and crevices in the ss surface were sufficiently large to harbor cells of *L. monocytogenes*. Initially, cells attached without evidence of visible adhesive structures. Extracellular material, however, appeared after 1 h incubation. No correlation was observed between the surface irregularities and the ability of the bacteria to attach to that particular surface.

In another study, 304 ss coupons with a No. 4 finish were suspended in raw milk bulk storage tanks or exposed to skim milk suspensions of *Pseudomonas fragi, Streptococcus lactis, Streptococcus cremoris, Staphylococcus aureus*, and *Lactobacillus bulgaricus*[9]. No attachment structures were observed for adherent cells of *S. lactis, S. cremoris, S. aureus*, and *L. bulgaricus*, suggesting that these bacteria were adhering through physical attractive forces. Adhesive molecules may exist, however, that elude surface examination by conventional electron microscopy. Fibril attachment structures were observed, however, for attached cells of *P. fragi*. Microorganisms were seen in depressions of the polished surface. Residue from the bulk liquid phase was also seen adsorbed to the ss surface. The above studies suggest that even though a No 4 finish is applied to the stainless steel surface, features still exist that facilitate bacterial attachment and biofilm development.

2.2 Selective Microbial Colonization of Surface Features on Stainless Steel

Surface features influence the colonization behavior of microorganisms on stainless steels and other metals [10]. Microorganisms accumulate preferentially at inclusions,creating a heterogeneous distribution of microorganisms across the

surface. This gives rise to biologically-active "hot spots" on the surface. The inclusions may arise from the presence of contaminants during fabrication or through poorly controlled heating either during fabrication or later on as a result of welding. Segregation of elements in the bulk material is a difficult phenomenon to control during welding.

Surface features of the oxide film appear to influence attachment and colonization of stainless steel surfaces [11]. Unpolished, mill-run 316L ss sheet cut from a coil of product that had been hot rolled (2300°F), pickled and passivated, cold-rolled, solution annealed at 2050°F, rapidly cooled and subjected to a final cold role to flatten and shape before receiving a 2B finish ("as received") exhibited a surface film that was characterized by islands of oxide grains separated by grain boundarie (Figure 1).

