

Inactivation of *Escherichia coli* O157:H7 in Poultry Chiller Water Using Combined Ultraviolet Light, Pulsed Electric Field and Ozone Treatments

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Abstract: A laboratory study was conducted to assess the efficacy of using combination of ultraviolet light (UV) irradiation, pulsed electric field (PEF), and ozone for inactivation of *Escherichia coli* O157:H7 in poultry chiller water. The *E. coli* cells showed high resistance when ozone was used alone in the study. Only about 0.6 log reduction was obtained at the ozone dose of 1 mg/ml of sample after 30 s contact time. UV treatment for 1 min at the intensities of 117 and 234 mW/cm² resulted in 2 and 3.5 log reductions, respectively. When the sample was treated with 200 pulses of electric field at 23 and 30 kV/cm, *E. coli* inactivation was 3.5 and 4.1 log, respectively. Combinations of ozone, UV and PEF yielded additional inactivation effect that was essentially sum of the different inactivation levels obtained separately for the different hurdles. Inactivation increased with increase in intensity of the treatments. For the treatment conditions studied, combined treatment using UV and PEF resulted in higher inactivation of *E. coli* O157:H7 in poultry chiller water.

Key words: Ultraviolet light, pulsed electric field, ozone treatments

Introduction

Water acquisition and wastewater treatment operations account for a significant portion of a poultry processing plant's operating costs. Therefore, there is great motivation to optimize the use of water. Decontamination and recycling of the poultry plant wastewater are important from the food safety, environmental and economical points of view (Nunes, 2003). Some chemical treatment methods, such as addition of chlorine, trisodium phosphate, organic acids, and ozone to the processing water, have been studied for control of microbial populations in poultry chiller water (Wabeck, 1994). However, chemical treatment methods are associated with undesirable residues, high cost or deleterious effects on poultry products. Some of these chemical residues may also have toxic effects on the environment.

Traditional disinfection strategies such as thermal treatment are problematic due to the subsequent cooling required after the treatment. During the past decade, a number of factors have rekindled interest in alternative physical treatments of food processing wastewater. These factors include economics, the need for pollution control, environmental concerns, etc. Newer non-thermal treatment technologies such as ultraviolet light irradiation, and pulsed electric field are "biodegradable" and produce minimal environmental problems. Ozonation at moderate dose levels is considered safe by the FDA in the USA, and offers several benefits over other chemical treatment (Nunes, 1998). But high doses of ozone are detrimental to the sensory attributes of foods (Unal *et al.*, 2001). Ultraviolet light (UV) is a cost effective method for treating clear or

filtered processing water (Diaz *et al.*, 2001). It is however limited by the turbidity and UV transmittance of the poultry chiller water. High intensity pulsed electric field (PEF) is another effective technique to inactivate microorganisms in several liquid media. The technology has not yet been used for treating poultry chiller water. By combining ozone with other innovative non-chemical treatments, the amounts of ozone required as well as the level of electric field strength may be reduced (Unal *et al.*, 2001). Since PEF, UV and ozone act on cell membranes, synergistic effects may be expected. Therefore, a laboratory study was conducted to evaluate the efficacy of combined treatment namely ozone-PEF, ozone-UV, UV-PEF for inactivation of *E. coli* O157:H7 and to determine the feasibility of their application as part of recycling processes for poultry chiller water.

Materials and Methods

Poultry Chiller Water Sample: Poultry chiller water samples were collected from a nearby local processing plant. The water had been filtered through screen to remove floating debris. Sufficient time was allowed for the processing plant system to reach steady state and to achieve a dynamic equilibrium between solids lost in the overflow water and solids gained from incoming carcasses before the water samples were collected. Collected water samples were stored in a refrigerator at 1°C until used for experiments. The sample did not exceed 10°C during any of the treatments studied. The water sample was tested for indigenous microbial load before each experiment.

Microbiological Analysis: A strain of *Escherichia coli*

O157:H7 (ATCC 35150) was used throughout this study. The *E. coli* cells were grown overnight in Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI) at 35°C for 24 h. The cells were harvested by centrifuging the overnight culture suspension at 10000 rev/min for 15 min (IEC 21000R, Needham Heights, MA) while temperature was maintained at 4°C. The *E. coli* O157:H7 cells were then washed twice by re-suspending in peptone water and centrifuging at the same conditions, and further suspended in poultry chiller water before subjected to various inactivation treatments.

