

## CHEMICAL TREATMENTS FOR INACTIVATION

### INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN BIOFILM ON STAINLESS STEEL BY TREATMENT WITH AN ALKALINE CLEANER AND A BACTERIOPHAGE (M. Sharma, J.-H. Ryu, and L.R. Beuchat)

Biofilms formed by *Escherichia coli* O157:H7 on inadequately cleaned and sanitized contact surfaces may be a source of contamination of ground beef and deli meat in processing facilities as well as in food service settings. Refrigeration temperatures in these environments provides opportunities for *E. coli* O157:H7 originating from fecal material on carcasses and hides to survive, attach to hydrophilic surfaces such as stainless steel, and become persistent. Cells attached to surfaces or enmeshed in biofilms may have altered sensitivities to cleaners and sanitizers compared to sensitivities of planktonic cells. *Salmonella* in biofilms has been reported to be spp. were more resistant than planktonic cells to acidic challenge, hypochlorite, and iodophors treatments. Biofilms may protect cells through a combination of mechanisms, including diffusional resistance of the EPS matrix, chemical and enzymatic inactivations of sanitizers and disinfectants, physiological changes in cells, and the induction of stress responses in cells. Strongly alkaline cleaners containing with hypochlorite have been shown to be effective in killing planktonic cells of *E. coli* O157:H7 but little is known about the ability of alkaline cleaners to inactivate biofilms of *E. coli* O157:H7 in biofilms.

Bacteriophages have been applied to various poultry products and fresh-cut produce for the purpose of inactivating foodborne pathogens. A lytic bacteriophage specific for *Salmonella* Enteritidis was shown to reduce populations of the pathogen counts on chicken skin. Reductions were increased as the multiplicity of infection (MOI, the number of phage particles needed to infect one bacterial cell) value increased. Treatment with bacteriophage has been reported to reduce populations of *Salmonella* on vegetable seed sprouts and fresh-cut honeydew melons.

We undertook a study to determine the effectiveness of an alkaline cleaner used in food processing plants and a lytic bacteriophage specific for *E. coli* O157:H7 in killing wild type and *rpoS*-deficient cells of the pathogen in a biofilm. Wild type and *rpoS*-deficient cells were attached to and developed biofilms on stainless steel coupons (ca. 7 – 8 log<sub>10</sub> cfu/coupon) at 22°C for 96 h in M9 minimal salts media (MSM) with one transfer to fresh medium. Coupons were treated with 100% (pH 11.9, with 100 µg/ml free chlorine) and 25% with working concentrations of a commercial alkaline cleaner used in the food industry (pH 11.9), chlorine solutions (50 and 100 µg/ml free chlorine), or sterile deionized water (control) free chlorine solutions at 4°C for 1 and 3 min. Treatment with 100% alkaline cleaners reduced populations by 5 – 6 log<sub>10</sub> cfu/coupon for a significantly ( $P \leq 0.05$ ) reduction compared to treatment with water more than other treatments. Initial populations (2.6 log<sub>10</sub> cfu/coupon) of attached cells of both strains attached to SSC's were reduced by 1.2 log<sub>10</sub> cfu/coupon when treated with bacteriophage KH1 (7.75 x 10<sup>7</sup> PFU/ml) for up to 4 days at 4°C. Low populations (2.7 – 2.8 log<sub>10</sub> cfu/coupon) of wild type and *rpoS*-deficient biofilms cells in biofilms that had developed for 24 h at 22°C were not decreased by more than 1 log<sub>10</sub> cfu/coupon when treated with KH1 (7.5 log<sub>10</sub> 3.4 x 10<sup>7</sup> PFU/ml) at 4°C. Results showed that higher numbers of cells of *E. coli* O157:H7 in biofilms are killed by treatment with an alkaline cleaner than with hypochlorite alone, possibly through a synergistic mechanism of alkaline pH and hypochlorite. Populations of cells attached on coupons were reduced by treating with bacteriophage but cells enmeshed in biofilms are protected. The alkaline involving pHs, in combination with hypochlorite, in a commercial cleaner are responsible for killing *E. coli* O157:H7 in biofilms. Treatment with bacteriophage KH1 may reduce populations of cells attached to coupons but not cells in biofilms.

