Influence of Surface Finish on the Cleanability of Stainless Steel

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ABSTRACT

Stainless steel for fabricating food processing equipment is available with various surface finishes. The objective of this research was to determine the effect of surface finish on cleanability. Nine samples of stainless steel, type 304, from various manufacturers including no finish (hot rolled and pickled), #4 finish, 2B mechanical polished, and electropolished were tested. Cleanability was assessed by using coupon samples soiled with either cultured milk inoculated with spores of *Bacillus stearothermophilus* or by growth of a *Pseudomonas* sp. biofilm. Samples were cleaned by immersion in a turbulent bath of 1.28% sodium hydroxide at 66°C for 3 min followed by a sterile water rinse, neutralizing in 0.1% phosphoric acid for 30 s, rinsing in phosphate buffer, sanitizing in 100 ppm hypochlorite, neutralizing in sodium thiosulfate, and drying. To determine residual milk soil, coupon samples were covered with PM indicator agar and incubated for 25 h at 58°C. Other coupons were subjected to an additional 10 soiling or cleaning cycles, and the residual protein was measured by using epifluorescent microscopy and image analysis. Results indicate that the spore count was more precise for measuring initial cleanability of the finished samples, and the protein residue determination was useful for determining the effect of repeated cleaning. Data on the removal of milk soil suggest that stainless steel should be purchased based on measures of surface defects rather than finish type. Surface defects, as determined using a surface roughness gauge, produced a correlation of 0.82 with spore counts. Data also indicated that biofilm was more difficult to remove than milk-based soil.

Stainless steel manufacturers sell product having different types of finishes and promote specific finishes for different applications. A standard milled or rolled (2B) finish is sometimes used for food applications, but because manufacturers cannot guarantee a pit-free rolled finish, most companies choose at least a #4 (150 grit) finish. Some processors prefer a #7 finish or 320-grit polish, while others prefer an electropolished surface. In the electropolishing process, surface metal is removed by anodic dissolution. Pitting and projections are minimized by leveling, that is, macropolishing and micropolishing. Because electropolishing reduces surface roughness, electropolished stainless steel surfaces may be more sanitary (7).

Previous research on the cleanability of stainless steel demonstrated that hot alkali detergent, rinsing, and sanitizer application were necessary for adequate removal of soil and microorganisms in a clean-in-place system (5, 15, 17). These studies suggest that surface defects reduce cleanliness because microbes in crevices are protected from cleaning chemicals by being coated with soil. Kauffman et al. (10) found that stainless steel with finishes 2B, #3, #4, and #7 soiled with milk were cleaned equally well. Milk soil is readily removed by chemical and physical cleaning procedures, so bacteria embedded in the milk soil are also removed because milk surrounds the bacteria preventing them from attaching directly on surfaces (12). On the other hand, biofilm may be more difficult to remove than some food soils because bacteria produce adhesive substances that firmly attach the cells to the substratum (16).

The purpose of this study was to determine the effect of surface finish on the ability of a clean-in-place process to remove milk-based soil and biofilm from stainless steel surfaces with different finishes. Our hypothesis is that surface roughness measures will correlate with the ease of soil removal.

MATERIALS AND METHODS

Stainless steel. Surfaces tested include two samples of hot rolled and pickled, three of #2B, three of #4, and one electropolished, all type 304. They were obtained from five different manufacturers. Stainless steel sheets were cut into coupons (7.5 by 11 cm).

Stainless steel coupon preparation. The stainless steel coupons were degreased with acetone, then washed in a dishwasher with alkali detergent (Lab-Dish I Concentrate, Labconco Corp., Kansas City, Mo.), and rinsed with deionized water. Coupons were submerged in deionized water then autoclaved.

Spore preparation. *Bacillus stearothermophilus* (ATCC 10149) was grown on nutrient agar with 0.082 g/liter of MnSO_4_ at 58°C for 2 to 3 days (1). Spores were harvested and suspended in phosphate buffer, then washed twice and concentrated by centrifugation (4,000 × g) for 15 min. The spore suspension (7 ml) was heated at 95°C for 5 min, cooled to 15 to 25°C, then stored at 5°C for 1 to 2 weeks prior to use. Spores were enumerated by plating on PM indicator agar (Difco, Detroit, Mich.) incubated at 58°C for 24 h.

Soiling procedure. *B. stearothermophilus* spore suspension was added (10^6 cells/ml) to buttermilk made from ultrahigh-temperature sterile milk. Inoculated buttermilk (0.2 ml) was spread over a 50-cm^2 area of the stainless steel coupon, which was dried at 90°C for 10 min and then cooled to room temperature.
Biofilm formation. *Pseudomonas* spp. (isolated from a meat plant environment) was transferred twice from tryptic soy broth (Difco) then inoculated (0.1%) into 500 ml tryptic soy broth. Coupons were submerged in broth, incubated at room temperature for 24 h, and rinsed in phosphate buffer to remove unattached cells.