To enumerate non-injured cell population before and after each treatment, 1 ml of sample was serially diluted with 0.1% peptone water (Difco Laboratories, Detroit, MI) and plated on Violet Red Bile Agar (VRBA) plates. They were then incubated at 35°C for 24 hrs. The initial inoculum's level of *E. coli* O157:H7 in the chiller water used for all the experiments was maintained at about 6.6×10^7 CFU/ml or 7.8 log (regarded as the control sample). Total viable (injured and non-injured) cell populations were enumerated using Trypticase Soy Agar (TSA) supplemented with 0.6% yeast extract (non selective medium).

Ozone Treatment: A schematic of the ozone generation system is shown in Fig. 1. A laboratory ozone generator (Ozomax Ltee, Granby, QC) was used to produce gaseous ozone of high concentration from an oxygen gas feed. All experimental work with ozone gas was done in a chemical fume hood. A 100 ml poultry chiller water sample in a glass flask was inoculated with *E. coli* O1547:H7 cells (7.8 log CFU/ml) and treated with gaseous ozone (20 mg O₃ per 1 L of O₂) for 30 s. The sample was continuously mixed at 10 rpm with a stirrer during the ozone treatment. The ozone concentrations used yielded an ozone dose of 1 mg/ml. Level of ozone dosage used in the study was fixed based on previous preliminary studies and review of literature. The ozonated samples were immediately kept in ice cooler and either analyzed for microbial load, or further treated with PEF and UV treatments.

UV Treatment: UV irradiation of samples was by using a collimated beam apparatus that allowed UV rays to be applied in parallel streamlines instead of the usual diffuse beam (Ngadi *et al.*, 2003). The apparatus consisted of a UV lamp (UV 722, Trojan Technologies, London, ON) with peak radiation at the 254 nm wavelength ranges. The UV intensity at the surface of the sample was measured using a radiometer (Model IL 1400A, International Light Inc., Newburyport, MA). The radiometer was placed at the same distance as the sample from the UV lamp. The UV lamp was switched on for at least 1 hr. prior to beginning sample treatment. This was to minimize fluctuations in the applied UV intensity. The lamp intensities were monitored before

each experiment with the radiometer to ensure fixed UV intensity levels. To irradiate a sample with UV, 6 ml poultry water in a 60 mm diameter Petri dish was placed directly below the collimated UV beam. Treatment time was 1 min. and UV intensities of 117 and 234±3 µW/cm² (UV1 and UV2, respectively) were applied.

Pulsed electric field (PEF) treatment: The PEF processing system was composed of a pulse generator, a treatment cell, and voltage and current measuring devices. The treatment cell consisted of two electrodes held in parallel by insulating material that formed an enclosure that contained the sample (approximately 5 ml) to be treated. A high voltage pulse generator (Samtech Ltd, Glasgow, UK) was used to provide rectangular shaped bipolar pulses. Pulse voltage and current were measured with a high voltage probe (P6015A, Tektronix TDS 3012, Tektronix Inc., OR) and a pulse current transformer (Model 411, Pearson Electronics, Inc., CA). The voltage and current data were recorded on a digital 2-channel oscilloscope (Tektronix TDS 3012, Tektronix Inc., OR). Pulse width and frequency were controlled via an external TTL device with a frequency trigger. Applied electric field strength was 23 and 30 kV/cm (PEF1 and PEF2, respectively). The number of pulses was varied between 50, 100 and 200 pulses of 2 ms each at a frequency of 1 Hz.

Combined Treatments: Combined treatments of poultry chiller water consisted of applying two different hurdles in tandem of each other. For combinations of ozone and UV (O3-UV) or ozone and PEF (O3-PEF), 100 ml of poultry water was first ozonated as previously described. One ml of the ozonated sample was then aseptically removed for microbial analysis. Similarly, appropriate amounts of the ozonated sample were quickly removed for either UV or PEF treatment. For the UV-PEF treatments, 6 ml of the poultry water sample was first irradiated for 1 min. at a given UV intensity (either 117 or 234±3 µW/cm²). Then 1 ml of the UV treated sample was aseptically removed for microbial analysis, whereas the remaining 5 ml was used immediately for PEF treatments as described earlier.

Experimental design: The experimental study consisted of evaluating individual applications of ozone, UV and PEF or their various combinations namely Ozone-UV (O₃-UV), Ozone-PEF (O₃-PEF) and UV-PEF (PEF-UV) for inactivation of *E. coli* cells suspended in poultry processing chiller water. The effectiveness of the different treatments was determined in terms of inactivation levels under the experimental conditions. All treatments were replicated twice in a completely randomized design. One-way analysis of variance was used to compare the effect of the treatments (SigmaPlot 2000, Ver. 6, SPSS Inc., Chicago, IL).

