Removal of Dairy Soil from Heated Stainless Steel Surfaces: Use of Ozonated Water as a Prerinse[1]

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ABSTRACT

Square (2.54 x 2.54 cm^2) 304 stainless steel metal plates were cleaned, passivated, and soiled by autoclaving (121°C at 15 psi for 15 min) with reconstituted nonfat dry milk (20% solids). Fifteen-minute treatments using either warm water (40°C) or ozonated cold water (10°C) were conducted to compare prerinse cleaning potential of soiled metal plates. The chemical oxygen demand determination was performed on extracted organic material from treated metal plates. Results indicated that the ozone treatment removed 84% of soil from metal plates versus 51% soil removal by the warm water treatment, but the effectiveness of the two treatments did not differ ($P > 0.05$). Cleaning effects were visualized using scanning electron microscopy at 200× and 2000× magnification. The amount of soil film present on stainless steel metal surfaces was visibly lower on ozonated treatments versus on warm water treatments.
(**Key words:** ozone, sanitation, dairy, chemical oxygen demand)

Abbreviation key: $\mathbf{ddH_2O}$ = distilled and deionized water; **COD** = chemical oxygen demand.

INTRODUCTION

Ozone (O_3) is formed by addition of an oxygen atom to molecular diatomic oxygen. The triatomic ozone is highly unstable and rapidly degrades by releasing the third oxygen atom. As a result, ozone is a strong oxidizing agent that has proven useful in cleaning and disinfecting operations (3, 4, 5, 7). The initial step in cleaning dairy processing equipment is typically a prerinse with warm water (c.a. 40°C). This prerinse is critical for removing the bulk of unbound soil materials and a significant portion of bound soil materials. Greater soil removal in the prerinse step aids further cleaning efforts by reducing detergent loading (2). The following study was devised to quantitate and visualize the effectiveness of ozonated water versus warm water as a prerinse for removing soil materials from stainless steel surfaces.

MATERIALS AND METHODS

Square (2.54 × 2.54 cm) number 304 stainless steel (A270; American Society for Testing Materials) metal plates polished to a number 4, 150-grit finish (Anbroco, Inc., Stanley, NC) were thoroughly cleaned using a paste made of a 50/50 mixture of sodium hydroxide and sodium carbonate. Each stainless steel metal plate was thoroughly rinsed five times with HPLC grade distilled and deionized water ($\mathbf{ddH_2O}$). Clean metal plates were passivated by overnight treatment in 1*N* nitric acid. After thoroughly rinsing five times with ddH_2O, metal plates were dried overnight 55°C and transferred with forceps to sterile Petri dishes for storage until needed.

On each of 5 d, two stainless steel metal plates were assigned as duplicate clean metal controls and were not treated beyond the cleaning, passivation, and drying procedure. On each of 5 d, six additional metal plates were soiled using heated reconstituted milk for subsequent use as duplicates for soiled controls, warm water treatment, and ozonated water treatment trials. Commercial nonfat dried milk was reconstituted to 20% total solids with ddH_2O. Each metal plate was wedged into a clean, polypropylene plastic tube (Falcon Sterile Blue Max 2198, 50-ml conical tubes; Becton Dickinson Labware, Franklin Lakes, NJ) such that only the plate edges were in contact with the tube wall. Each tube was filled with sufficient reconstituted milk (20 ml) to completely cover the metal plates. Tubes were autoclaved for 15 min at 121°C and 15 psi. After being autoclaved, the tubes were allowed to cool to room temperature. Each tube was decanted, and stainless steel plates were gently rinsed three times with approximately 40 ml of ddH_2O per rinse to remove unbound milk solids. The soiled metal plates were transferred from the soiled tubes into clean tubes using extreme care to prevent scratching or disturbance of the milk

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soil. Metal plates were dried overnight in the plastic tubes at 55°C.

Duplicate soiled metal plates in tubes were selected at random to be soiled control samples, warm water-treated samples, or ozone-treated samples. Soiled control metal plates received no treatment beyond the overnight drying at 55°C.

For ozone-treated samples, 20 ml of ddH_2O (28°C) was added to each tube containing a soiled metal plate. Ozone was generated by pumping air (Neptune Dynapump; Neptune Products, Inc., Dover, NJ) through a Pure Power O3 ozonator (Longmark Ozone Industries, Yreka, CA). Ozone was bubbled into each tube with Pasteur pipettes as bubbling tips. Extreme care was taken to prevent accidental scratching of the soil film by the glass bubbler tips. Because ozone is more soluble at lower temperatures, the tubes were placed into an ice bath and were ozonated for 15 min. Ozonated water temperature was 10°C after 15 min of ozonation. After treatment, water was decanted, and the metal plates were dried overnight at 55°C.