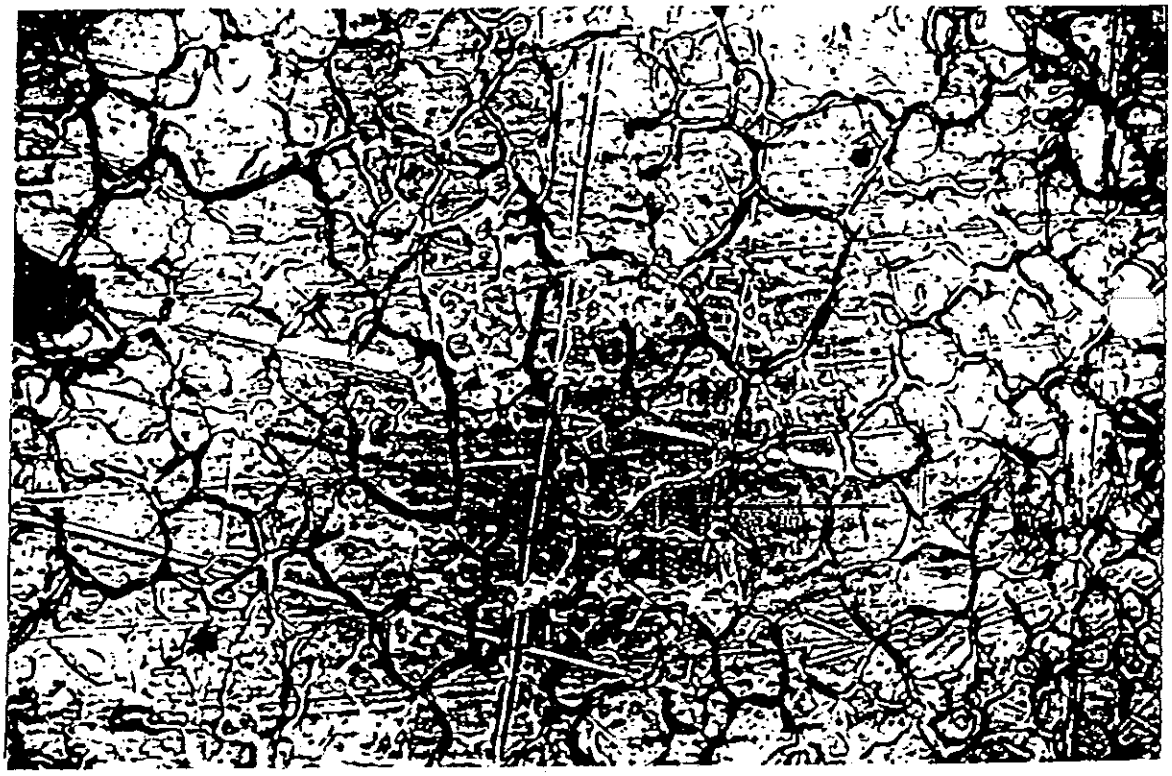

Figure 1

Scanning laser confocal micrograph of surface of "as received" 316L stainless steel coupon from rolled coil which received a 2B finish. Note the islands (i) of the surface oxide film and the surrounding grain boundaries (GB) which, along with the milling lines, make up the dominant surface features of the alloy. Bar equals 25 μm.

The oxide grain boundaries appeared as 1.6 μm wide,0.6 μm deep depressions in

the surface film when evaluated by atomic force microscopy [11]. These depressions were sites of selective attachment and colonization by bacteria recovered from tubercles of corroding service water piping in a nuclear plant (Figure 2).

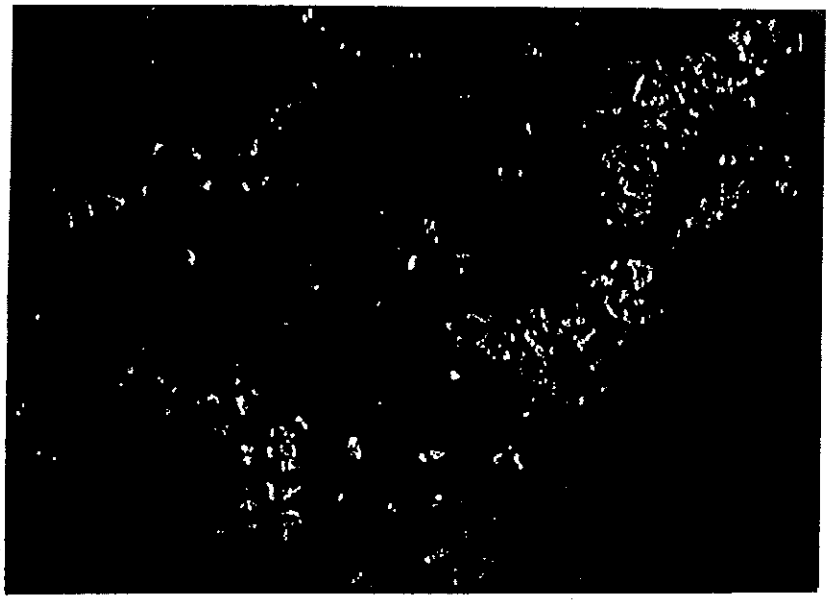

Figure 2

Scanning laser confocal micrograph of "as received" 316L stainless steel coupon cut from rolled coil which received a 2B finish. Red spots represent individual bacterial cells which have colonized the surface of the alloy following submersion in aqueous medium inoculated with bacteria isolated from a tubercle on a corroding stainless steel pipe. The bacteria were stained with the fluorochrome acridine orange and visualized by epiillumination.

Thirty to ninety five percent of the total bacteria associated with the surface of the metal were concentrated in or adjacent to the oxide grain boundaries, which represented 10-30% of the total surface area (Figure 3).

These differences were significant at the confidence level of $p<0.0001$. Selective colonization of the depressions in the ss surface defined by the oxide grain boundaries was maintained for periods of at least 1 month exposure to bacteria-containing, bulk aqueous phase [11]. These surface features are removed by mechanical or electropolishing producing a surface that is colonized randomly by adherent bacteria [12].

