A Comparison of Ozonation and Chlorination for the Disinfection of Stainless Steel Surfaces[1]

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ABSTRACT

Ozonated water and chlorinated sanitizer were compared for effectiveness against biofilms of milk spoilage bacteria. Stainless steel plates were incubated in UHT-pasteurized milk inoculated with pure cultures of either *Pseudomonas fluorescens* (ATCC 949) or *Alcaligenes faecalis* (ATCC 337). After incubation, the plates were removed and rinsed in sterile PBS. A control rinsed stainless steel plate was swabbed and plated on standard plate count agar. A second rinsed stainless steel plate was covered and treated for 2 min with a commercial chlorinated sanitizer (dichloro-s-triazinetrione), prepared according to the manufacturer's recommendations; after treatment, the plate was rinsed twice in sterile PBS, swabbed, and plated on standard plate count agar. A third rinsed stainless steel plate from the culture was placed in ozonated deionized H_2O (.5 ppm of ozone) for 10 min, rinsed twice as described, swabbed, and plated. Both ozonation and chlorination reduced bacteria populations by >99% at initial cell densities in the range of approximately 1.24×10^5 to 8.56×10^5 cfu/cm^2 for *P. fluorescens* and 1.53×10^4 to 8.56×10^5 cfu/cm^2 for *A. faecalis* in milk films on stainless steel surfaces.

(Key words: ozone, chlorine, sanitization, milk spoilage organisms)

Abbreviation key: **dH_2O** = deionized water, **SS** = stainless steel.

INTRODUCTION

Chlorinated sanitizers have been used extensively in the dairy industry for many years. These sanitizers are effective in destroying bacteria provided that excessive organic material is not present. Recently, questions have arisen concerning environmental effects of chlorine-based sanitizers. Chlorination can pose health risks to humans and wildlife because of the formation of trihalomethanes and other carcinogenic halo-organic compounds (4). A possible alternative to chlorinated sanitizing agents is ozonated water. Ozone is produced when energy in the form of radiation, electricity, or heat is applied to gaseous oxygen. Ozone is usually prepared by passing gaseous oxygen through a high voltage electrical field (3). Ozone is a powerful oxidant that may be used as a disinfectant. Disinfecting agents for water have been ranked in the following order of decreasing efficiency: ozone, chlorine dioxide, hypochlorous acid, hypochlorite ion, dichloramine, and monochloramine (10).

Ozone first attacks the bacterial membrane at the glycoproteins, glycolipids, or at certain amino acids such as tryptophan. Ozone also acts on the sulfhydryl groups of certain enzymes, resulting in disruption of normal cellular enzymatic activity. Bacterial death is rapid and is often attributed to changes in cellular permeability followed by cell lysis. However, lysis is probably not the primary inactivation mechanism, but a consequence of high oxidant concentration. Ozone further acts on the nuclear material of the bacterial cell by modifying the purine and pyrimidine bases of nucleic acids. Ozone was effective against Gram-positive (including sporeformers) and Gram-negative bacteria, viruses, and amoebae (10). The Safe Drinking Water Committee of the National Research Council concluded in 1980 that ozone was effective in destroying bacteria and viruses at pH 6.0 to 8.5 (9). Other data

Received October 25, 1992.
Accepted May 28, 1993.
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indicate that ozone destroys bacteria and viruses at pH 5.6 to 9.8 (7).

Ozone has been used since 1906 to treat municipal drinking water (10). Recently, ozonated water has been used as a sanitizer by soft drink bottlers in South Carolina and elsewhere. Ozonation has been approved by the USDA Food Safety and Inspection Service for use in treating poultry chilling water. Approval for use of ozone as a direct food additive is still under investigation (1). Because ozone is extremely labile, it is not persistent and, consequently, may pose minimal health risks unless directly inhaled in large quantities (4). Respiratory risks can be minimized by strategically placing fans in the work environment (8). Ozone destruction units are also available to destroy the labile ozone.

Recently, small-scale ozonation units have been developed that can be used in food and dairy processing plants. These units use ambient air as an oxygen source, require only routine replacement of desiccant, and recirculate water through existing clean-in-place systems. Ozone tends to be unstable in water. However, recirculating water through the ozonator maintains sufficient ozone concentration for sanitization. Ozonators are available in a wide range of sizes. A typical unit used in a clean-in-place system fully ozonates 1818 L (400 gal) of water in approximately 15 min. Larger ozone-generating units ozonate the water more quickly. In many commercial food applications, a circulation time of 20 min has been used. For milk contact surfaces, a contact time of 10 min was suggested to be sufficient for sanitization (F. Biggs, 1992, personal communication). However, according to published data (7), ozone inactivates microorganisms within 10 s to 5 min of exposure at concentrations of .1 to .4 ppm.