For warm water-treated samples, 20 ml of ddH_2O (40°C) was added to each tube containing a soiled metal plate. The tubes were placed into a preheated 45°C water bath for 15 min to maintain the cleaning water temperatures at 40 ± 2°C. To account for the possible scrubbing action derived from the bubbles generated in the ozonation trials, warm water-treated plates were aerated using identical pumps and glass bubbling tips as used in the ozone treatment experiment. After treatment, water was decanted and metal plates were dried overnight at 55°C.

The chemical oxygen demand (**COD**) determination (1, 6) was used to compare the cleaning power of an ozonated water treatment with a warm water treatment. Soil material was extracted from metal plate surfaces by shaking (c.a. 80 to 90 rpm) the plates for 24 h at room temperature on a rotary shaker (American Rotator V; American Dade, Miami, FL) with 20 ml of H_2SO_4/Ag_2SO_4 COD reagent. The COD determinations were performed on extracted samples. Samples included a reagent control containing H_2SO_4/Ag_2SO_4 reagent only and no contact with metal plate; a clean metal control containing a clean metal plate with no soil material, extracted with H_2SO_4/Ag_2SO_4 reagent; soiled metal samples comprising a soiled metal plate that was extracted with H_2SO_4/Ag_2SO_4 reagent; ozonated water-treated samples comprising a soiled metal plate treated with ozonated water, extracted with H_2SO_4/Ag_2SO_4 reagent; and warm water-treated samples comprising a soiled metal plate treated with warm water, extracted with H_2SO_4/Ag_2SO_4 reagent.

The COD determination was performed on extracted organic material from treated metal plates, using a randomized complete block design of two blocks, four treatments per block (clean, soiled, ozonated water, or warm water), and five replicates per treatment. Experimental results were analyzed by ANOVA. The least significant difference test (LSD) was used to test significant differences among means at $P < 0.05$ (SAS Institute, Cary, NC).

Scanning electron microscopy was used to visualize soil residue on metal plates. Metal plates, selected at random, were dried at 55°C overnight. Metal plates were gold-coated (300 s) with a HummerX gold sputter-coater (Anatech, Ltd, Springfield, VA). Samples were viewed using a scanning electron microscope (JSM-IC848; Jeol, Inc., Tokyo, Japan) at 200× and 2000× magnification.

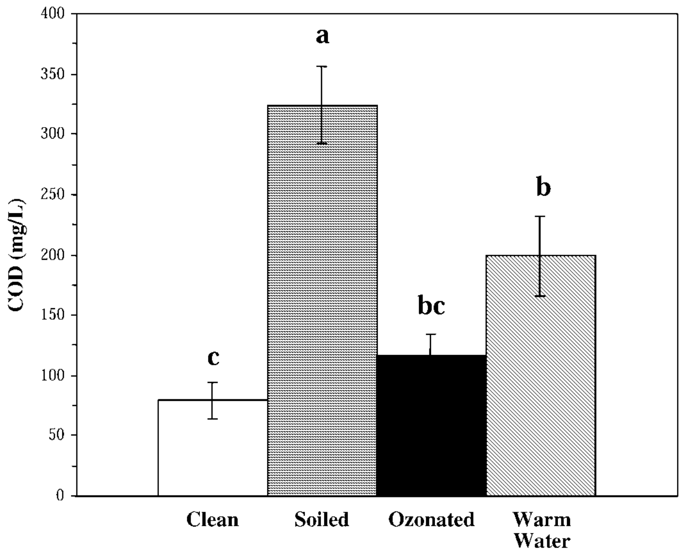


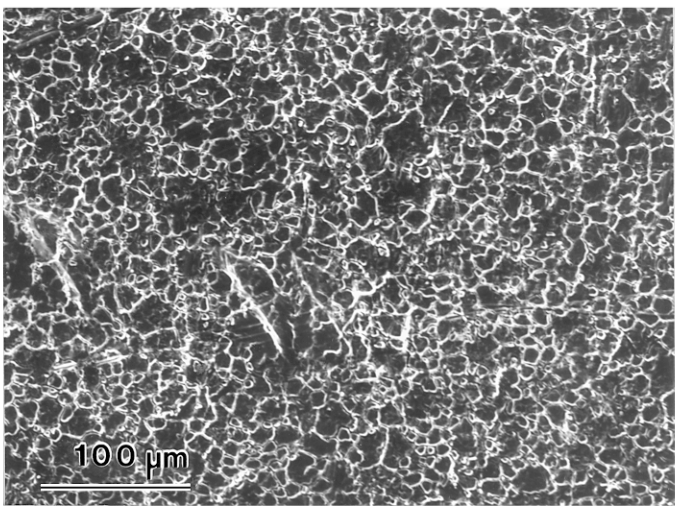
Figure 1. Mean chemical oxygen demand (COD) values (mg/L; ±SE) for heated milk components extracted from metal plates. [a,b,c]Means designated by the same letter do not differ ($P > 0.05$).