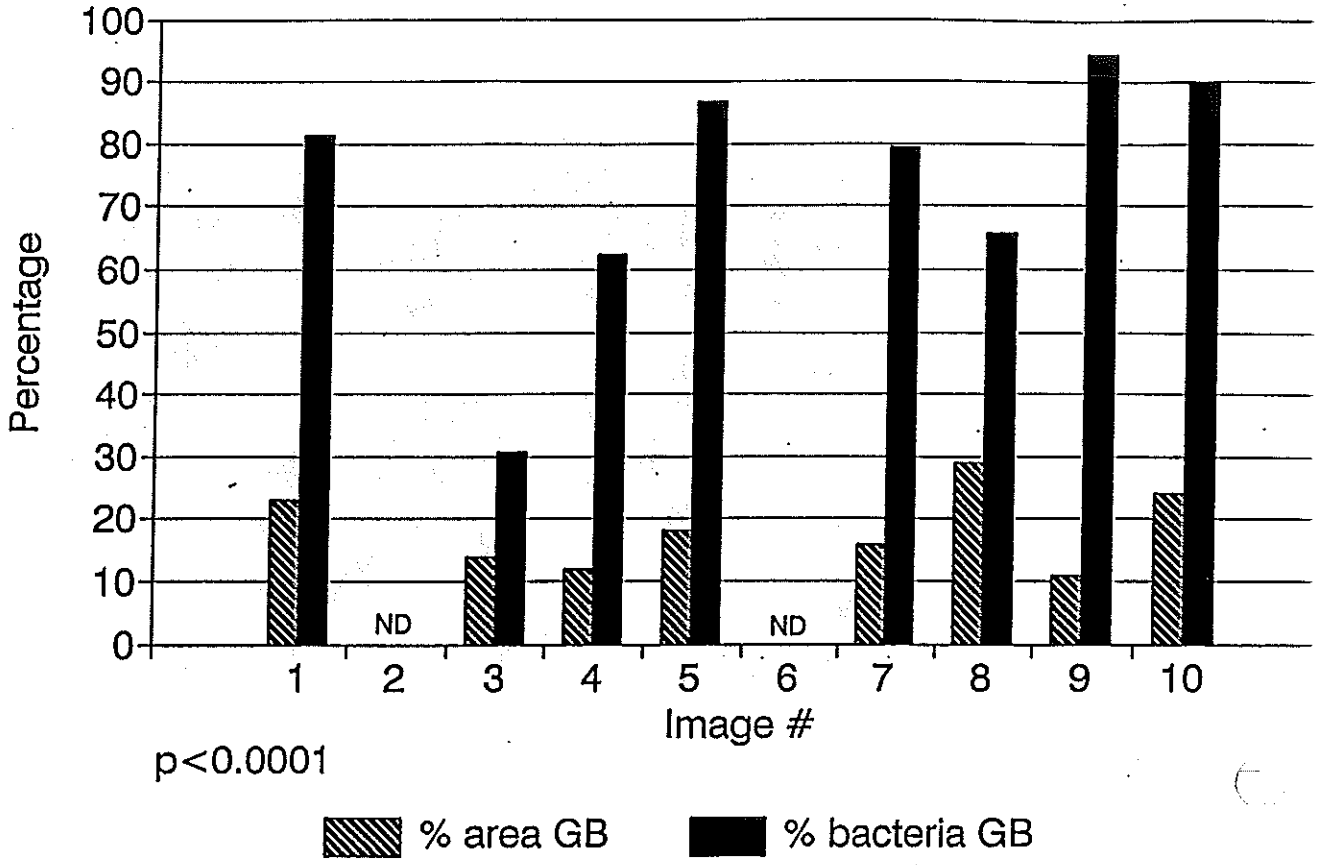

Figure 3

Comparison of fraction of total area of 316L ss coupon, prepared as in previous figures, that was contributed by grain boundaries around the islands of oxide formed on the surface of the alloy with the fraction of total bacteria (*Citrobacter freundii*) attached to the surface that were in or immediatly adjacent to the grain boundaries. Data is presented for 8 separate areas (image #) of the coupon. Fractions are expressed as percentage. The differences are significant at the p value indicated.