**TREATMENTS FOR CONTROL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN DRINKING WATER CONTAMINATED WITH RUMEN CONTENT OR FECES** (P. Zhao, M. P. Doyle, T. Zhao, J. W. West, J. Bernard, and H. Cross)

*E. coli* O157:H7 has emerged in the last 10 years as an important foodborne pathogen with an estimated 73,000 cases annually in the U.S. Cattle are the major reservoir and studies revealed that when present in cattle drinking water, *E. coli* O157:H7 was disseminated to other cattle using the contaminated water source. Hence, drinking water for cattle is an important vehicle of *E. coli* O157:H7 transmission. Studies indicate that once contaminated in the drinking water of a cattle farm, *E. coli* O157:H7 can survive for many months.

A variety of treatments have been evaluated for their efficacy in killing *E. coli* O157:H7 in drinking water contaminated with rumen content or cattle feces. Results revealed that most had minimal effect on killing *E. coli* O157:H7 because these treatments were neutralized by organic materials present in the rumen content or feces. The objective of this study was to identify practical treatments to eliminate or control *E. coli* O157:H7 in drinking water simulating on-farm conditions.

Survival of *E. coli* O157:H7 in water containing rumen content at different water:rumen content, *E. coli* O157:H7 cell numbers, and temperatures was determined. At 21°C, *E. coli* O157:H7 inoculated at a high inoculum ( $10^{5.8}$  cfu/ml) survived for 8, 15, 23, >56 and 24 weeks and at a low inoculum ( $10^{2.9}$  cfu/ml) survived for 8, 11, 10, 11 and 10 weeks at a water:rumen content ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively.

Different treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate and competitive inhibition *E. coli* were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm) and ozone treatment (22-24 ppm at 5°C or 8-12 ppm at 21°C) of water had minimal effect on killing *E. coli* O157:H7 in the presence of rumen content at ratios of 50:1 and higher. Treatment by competitive inhibition *E. coli* in water with rumen content also had minimal effect on *E. coli* O157:H7 counts compared with untreated controls. Four chemical treatment combinations including: (a) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid (Treatment A); b) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate (Treatment B); (c) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid (Treatment C); (d) 0.1% lactic acid, 0.9% acidic calcium sulfate and 100 ppm chlorine dioxide (Treatment D) were highly effective at 21°C in killing *E. coli* O157:H7, O26:H11 and O111:NM/ml in water heavily contaminated with rumen content (ratio of 10:1 water:rumen content, v/w) or feces (ratio of 20:1, water:feces, v/w). Among them, Treatments A, B and C killed >5 log<sub>10</sub> *E. coli* O157:H7, O26:H11 and O111:NM/ml within 30 min in water containing rumen content. For Treatment D, *E. coli* O157:H7, O26:H11, and O111:NM were reduced within 30 min by 2.8, 4.3, and 3.2 log cfu/ml in water containing rumen content, respectively, and by 3.5, 4.9, and 4.6 log cfu/ml in water with feces, respectively.

Cattle fed ad libitum water containing Treatment A, C, or control (untreated water) for two treatment periods at 7-day increments drank an average of 15.2, 13.8, and 30.3 L/day, respectively. Cattle provided water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 L/day. The amount of water consumed for all water treatments was significantly different from the control, and there were no significant differences among water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow body weight (BW) was converted to MBW ( $BW^{0.75}$ ), and intake of water per MBW was calculated. Treatment effects for water intake/MBW were similar to those for total water intake. Because water intake was

substantially reduced when treated with the chemicals described above, optimal on-farm use of such treatments would be periodic, rather than continuous. In addition, application of chemicals to drinking water systems followed by flushing to remove or dilute the chemicals after 30 minutes of exposure is recommended.

**EFFICACY OF GASEOUS CHLORINE DIOXIDE AS A SANITIZER FOR KILLING *SALMONELLA*, YEASTS, AND MOLDS ON BLUEBERRIES, STRAWBERRIES, AND RASPBERRIES** (K. V. Sy, K. H. McWatters, and L. R. Beuchat)

Sanitizers such as gaseous chlorine dioxide ( $\text{ClO}_2$ ) have been explored as alternatives to aqueous chemicals for sanitizing fruits and vegetables eaten raw. Gaseous  $\text{ClO}_2$  has some advantages over chlorinated water in that it can break down phenolic compounds and remove phenolic tastes and odors from the water, does not react with ammonia, and has 2.5 times the oxidation capacity of chlorine. The bactericidal efficacy of gaseous  $\text{ClO}_2$  is also not markedly affected by pH as well as it has greater sporicidal activity. Several studies have shown gaseous  $\text{ClO}_2$  to be effective in killing enteric pathogens on several fruits and vegetables. However, the efficacy of gaseous  $\text{ClO}_2$  gas in killing or removing *Salmonella*, yeasts, and molds on small fruits has not been reported. The objective of this study was to evaluate gaseous  $\text{ClO}_2$  for its effectiveness in killing *Salmonella* inoculated onto the surface of blueberries, strawberries, and red raspberries. Inactivation of yeasts and molds naturally occurring on the fruits was also determined.