RESULTS AND DISCUSSION

Mean COD values for all metal plates are shown in Figure 1. There was sufficient milk soil on the metal plates to detect differences between treatments. Results indicate that the ozonation treatment was effective in removing milk soil from metal plates, but there was no significant difference ($P > 0.05$) between the 40°C warm water treatment and the ozonation treatment. Ozonation treatment removed approximately 84% soil from metal plates, whereas the 40°C warm water treatment removed approximately 51% soil from metal plates. Significant differences ($P < 0.05$) occurred between soiled metal plates and ozonated water-treated metal plates and between soiled metal plates and warm water-treated metal plates. There was no difference ($P > 0.05$) between clean metal plates and ozonated water-treated metal plates. However, there was a difference ($P < 0.05$) between clean metal plates and warm water-treated metal plates.

As visualized under the scanning electron microscope, the clean, untreated metal plates were free of surface soil materials (Figure 2). The lacy appearance of the clean stainless steel shown on the 200× clean metal plate (Figure 2a) was due to the granular nature of the stainless steel; the granular structure of the stainless steel is demonstrated at the 2000× magnification (Figure 2b). On the soiled metal plates (Figure 3), the heat-treated milk components adhered to the

2a



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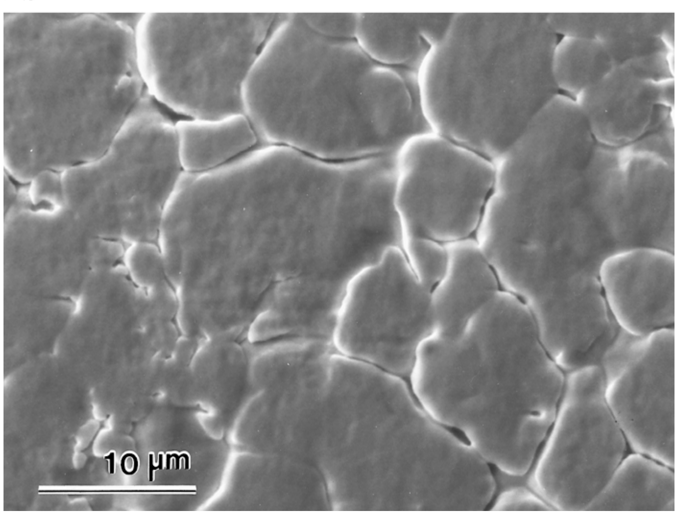
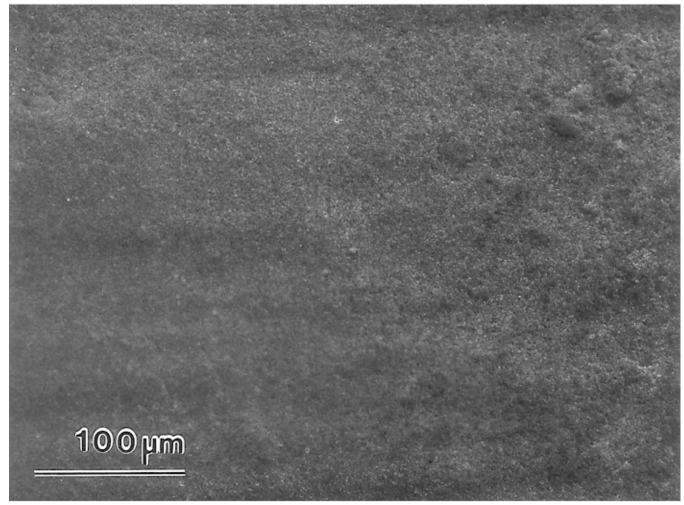


Figure 2. a) Scanning electron micrograph of clean metal plate at 200× magnification (bar = 100 μm); b) scanning electron micrograph of clean metal plate at 2000× magnification (bar = 10 μm).