2.3 Elimination of Biofilms from Stainless Steel Surfaces

304 stainless steel was found to be more easily cleaned and disinfected by a variety of products than synthetic polymer surfaces [2,13]. Populations of *L. monocytogenes* attached to ss surfaces were effectively removed and inactivated

by the various treatments studied. Studies have shown that mechanical means are the most effective way to eliminate biofilms from surfaces [6]. Materials that resist surface changes like stainless steels, will remain more hygienic when subjected to natural wear than material that becomes more readily damaged by cleaning and disinfection. The smoother the surface, the more effective the mechanical cleaning process can be. Mechanical cleaning in the food industry most frequently takes the form of brushing or swiping the surface with a cloth. Neither of these cleaning methods will induce changes to stainless steel surfaces. However, chemical cleaning procedures used in conjunction with mechanical treatments, may alter the surface roughness and chemistry. Acid-alkali or alkali-acid treatment can, over time, influence the integrity of the stainless steel surface.

2.4 Influence of Surface Features Contributed by Welding on Microbial Colonization of Stainless Steel Surfaces

Under-deposit pitting of stainless steels has been frequently observed at weldments. Because microorganisms are found in and under the deposits, they have been implicated in the corrosion process [14]. A question that has been asked is why are the deposits more inclined to develop at welds than randomly across the metal surface? Based on the assumption that microorganisms, in general. control deposit formation, it has been proposed that microorganisms are preferentially attracted to the welded areas.

The Dairy Research Institute (DRI) of New Zealand compared the biofouling resistance of surfaces of stainless steels subjected to different welding procedures (gas tungsten arc welding, gas metal arc welding, flux cored arc welding manual metal arc welding, plasma arc welding and submerged arc welding) as well as those subjected to grinding and polishing [15]. There was no difference in the numbers of bacteria detected between welded sites and adjacent unaffected parent metal. Similarly, there was no difference in the numbers of bacteria at welded areas and polished areas. However, numbers of bacteria alone do not necessarily reflect the diversity of bacteria present nor their activities.

Biofilm accumulation was found to be independent of the welding process. In this study, biofilm biomass was based on recoverable viable cells and ATP measurements from swabs drawn across standardized areas of test coupons suspended in raw milk for 72 h or in monocultures of test bacteria (*Pseudomonas fluorescens, Bacillus subtilis* or *Bacillus cereus*) for 18 h. It was concluded that the difference in roughness between polished stainless steel and welds <1 μm vs 6-10 μm) may not be enough to affect kinetics of biofilm development [15]. These measures of biofilm biomass have limitations that could make them insensitive to roughness-related surface parameters.

Dowling et al. [16] could not distinguish between a chemical or microbiological source for initiation of 304/308 or 316/308 welds. They did demonstrate selective

recruitment of iron-oxidizing bacteria to areas of the surface undergoing active corrosion, however. Total culturable heterotrophic bacterial densities were shown to be approximately 10-fold higher on corroding surfaces than non-corroding surfaces. Thus, it remains to be determined whether microorganisms selectively colonize welded areas before initiation of corrosion. Hence, their role in the initiation stages of corrosion has yet to be demonstrated.

3 CORROSION RESISTANCE OF STAINLESS STEELS

3.1 Passivating Oxide Film

The superior corrosion resistance of ss is believed to be due to its passivity. The oxide film theory has been proposed as an explanation for the passivie behavior. Thin metal oxides, primarily composed of hydrated chromium oxyhydroxide, form a passive coating, creating a barrier to diffusion and preventing exposure of the base metal to the environment, thus, reducing the corrosion rate of the alloy [17]. The passive coating may be only a few atoms thick, but thickness can vary depending on surface preparation and history. The abundance of elements comprising the oxide film can also vary depending on pretreatment [17]. In some cases, Cr is depleted at the outermost portion of the film while in other situations, this element can be enriched in this region. In general, as the Cr content of the film increases, film thickness decreases.