An inoculum (100  $\mu\text{l}$ , 6.0 - 6.8  $\log_{10}$  cfu/g of fruit) containing five serotypes of *Salmonella enterica* was deposited on the skin, calyx tissue, or stem scar tissue of blueberries, skin or stem scar tissue of strawberries, and skin of red raspberries, dried for 2 h at 22°C, then held for 20 h at 4°C and 2 h at 22°C before treatment. Sachets containing reactant chemicals were formulated to release gaseous  $\text{ClO}_2$  at concentrations of 4.1, 6.2, and 8.0 mg/L of air within treatment times of 30, 60, and 120 min, respectively, at 23±1°C. Treatment with 8.0 mg of  $\text{ClO}_2/\text{L}$  significantly ( $\alpha = 0.05$ ) reduced the population of *Salmonella* on blueberries by 2.4 - 3.7  $\log_{10}$  cfu/g. Lethality was higher to cells in inoculum placed on the skin, compared to the stem scar tissue. Populations of *Salmonella* on strawberries treated with 8.0 mg of  $\text{ClO}_2/\text{L}$  were reduced by 3.8 - 4.4  $\log_{10}$  cfu/g. A significant reduction of 1.5  $\log_{10}$  cfu/g of raspberries was also achieved. Treatment with 4.1 - 8.0 mg of  $\text{ClO}_2/\text{L}$  caused reductions in populations of yeast and molds on blueberries, strawberries, and raspberries of 1.4 - 2.5, 1.4 - 4.2, and 2.6 - 3.0  $\log_{10}$  cfu/g, respectively. Lethality of  $\text{ClO}_2$  to *Salmonella*, yeasts, and molds was higher when fruits were treated at 75 - 90% relative humidity than at lower relative humidity. Treatment with 4.1 mg/L  $\text{ClO}_2$  did not markedly affect the sensory quality of fruits stored for up to 10 days at 8°C. Results indicate that gaseous  $\text{ClO}_2$  has promise as a sanitizer for small fruits.

**LETHALITY OF CHLORINE, CHLORINE DIOXIDE, AND A COMMERCIAL FRUIT AND VEGETABLE SANITIZER TO VEGETATIVE CELLS AND SPORES OF *BACILLUS CEREUS* AND SPORES OF *BACILLUS THURINGIENSIS*** (L. R. Beuchat, C. A. Pettigrew, M. E. Tremblay, B. J. Roselle, and A. J. Scouten)

Concerns about international bioterrorism have rekindled an interest in developing and refining technologies to kill *Bacillus anthracis* spores in urban environments and in foods. While spores of several *Bacillus* species known to cause spoilage of foods and foodborne disease have been studied extensively to determine conditions affecting growth and sporulation, as well as their sensitivity to physical treatments and sanitizers, comparatively little is known about conditions affecting survival and growth of *B. anthracis* in foods and the effectiveness of sanitizers in killing spores of the organism on food-contact surfaces and in foods. *Bacillus anthracis* is closely related to *Bacillus cereus* and *Bacillus thuringiensis*, the principle distinguishing difference being the presence of virulence genes on plasmids in *B. anthracis*. Direct comparisons of the sensitivity of spores of *B. anthracis* and spores of other *Bacillus* species to sanitizers used to decontaminate food-contact surfaces and foods have not been described.

Information on the sporicidal activity of chemical treatments using *B. cereus*, *B. thuringiensis*, and perhaps other *Bacillus* species as potential surrogates for *B. anthracis* would provide insights to the relative sensitivity of *B. anthracis* spores to the same treatments.