3a



3b

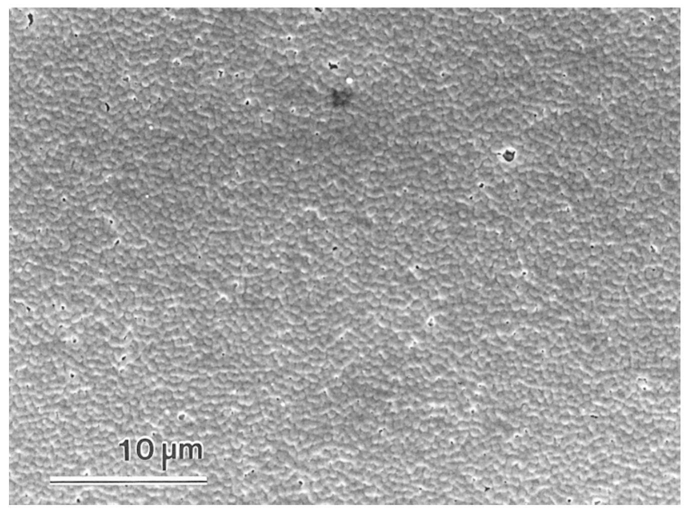
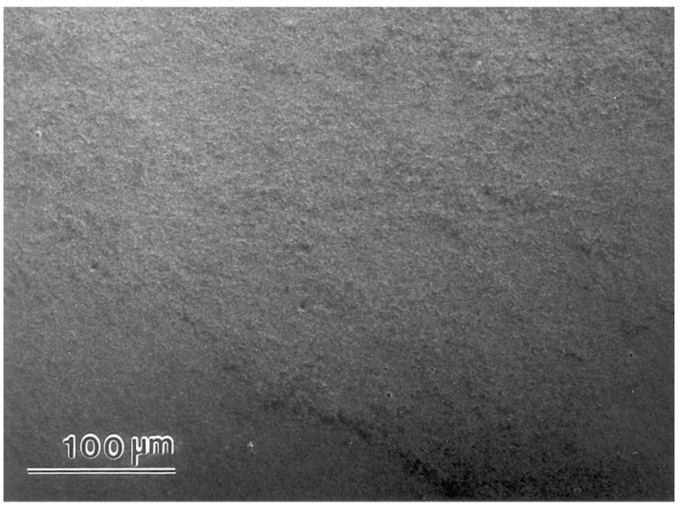


Figure 3. a) Scanning electron micrograph of soiled metal plate at 200× magnification (bar = 100 μm); b) scanning electron micrograph of soiled metal plate at 2000× magnification (bar = 10 μm).

surface, totally obscuring the fine structure of the stainless steel. On the warm water-treated metal plates, considerable soil material remained (Figure 4), and the milk film obscured the structure of the stainless steel. On the 200× magnifications of the soiled metal plate (Figure 3a) and the warm water-treated metal plate (Figure 4a), few differences were detectable in the milk films. However, in comparing the 2000× magnification of the soiled metal plate (Figure 3b) versus the 2000X magnification of the warm water-treated plate (Figure 4b), some slight differences were noted. The particulate matter on the warm water-treated plate (Figure 4b) appeared to be more variable with areas of dense and thin soil. The milk film on the soiled metal plate appeared to be more uniform in density (Figure 3b). This observation seems to be indicative of the cleaning ability of the warm water treatment and correlates with the observation noted in the COD experiment in which warm water removed 51% of heated dairy soil materials that adhered to stainless steel surfaces. On the 200× magnification of the ozone-treated metal plates (Figure 5a), patches of soil material were noted (as indicated by arrows). However, even at the 200× magnification, it is apparent that the stainless steel surface was cleaned better by ozonation than by the warm water treatment (Figure 4a). The lacy structure of the stainless steel was visible in areas on the ozonated surface, whereas it was completely obscured by milk film on the warm water-treated surface. These observations correlate with the COD study that concluded that ozonated water removed 84% of soil materials from stainless steel surfaces. At 2000× magnification of the ozone treated metal plate (Figure 5b), in an area that was selected at random, the surface of the stainless steel appears clean and similar to the clean metal surface (Figure 2b). No such clean areas could be found in any microscopic field on the warm water-treated metal plates.