Cleaning procedure. The cleaning system consisted of a Buchi 461 waterbath (Fisher Scientific, Norcross, Ga.) with a spindle mixer, model 1750 (VWR, Atlanta, Ga.) with an impeller 6.35 cm in diameter. The coupon holder had a radius of 11.75 cm. Six coupons, spaced 2.54 cm apart, were placed on the circumference of the holder. Soiled coupons were placed vertically on a stainless steel holder in a bath circulating at a turbulent flow of Reynolds number 14680, in 1.28% sodium hydroxide (pH 13.0), at 68°C for 3 min, then were rinsed in distilled water for 10 s, immersed in 0.1% phosphoric acid (pH 2.5) for 30 s, and immediately rinsed in phosphate buffer. The washed coupons were immediately sanitized in 100 ppm chlorine for 20 s, which was neutralized by placing the coupons in 2 to 10 ppm sodium thiosulfate. After a final rinse in phosphate buffer, the coupons were allowed to air dry. After initial evaluation of cleaning, stainless steel coupons were subjected to 10 additional soil and clean cycles in the clean-in-place system.

Determination of residual soil by direct surface plating. Residual milk soil was determined by pouring PM indicator agar over 50 cm² of each cleaned, dry surface as described previously (6). Coupons with agar overlay were incubated for 24 h at 58°C. Counts were recorded as CFU/cm².

Determination of residual protein and biofilm by microscopic observation. Samples were evaluated for residual protein after 11 soiling and cleaning cycles by using direct microscopic observation. Two samples each, from the control and soiled stainless steel surfaces, were stained with fluorescein-5-isothiocyanate (0.1 mg/ml, pH 8; Sigma Chemical Co., St. Louis, Mo.) and incubated in the dark for 30 min at room temperature, rinsed with deionized water, and allowed to air dry for 30 min. The stained surfaces were viewed under a Nikon digital fluorescent microscope with a ×20 objective. The digital microscope was equipped with a 100-W halogen lamp and a blue fluorescein-5-isothiocyanate filter. The images were collected and the data analyzed using a Sony digital image analyzer. Fifteen fields per sample, of size 0.91 mm², were observed and analyzed. Results are reported as percent area covered after subtracting fluorescence associated with unsoiled controls.

Surface roughness determination. Surface roughness profile was determined by using a Hommel Tester, model T500 (Hommel America Inc., New Britain, Conn.). Data were collected for the following parameters: average roughness (Rₐ); mean peak to valley height (Rₛ[DIN]); maximum peak to valley height within one cutoff zone (Rₘₐₓ); and root mean square of the deviation of the profile from the mean line (R₉). Data were reported as the means of four scans.

Experimental design. In the protein residue experiment, there were three replicates in each trial, with three trials in the experiment. Surfaces were cleaned and evaluated after first cleaning and again after 10 times cleaning. In the biofilm experiment, there were two replicates in each trial with five trials in the experiment. All data were analyzed using the general linear model and correlation procedures of the Statistical Analysis Systems (SAS Institute, Cary, N.C.). Duncan’s multiple range test was used to determine differences among means when significant effects were observed. A significance level of 5% was employed.

RESULTS AND DISCUSSION

Researchers have used various methodologies to evaluate cleaning effectiveness including direct plating for seeded spores, agar contact methods, and radiolabeling of microorganisms (4, 10, 14). In this study, direct agar overlay for determining residual spore counts was found more useful than direct microscopic examination of stained samples for determining removal of milk soil (after one soiling and cleaning cycle), because the spore count method provided greater differentiation among samples (data not shown). After 11 soiling and cleaning cycles with milk-based soil, direct staining for protein was used to evaluate soil residual because repeated cleaning inactivated the spores. Similarly, biofilm soil was evaluated by direct microscopic observation of stained samples because the spores are not readily incorporated into the biofilm.

Evaluation of unpolished stainless steel. Methods employed in this study for evaluating cleaning effectiveness were found unsuitable for use with unpolished samples (hot rolled and pickled). Residual spore counts did not reflect the amount of residual soil observed by direct staining. This may be because thicker layers of residual protein on the unpolished surfaces caused inconsistent spore germination. Direct staining of residual protein or biofilm also did not provide accurate data. Photomicrographs of the stained coupons are present in Figure 1A and 1B. The photomicrographs show that much of the soil is out of the focal plane. The roughness of the unpolished surfaces provides multiple focal planes for soil to accumulate, the result being that determination of percent area covered based on one focal plane underestimated the total amount of soil coverage. Our judgment is that the amount of this underestimation was too inconsistent for data to be of value. Therefore, we are providing no quantitative evaluation of the two unpolished (hot rolled and pickled) samples included in this study.