While the response of the oxide film to abiotic environmental factors has been extensively studied, little is known of the effect microbial biofilms have on its passivity, chemistry, and structure. "As received", mill-run 316L ss possesses an oxide layer that may be characterized as a mozaic of islands of oxide grains with a mean surface area of 92 μm^2 separated by grain boundaries with dimensions previously described. Such surface oxide layers are susceptible to microbially-induced chemical changes that render the underlying bulk alloy vulnerable to localized attack at the oxide grain boundaries [11]. Oxide films colonized by the facultative anaerobe *Citrobacter freundii* and the sulfate-reducing bacterium (SRB) *Desulfovibrio gigas*, two bacteria isolated from a tubercle on a corroding stainless steel pipe, exhibit subsurface depletion of Cr and Fe relative to Ni at the oxide grain boundaries where biofilms of these bacteria seem to initiate. The sulfate-reducing bacteria were only found in significant numbers in areas that contained high densities of *C. freundii.* Studies using anaerobic batch cultures demonstrated that *D. gigas* required *C. freundii* or other bacteria for growth (Figure 4) [18]. The presence of the SRB enhanced the depletion of Fe relative to Ni over that observed in the presence of *C. freundii* alone [11]. No significant depletion of either Cr or Fe occurs at the predominantly uncolonized grains or at the grain boundaries of material exposed to sterile but otherwise identical conditions [11]. Depletion of Cr at bulk metal grain boundaries has been suggested to compromise the corrosion resistance of this alloy and may facilitate localized attached and subsequent pitting