4a



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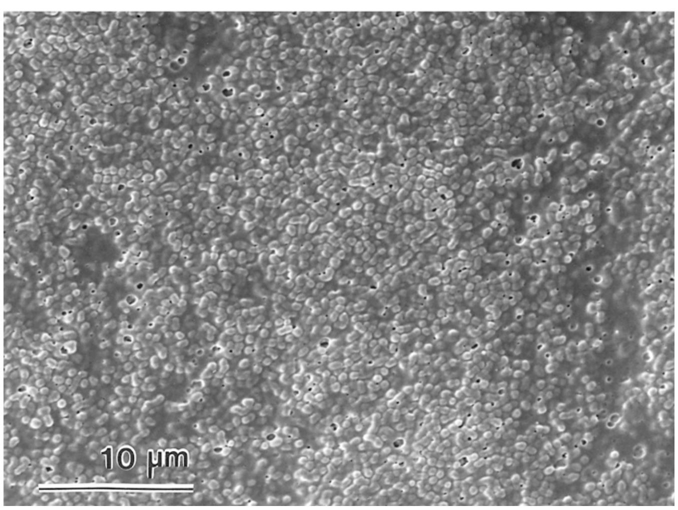
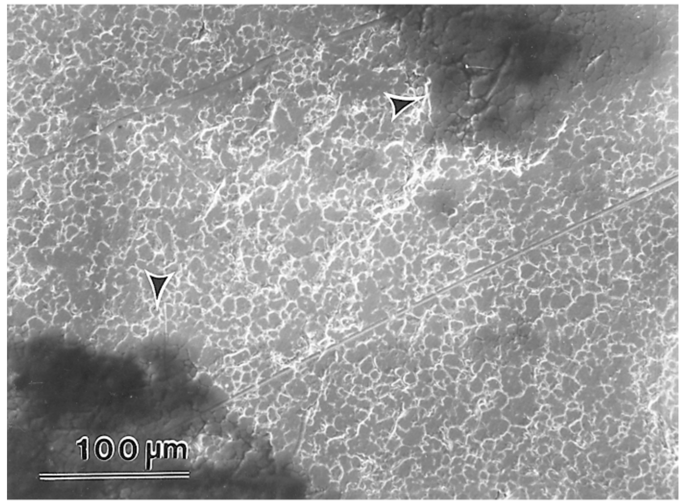


Figure 4. a) Scanning electron micrograph of warm water-treated metal plate at 200× magnification (bar = 100 μm); b) scanning electron micrograph of warm water-treated metal plate at 2000× magnification (bar = 10 μm).

5a



5b

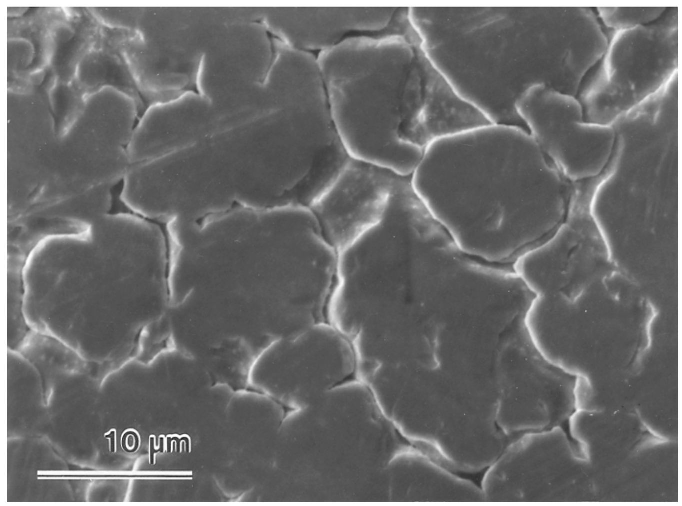


Figure 5. a) Scanning electron micrograph of ozonated water-treated metal plate at 200× magnification (bar = 100 μm). Arrows indicate patches of soil materials. b) Scanning electron micrograph of ozonated water-treated metal plate at 2000× magnification (bar = 10 μm).

CONCLUSIONS

Results of the COD experiment indicated that ozonated water cleaned the metal plates equally as effective as the warm water method. Circulating warm water prerinse is commonly used in the dairy industry to remove soil from equipment. However, the cleaning effect of water flow rate was not examined in this study. The ozonation process created bubbling that may have caused some cleaning action. Therefore, to minimize error, air was bubbled through the warm water treatment to mimic the ozone bubbling action. This experiment was a preliminary experiment to determine the cleaning power of ozone on heated milk stainless steel surfaces. We concluded from this study that ozonated water is effective in removing heated milk soil materials.

Microscopic evaluation of metal surfaces indicated that there were abundant soil materials on dirty metal plates and, to a lesser degree, on warm water-treated metal plates. Although residual soil materials were noted on ozone treated metal plates, soil material did not cover the entire surface as noted for the soiled and the warm water-treated metal samples.

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