Polished stainless steel with milk soil evaluated after one cleaning cycle. Residual spore count data (after one cleaning cycle) (Table 1) indicated that #4 and both 2B finishes from manufacturer E differed in cleanability compared to surfaces from other manufacturers. These surfaces also had low Rₘₐₓ and Rₛ[DIN] values, indicating that this manufacturer produced a surface lower in defects than others. Our study was limited because all finish types were not provided by each manufacturer. Although we are not able to compare manufacturers directly, our data indicate that surface defect measurements (Rₘₐₓ and Rₛ[DIN]) are more reliable predictors of cleanability than the designated finish type (#4, 2B, electropolished). This conclusion corroborates the results of Kauffman et al. (9) who found no differences in cleanability among electropolished, 2B and #4 finishes.

Electropolished surfaces are often marketed on the premise that surface polishing reduces surface roughness and thereby provides a more cleanable surface. The electropolished surfaces tested in this study were manufactured from steel using a 2B finish. A limitation of electropolishing is that it may not be able to smooth over the deeper valleys found on a 2B finish, the result being a rougher
surface than if a #4 finish is electropolished. Our data indicate that electropolishing provides no guarantee of greater cleanliness because defects in electropolished surfaces originate with the initial mechanical polishing process. Therefore, the surface roughness profile (Table 1) of the electropolished sample (source C) used in this study may reflect more a poor quality of the preelectropolished surface than a poor quality electropolishing process. This surface was noted to have visible scratches, and the surface roughness profile indicated that although peaks were flattened, there were numerous valleys.

**Polished stainless steel with milk soil evaluated after repeated cleaning cycles.** Repeated exposure of stainless steel surfaces to soiling and cleaning creates soil buildup; soils such as milk protein and fat have protective effects on certain microbes and also inactivate chemical sanitizers (2, 3, 13). Therefore, it is useful to determine surface cleanability after repeated soiling and cleaning. Residual spore determination after 11 soiling and cleaning cycles showed a lower level of spores remaining on surfaces than after one cycle cleaning (data not shown) even though direct microscopic observation indicated increased soil accumulation. Kauffman et al. (9) observed a similar phenomenon. The repeated soiling and cleaning cycles may stimulate heat activation and inactivation of spores. Direct microscopic measurement of percent area covered by protein demonstrated soil buildup under with repeated soiling and cleaning cycles (Fig. 1C through 1F). These measurements, however, have limited ability to distinguish different levels of cleanability as statistical analysis places all surfaces into one grouping (Table 1). One interpretation of the protein residual data is that surface finish does not have a significant effect on cleanability; however, there was a high variation associated with this data (coefficient of variation = 88.1), probably a
TABLE 1. Relative cleanability of stainless steel surfaces soiled with milk protein and Pseudomonas sp. biofilm and associated surface roughness values

<table>
<thead>
<tr>
<th>Sources of stainless steel</th>
<th>Surface finish</th>
<th>Residual spore count&lt;sup&gt;b&lt;/sup&gt; (CFU/cm²)</th>
<th>Residual protein&lt;sup&gt;c&lt;/sup&gt; (% area covered)</th>
<th>Residual biofilm&lt;sup&gt;d&lt;/sup&gt; (% area covered)</th>
<th>Surface roughness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R&lt;sub&gt;p&lt;/sub&gt;</td>
</tr>
<tr>
<td>B</td>
<td>#4</td>
<td>29.24 A</td>
<td>0.81 A</td>
<td>6.0 B</td>
<td>0.40 B</td>
</tr>
<tr>
<td>B</td>
<td>#4</td>
<td>25.68 AB</td>
<td>7.36 A</td>
<td>10 AB</td>
<td>0.34 BC</td>
</tr>
<tr>
<td>C</td>
<td>EP&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.71 AB</td>
<td>1.42 A</td>
<td>8.0 B</td>
<td>0.55 A</td>
</tr>
<tr>
<td>A</td>
<td>2B</td>
<td>18.42 B</td>
<td>5.1 A</td>
<td>5.0 B</td>
<td>0.20 CD</td>
</tr>
<tr>
<td>E</td>
<td>2B</td>
<td>5.49 c</td>
<td>5.12 A</td>
<td>9.0 B</td>
<td>0.13 D</td>
</tr>
<tr>
<td>E</td>
<td>2B</td>
<td>4.83 c</td>
<td>2.74 A</td>
<td>16 A</td>
<td>0.22 CD</td>
</tr>
<tr>
<td>E</td>
<td>#4</td>
<td>4.20 c</td>
<td>3.13 A</td>
<td>4.0 B</td>
<td>0.32 B</td>
</tr>
</tbody>
</table>

<sup>a</sup> Letters in columns that are not similar represents data that are significantly different (P ≤ 0.01).
<sup>b</sup> Residual protein stained with fluorescein isothiocyanate. Results after one cleaning cycle.
<sup>c</sup> Residual protein stained with fluorescein isothiocyanate. Results after 11 cleaning cycles.
<sup>d</sup> Residual biofilm stained with fluorescein isothiocyanate. Results after one cleaning cycle.
<sup>e</sup> EP, electropolished.