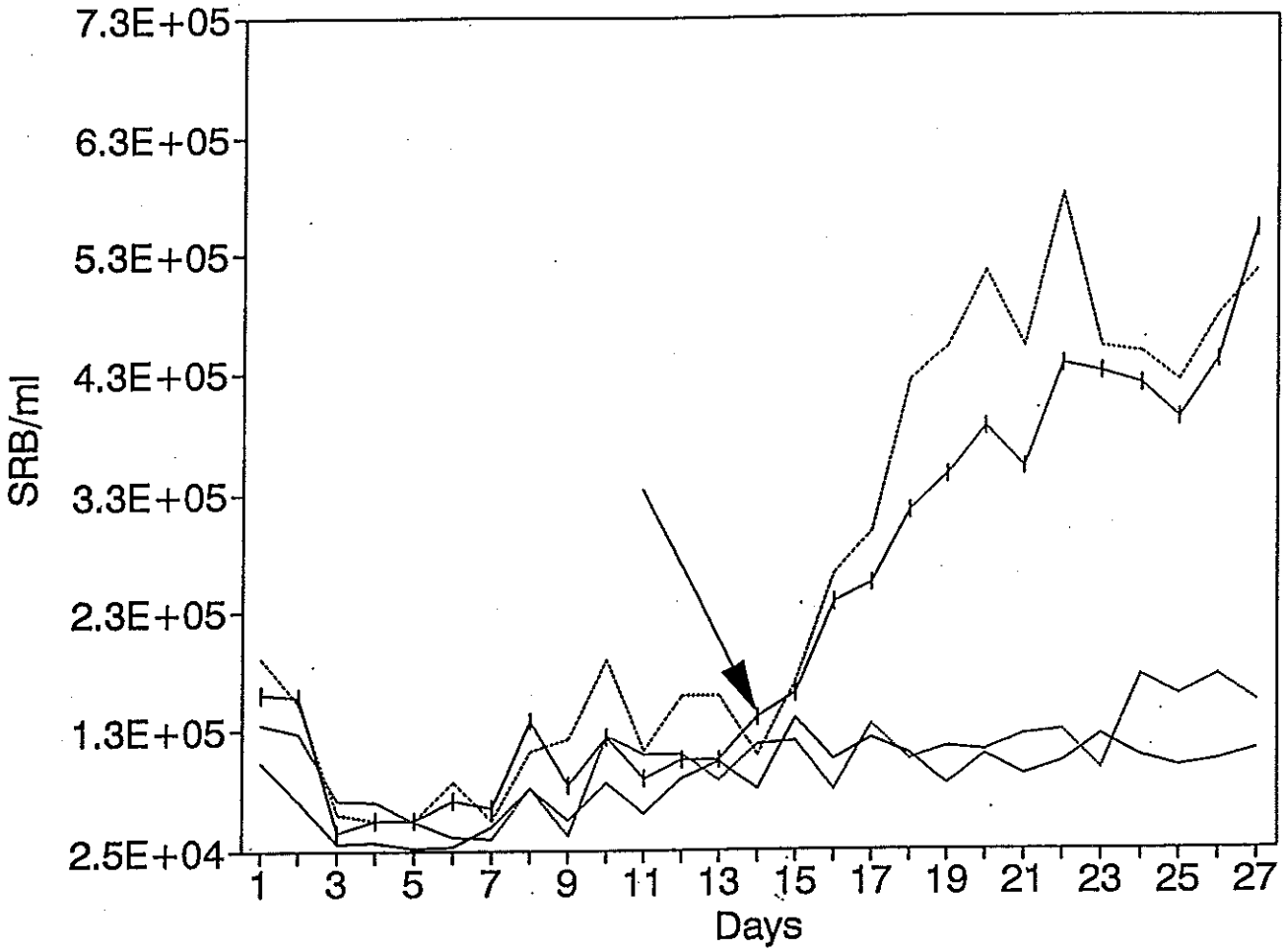

Figure 4

Anaerobic batch cultures of *D. gigas* in the presence (--- , ——) and absence (·····,—) of *C. freundii.* Arrow indicates time at which *C. freundii* was introduced to the culture containing *D. gigas.* Note the immediate increase in *D. gigas* (SRB) cell densities upon inoculation of *C. freundii.*

corrosion at these surface features [19].

Mechanically or electrochemically polished material exhibits a homogeneous oxide film with no evidence of grains and grain boundaries [12]. Chemical passivation ensures a more-or-less thin but continuous oxide film resistant to localized attack and pitting corrosion.

3.2 Pitting Corrosion

If the surface of ss is in contact with aqueous solutions that contain Cl^- ions and

oxidizing agents such as oxygen, pitting corrosion may occur [20]. The Cl^- ions replace the oxygen atoms at defects in the passive film, thereby promoting the destruction of the film. Experience has demonstrated that susceptibility to pitting corrosion is related to the critical pitting potential E_{cpp}, which is the most noble potential above which pitting will initiate. E_{cpp} has been used to predict pitting corrosion [21,22]. The pitting potentials increase to higher values as the Cr and Mo content of the ss is increased. It is thought that the Cr and Mo content of the bulk alloy influences the abundance of these elements in the oxide film and its subsequent passivity and resistance to breakdown. A number of other factors such as temperature, aeration (flow) and the presence of a biofilm also influence the pitting potential, possibly through the effect they have on the access of bulk solution oxygen to the oxide film. Surface preparation is reported to have little effect on resistance to pitting [23].

3.3 Influence of the Presence of Microbial Biofilms on Electrochemical Properties of Stainless Steel

It is well-known that biofilms increase (enoble) the open circuit potential (OCP) or corrosion potential toward the critical pitting potential (E_{cpp}) for passive metals such as stainless steels when submerged in seawater [24]. There is a rough correlation between the amount of extracellular polymeric substance (EPS) that is produced and the OCP. Activities of specific biofilm populations rather than biofilm biomass seem to control the OCP. Mixed population biofilms are able to maintain a higher corrosion current than monoculture biofilms [25]. A positive correlation has been reported between the presence of sulfate-reducing bacteria at anodic sites and corrosion current [25].

Theoretically, potential enoblement caused by biofilms should increase the probability of initiation of pitting and crevice corrosion. Biofilms significantly increased the E_{cpp} for 304 ss but not 316 ss in seawater [21,22]. E_{cpp} has been proposed to be a useful parameter to predict the initiation of pitting for low resistance stainless steels like 304 when biofilms are present [21,22].

3.4 Susceptibility of Weld Metal to Corrosion and Remedial Measures to Minimize Attack

Much of the ss employed by the food processing industry has been welded to meet specific design requirments. Although 304 ss, 316 ss, and other 18% Cr-8% Ni stainless steels have a fully austenitic structure in the solution treated state, their structure is unstable when cold-worked or welded.