We conducted a series of experiments to determine the effectiveness of chlorine, ClO<sub>2</sub>, and a commercial raw fruit and vegetable sanitizer in killing vegetative cells and spores of *B. cereus* and *B. thuringiensis*. The goal is to eventually test the sensitivity of vegetative cells and spores of *B. anthracis* to treatments causing the highest reductions in populations of these potential surrogates. Insights to the sensitivity of *B. cereus* and *B. thuringiensis* to these sanitizers will be valuable in achieving that goal. Treatment with alkaline (pH 10.5 - 11.0) ClO<sub>2</sub> (200 µg/ml) produced by electrochemical technologies reduced populations of a five-strain mixture of vegetative cells and a five-strain mixture of spores of *B. cereus* by >5.4 and > 6.4 log<sub>10</sub> cfu/ml, respectively, within 5 min. This compares to respective reductions of 4.5 and 1.8 log<sub>10</sub> cfu/ml resulting from treatment with 200 µg/ml chlorine. Treatment with a 1.5% acidified (pH 3.0) solution of Fit<sup>®</sup> powder product (FPP) was less effective, causing 2.5 and 0.4 log<sub>10</sub> cfu/ml reductions in the number of *B. cereus* cells and spores, respectively. Treatment with alkaline ClO<sub>2</sub> (85 µg/ml), acidified (pH 3.4) ClO<sub>2</sub> (85 µg/ml), and a mixture of ClO<sub>2</sub> (85 µg/ml) and FPP (0.5%) (pH 3.5) caused reductions in vegetative cell/spore populations of >5.3/5.6, >5.3/5.7, and >5.3/6.0 log<sub>10</sub> cfu/ml, respectively. Treatment of *B. cereus* and *B. thuringiensis* spores in a medium (3.4 mg of organic and inorganic solids/ml) in which cells had grown and produced spores with an equal volume of alkaline (pH 12.1) ClO<sub>2</sub> (400 µg/ml) for 30 min reduced populations by 4.6 and 5.2 log<sub>10</sub> cfu/ml, respectively, indicating high lethality in the presence of materials other than spores that would potentially react with and neutralize the sporicidal activity of ClO<sub>2</sub>.

**EFFECTIVENESS OF CLEANERS AND SANITIZERS IN KILLING SALMONELLA NEWPORT IN THE GUT OF A FREE-LIVING NEMATODE, CAENORHABDITIS ELEGANS** (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Large microbial populations in the soil matrices, such as those amended with manure, have been reported to attract free-living nematodes. *Caenorhabditis elegans*, a free-living, microbivorous nematode found in the soil of temperate regions, has been reported to ingest *Escherichia coli* O157:H7, *Listeria monocytogenes*, several serotypes of *Salmonella enterica*, *Bacillus cereus*, and *Staphylococcus aureus*. Depending on environmental conditions, bacteria may persist within the gut of *C. elegans* for several days after consumption. Release of pathogens as a result of rupturing of the cuticle or defecation are ways that infected nematodes can contaminate the soil environment. Commercial cleaners and sanitizers used by the produce industry may contain surfactants to aid in the release of microorganisms, and perhaps also nematodes, from the surface of produce. Nematodes may subsequently become resident in or on produce contact areas such as water baths, belts, tables, and sorters in processing facilities. Pathogens from a single worm released onto processing equipment could theoretically release ingested cells of a pathogen and contaminate large amounts of produce. The plausibility of this series of events happening on a commercial level can be more easily assessed if the effectiveness of cleaners and sanitizers in killing pathogens ingested by nematodes is known. A study was undertaken to determine the effectiveness of two commercial cleaners and four sanitizers in killing *E. coli* OP50 and *S. Newport* in the gut of *C. elegans*. The effectiveness of these treatments in killing planktonic cells of *E. coli* OP50 and *Salmonella Newport* was also evaluated.

The efficacy of cleaners and sanitizers in killing *S. Newport* in the gut of *C. elegans* was studied. Adult worms were fed nalidixic acid-adapted cells of *E. coli* OP50 (control) or *S. Newport* for 24 h, washed, placed on paper discs, and incubated at 4 or 20°C and relative humidities of 33 or 98% for 24 h. Two commercial cleaners (Enforce<sup>®</sup> and K Foam Lo<sup>®</sup>) and four sanitizers (2% acetic acid, 2% lactic acid, Sanova<sup>®</sup>, and chlorine [50 and 200 µg/ml]) were applied to worms for 0, 2, or 10 min. Populations of *E. coli* and *S. Newport* (cfu/worm) in untreated and treated worms were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic

acid. Populations of *S. Newport* in worms exposed to 33 or 98% relative humidity at 4°C or 33% relative humidity at 20°C were significantly ( $P \leq 0.05$ ) lower than the number surviving exposure to 98% relative humidity at 20°C. In general, treatment of desiccated worms with cleaners and sanitizers was effective in significantly ( $P \leq 0.05$ ) reducing the number of ingested *S. Newport*. Results indicate that temperature and relative humidity influence the survival of *S. Newport* in the gut of *C. elegans*, and cleaners and sanitizers may not eliminate the pathogen.