Ozone is a more powerful sanitizer than chlorine (10). With concerns about the formation of chlorination by-products such as trihalomethanes, ozonation may have potential as a sanitizing agent in the food and dairy industries. Although it has been used for many years in water disinfection, ozone has not been used extensively for dairy sanitization. In addition to improved sanitization, ozonation may reduce sanitization costs because the cost of the unit and routine maintenance will likely be less than the cost of chlorine compounds. Some food plants depend on heat to assist sanitization. Because ozonation does not require heat, power consumption is reduced.

The purpose of this study was to determine whether ozone would effectively destroy attached milk spoilage bacteria. Numerous studies (5, 6, 11) have demonstrated the attachment of microorganisms to milk contact surfaces. These attached organisms are difficult to destroy and may contribute to deterioration in microbiological quality of milk. A method for destroying these organisms effectively in the presence of heavy organic material without the concurrent formation of trihalomethanes could be extremely beneficial to the dairy and food industries.

MATERIALS AND METHODS

Square (2.24 cm × 2.24 cm) number 304 stainless steel (SS) A270 American Society for Testing Materials plates polished to a number 4, 150 grit finish (Anbroco, Inc., Stanley, NC) were cleaned with Delvak® (Diversey Corporation, Wyandotte, MI) and passivated in 1.0*N* HNO_3 for 30 min. After being rinsed in deionized H_2O (dH_2O), the SS plates were autoclaved and placed in sterile plastic Petri dishes (three plates per dish).

Sterile UHT milk, which had been inoculated with either *Pseudomonas fluorescens* (ATCC 949) or *Alcaligenes faecalis* (ATCC 337), was added to the Petri dishes to cover the SS plates. Cultures were incubated at 32°C for 4 to 24 h. After incubation, SS plates were aseptically transferred with Triceps® (The Texwipe Co., Upper Saddle River, NJ) to sterile 60- × 20-mm Petri dishes containing ca. 20 ml of sterile PBS. Care was taken to avoid scratching the biofilm on the SS plate surface. The SS plates were rinsed in the PBS with shaking on a model R2 New Brunswick Scientific Shaker (New Brunswick Scientific, Edison, NJ) at 100 rpm for 1 min. Plates were immediately removed to dry, sterile Petri dishes after being rinsed. A control plate was thoroughly swabbed with a sterile cotton swab. The swab was aseptically broken into a 5-ml PBS dilution blank. Serial dilutions were plated on standard plate count agar.

A second rinsed SS plate was covered and treated for 2 min with the chlorinated sanitizer, Antibac B® (Diversey Corporation), prepared

according to the manufacturer's recommendations using dH_2O (24°C). In this solution, the final concentration of chlorine was 100 ppm. The active ingredient in Antibac B® is sodium dichloro-s-triazinetrione. The manufacturer recommended 2 min of contact time for sanitizing dairy equipment. After treatment, the chlorinated SS plate was rinsed twice in sterile PBS to remove residual chlorine (20 ml of sterile PBS in a sterile 60- × 20-mm Petri dish, shaken at 100 rpm for 1 min), swabbed, and plated by serial dilution on standard plate count agar. To simulate industry conditions, the chlorinated sanitizer was not inactivated.

A third rinsed SS plate was placed in ozonated dH_2O (Pure Power O_3; Longmark Ozone Industries, Yreka, CA) at .5 ppm of ozone for 10 min, rinsed twice as described, swabbed, and plated by serial dilution on standard plate count agar. Ten minutes was the exposure time for sanitization recommended by the ozonator supplier. Although ozone will readily dissipate, ozone-treated SS plates were also rinsed twice after treatment to ensure removal of residual ozone. Ozone concentration in dH_2O was tested with a model 03-T test kit (Longmark Ozone Industries). These procedures were repeated 30 times each for *P. fluorescens* and *A. faecalis*.

A preliminary study was performed to test whether the additional soaking times and rinses used for the sanitized samples affected bacterial cell densities. Cultured SS plates were initially rinsed in PBS (20 ml, 100 rpm, 1 min). One plate was immediately swabbed, one plate was soaked in sterile PBS for 2 min and rinsed twice (20 ml, 100 rpm, 1 min) to stimulate the chlorination treatment, and one plate was soaked in sterile PBS for 10 min and rinsed twice (as described) to simulate the ozone water treatment.