Scale, heat tint, welding slag residues and contamination disturb the formation of the passive oxide film, thus reducing corrosion resistance. Therefore, the last step in the manufacturing process should be cleaning of the stainless steel surface to remove these surface features before the material is placed in service. Corrosion

resistance of austenitic stainless steels followed the order of surface treatments: pickling process > grinding > blasting [23]. Increasing surface roughness correlated with decreasing corrosion resistance [23].

3.5 Infuence of Biofilms on Corrosion-Resistance of Welded Stainless Steels

OCP and polarization resistance (R_p) were used to correlate corrosion susceptibility and bacterial activity with alloy welding parameters [26]. R_p of "as received" welded samples in a sulfate-reducing bacteria-enriched lake water was significantly lower than the comparable sample in sterilized lake water. The authors proposed that the R_p was due to a reaction other than corrosion potential; possibly charge transfer reactions within biofilms on the surface. It was not possible to distinguish between charge transfer reactions related to corrosion and those related to biofilm bacterial activities.

Exposure of 304L ss and 316L ss to microbially-contaminated groundwater for 140 days led to nodule formation on heat-tint regions of "as received" welded metal at locations where crevice corrosion was encouraged [27]. No nodules were detected where the thermal oxides, formed at heat tint regions as a result of welding, were removed by pickling, electopolished or mechanical grinding.

Nodules developed on surfaces of 304L and 316L ss following exposure to simulated river water in the presence and absence of bacteria isolated from previously corroded metal [28]. Nodule formation was observed at crevice sites within weldment heat-tint areas of these alloys. The alloys were welded using a gas-tungsten-arc in the down-hand position using 308L or 316L filler metal. Major differences in corrosion potential and anodic polarization behavior were evident, however, between specimens exposed to biotic and abiotic conditions. Fluctuations in OCP were only observed under biotic conditions [28]. Control studies showed, however, that nodule formation was not the cause of the changes in electrochemical behavior. Corrosion rates were higher for the specimens exposed to bacteria. Chloride concentration in the bulk solution was found to play a key role in the formation of nodules. Removal of the oxide layer by chemical or electrochemical means made the unwelded surface as well as the welded area susceptible to nodule formation regardless of the presence of bacteria. The nodules were believed to be crevice-related and the bacteria were thought to only accelerate the rate of corrosion through their activities.

A study of the susceptibility of various weld metals deposited on 304 ss by the shielded metal arc welding process was conducted in a sewage wastewater effluent [29]. After 10 days tubercles of soft corrosion product appeared on the weld and grew slowly with time. Pitting corrosion was observed under the tubercles. Under some tubercles the austenitic phase had been preferentially attacked leaving the ferritic dendritic structure intact with no pit formation. The latter contained a high Cr content. In long-term exposure studies (240 days),

bacteria were observed on the weld metal surface around the pits. Tubercles did not develop on the surface of weld metal subjected to sterile but otherwise similar conditions. It was concluded that localized corrosion on weld metal surfaces exposed to nutrient-rich sewage water was influenced by microorganisms [29].

In another study, pits in 308 ss weld metal induced by microorganisms displayed concurrent attack of the ferrite phase and austenite phase and interfaces between these phases [30]. Whether there is a relationship between biofilm population structure and activity and phase stability remains to be determined.

CONCLUSIONS

Stainless steels are widely used in the fabrication of equipment for the food preparation industry due to the ease in which it can be cleaned and disinfected of biofilm-forming microorganisms. Surface smoothness is a key attribute in this regard. Surfaces should be pickled and polished to achieve a uniform, continuous, protective oxide film. Failure to produce a continuous oxide film will lead to selective colonization of oxide film grain boundaries by microorganisms that promote selective depletion of Cr and Fe at these features. Depletion of Cr is believed to decrease resistance of the bulk alloy to localized corrosion. These type of influences by surface colonizing microorganisms may typify their role in pit formation in stainless steels. Microbially influenced corrosion of stainless steel also increases surface roughness, making it more difficult to clean and disinfect, compromising the hygienic character of the material.

ADDENDUM

More information on biofilms and their consequences in the food industry can be found in recent reviews on the subject [31,32].

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