Consequence of the small amount of area sampled. The use of automated image collection and analysis techniques would provide direct microscopic data that are more sensitive to changes in cleanability because a larger area could then be sampled.

**Biofilm removal from polished stainless steel.** Surface finish had no significant influence on biofilm removal (Table 1) as determined by direct microscopic observation. A 2B finish (source E) was least cleanable with 16% of area remaining covered with biofilm, and a #4 finish (source E) was most cleanable with 4% area remaining covered. The electropolished surface was of intermediate cleanability. Biofilm formation is the result of an active metabolic process during which bacteria produce adhesive substances that firmly attach the cells to surfaces. This may result in a soil that is more difficult to remove than normal food residues. Evidence for this is the large amount of surface (4 to 16%) still covered by biofilm after one cleaning compared to the protein soil remaining after repeated cleaning. The ineffectiveness of the cleaning procedure toward biofilm soil may have obscured any differences in cleanability between surfaces, because biofilm likely remained on relatively smooth areas of the surface. These data suggest that cleaning regimens designed to remove food residues may not be effective in biofilm removal. Additional research is needed to determine if increased exposure times, temperatures, and alkali concentration are required to remove biofilm residue from stainless steel.

**Surface roughness profile determination of polished stainless steel.** Surface roughness parameters determined included R<sub>p</sub>, R<sub>z(DIN)</sub>, R<sub>max</sub>, and R<sub>q</sub>. R<sub>p</sub>, also known as root mean square value, is the square root of the average of the square of the deviation of the mean line. These do not differentiate between peaks and valleys of a surface but are useful for measuring gradual change in roughness due to cutting tool wear. R<sub>q</sub> is more sensitive to peaks and valleys than R<sub>p</sub>. Defects in the surface finish do not greatly influence these values because they are means. R<sub>max</sub> is the maximum peak to valley height within a zone of measurement. This value is useful in assessing the amount of major defects associated with a surface finish. R<sub>z(DIN)</sub> is the mean peak to valley height measurement. To obtain this value, the zone of measurement is divided into five equal zones, the maximum peak-to-valley height is determined for each zone, and the mean of these five values is calculated. This measurement is generally more sensitive to changes in surface finish than R<sub>p</sub>, R<sub>z(DIN)</sub>, and R<sub>max</sub> together are useful to monitor variations in surface finish in a production process (11).

Surfaces with the lowest mean roughness (R<sub>p</sub> and R<sub>q</sub>) values were the 2B samples from source E (Table 1). These surfaces were also among those evaluated as easiest to clean by the residual spore counts (Table 2). The electropolished surface exhibited the highest mean roughness, probably associated with the high amount of defects as determined by the R<sub>max</sub> and R<sub>z(DIN)</sub> values.

R<sub>max</sub> and R<sub>z(DIN)</sub> but not R<sub>p</sub> and R<sub>q</sub> were significantly correlated with residual spore counts (Table 2). These correlations indicate that at the level of cleanliness achieved in this study, residual soil was more closely associated with surface defects rather than mean surface roughness. Abrasion or scratches have been shown previously to affect cleanability adversely (8). This association was not observed for biofilm soil, probably because a lower degree of clean-
liness was achieved when using this soil. Although the number of surfaces we examined is limited, these data suggest that $R_{\text{max}}$ and $R_{z(DIN)}$ are indicators of cleanability.

In conclusion, those surface roughness measurements of finished stainless steel that are associated with defects are significantly correlated with cleanability of buttermilk-soiled surfaces (Table 2), whereas surface finish types (2B, #4, electropolished) did not provide a good prediction of cleanability. The spore count indicator test was useful for assessing cleanability of stainless steel after one but not multiple soiling and cleaning cycles. Direct microscopic determination of protein maybe useful for evaluating residual soil after repeated cleaning, but our methodology was not sufficiently sensitive for differentiating cleanability among the polished surfaces included in this study. High levels of biofilm soil remained on all finished surfaces after cleaning, with no specific surface finish being more difficult to clean of this soil type. Surface roughness did not correlate with biofilm removal under the conditions of this study. Biofilm removal is an important aspect of equipment sanitation and should be considered in equipment and cleaning process design. When purchasing stainless steel, it may be desirable to specify $R_{z(DIN)}$ and $R_{\text{max}}$ to obtain a product of good quality especially if ease of cleaning is an important consideration.

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REFERENCES