To determine the efficiency of swabbing, swabbed SS plates were aseptically inverted and pressed onto the surface of standard plate count agar. After a preliminary incubation of 1 h at 32°C, SS plates were aseptically removed, and the Petri dishes were returned to the incubator. Direct microscopic examination of the swabbed SS plates revealed no detectable milk films with reflected light. However, because reflected light was required to examine the solid SS surfaces, a quick and easy method to examine the surfaces microscopically for attached bacteria was not readily available.

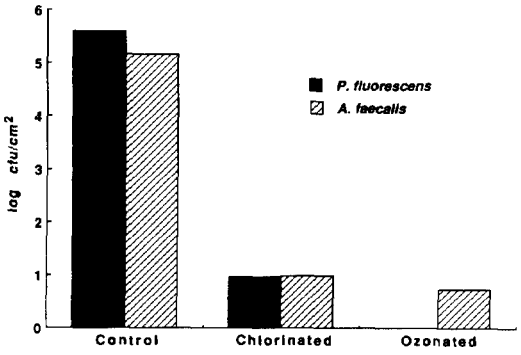

Figure 1. Logarithm of colony-forming units of *Pseudomonas fluorescens* and *Alcaligenes faecalis* per square centimeter, before and after treatment with chlorinated sanitizer (2 min) and ozone (10 min).

RESULTS AND DISCUSSION

Bacterial cell densities of approximately 1.24×10^5 to 8.56×10^5 cfu/cm^2 for *P. fluorescens* and 1.53×10^4 to 8.56×10^5 cfu/cm^2 for *A. faecalis* were produced in milk films on SS surfaces. Results indicate that both chlorine and ozone destroyed or inhibited >99% of the bacteria on the SS plates. As shown in Figure 1, this bacterial inhibition was equivalent to a 4.6 log reduction for chlorinated sanitizer and a 5.6 log reduction for ozone for *P. fluorescens* and equivalent to a 4.2 log reduction for chlorinated sanitizer and a 4.4 log reduction for ozone for *A. faecalis*. These represent >99% destruction of the bacteria.

In preliminary studies, extended soaking times and additional rinsing steps caused no significant difference in biofilm cell densities. Consequently, the reduction in cell densities after sanitization treatment was due to the action of chemical sanitizers and not due to the physical action of rinsing. This study indicates that both ozone and chlorine destroyed or inhibited >99% of these common milk spoilage bacteria.

Studies indicated that swabbing effectively removed bacteria from SS surfaces; <20 cfu per swabbed SS plate were recovered by incubation of SS plate imprints.

This experiment was designed to test the effect of a chlorinated sanitizer and ozonated water on heavily contaminated SS surfaces in a worst case situation, such as may accidentally

occur if cleaning were insufficient. Known psychrotrophic milk spoilage bacteria were selected because these organisms can present problems in the dairy industry. Concentration of the chlorine-based sanitizer was consistent with the manufacturer's maximum recommendation for no-rinse applications (5). The standard procedure for evaluation of sanitizers is the AOAC (2) method: Germicidal and Detergent Sanitizing Action of Disinfectants, 4.020 to 4.029. However, because this experiment was designed to compare the no-rinse effect of ozone versus chlorine-based sanitizer on bacteria trapped in a milk-based biofilm, the standard procedure was inappropriate.

CONCLUSIONS

Ozonation has been used for many years to disinfect drinking water. Results presented herein indicate that ozone is effective in destroying surface-attached bacteria, even at high cell densities and in the presence of high organic material. Ozone requires no heat and, consequently, uses less energy than sanitizing systems that use steam or hot water. Costs of chemical sanitizers would be reduced or possibly eliminated by use of ozone as a sanitizing agent. Furthermore, release of chlorinated chemical residues to the environment would be reduced. Because ozone is extremely reactive, it would not be persistent. In certain operations, current recommendations for ozonation would require longer sanitization procedures than those now used for chemical sanitization. However, because ozone is a more powerful oxidizer than chlorine, the contact time necessary for complete sanitization by ozone likely would be less than that currently recommended. Further research is needed to determine optimal ozone contact time and the safety of ozone to workers, consumers, food, equipment, and the environment. However, results presented herein indicate that, concerning lethality to spoilage bacteria, ozonation is an effective sanitization method that may have potential use in the dairy industry.

ACKNOWLEDGMENTS

The researchers thank Fletcher Biggs and Perry Strait of Anbroco, Inc., Stanley, NC for providing the Pure Power O_3, and the SS plates and for financial support of this project. The researchers also thank A. B. Bodine for his technical assistance.

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