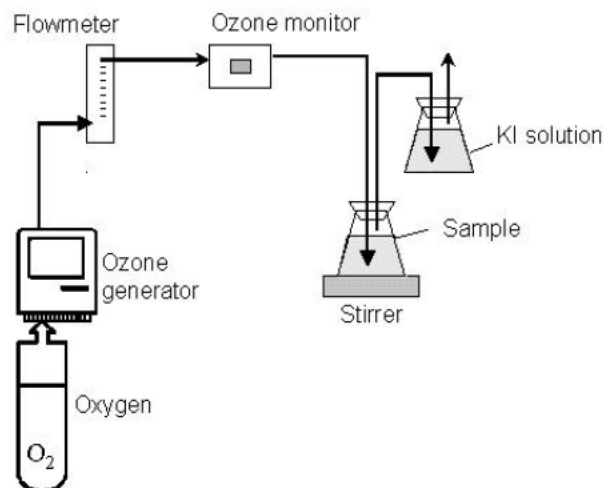


Fig. 1: Illustration of experimental setup for ozone treatment of poultry chiller water

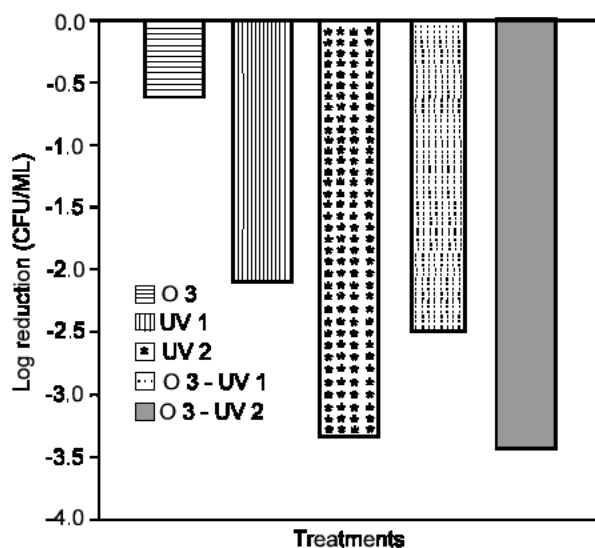


Fig. 2: Inactivation of *E. coli* O157:H7 in poultry chiller water using combined ozone (O₃, at 1 µg/ml) and ultraviolet light (UV, at 117 and UV2 - 234 µW/cm²)

In the legend: O₃ represents O₃; UV1 - UV at 117µW/cm²; UV2 - UV at 234 µW/cm²; O₃-UV1 - Combined O₃ and UV1; O₃-UV2 - Combined O₃ and UV2

Results and Discussion

Levels of *E. coli* O1547:H7 reduction in poultry chiller water using separate individual treatments of ozone, UV and combinations of ozone and UV are shown in Fig. 2. The ozone treatment was applied at the concentration of 1 µg/ml for 30 s. The UV irradiation was at the wavelengths of 117 (UV1) and 234 µW/cm² (UV2) for a

treatment time of 1 min. The reduction levels were significantly (at the 5% level) different for all the various treatments compared to the control treatment. Using the 1 µg/ml ozone dose alone, resulted in only about 0.7 log reduction of *E. coli* in the poultry chiller water. Unal *et al.* (2001) reported 3.7 log reduction of *E. coli* O157:H7 in 0.1% NaCl solution using similar 1 µg/ml ozone dose. The lower inactivation obtained in this study was due to the difference between NaCl solution and poultry chiller water. Ozone is typically more effective in pure and clear cell suspensions. For more complex organic solutions such as poultry chiller water, there may have been competition between bacteria cells and dissolved organic matters resulting in possible shielding of the cells from ozone by the organic solids. Other authors (Diaz *et al.*, 2001; Diaz *et al.*, 2002) also reported that ozone treatment was not effective for *E. coli* inactivation in poultry chiller water even at higher ozone concentrations. The concomitant consequence of using higher concentrations of ozone includes problems with product quality and acceptability.

Fig. 2 also shows that UV treatments were more effective than ozone treatment in reducing the *E. coli* population in poultry chiller water. UV treatment at the intensities of 117 and 234 mW/cm² resulted in 2.1 and 3.4 log reduction, respectively compared to the control sample. Diaz *et al.* (2001) reported about 1.1 log reduction in *E. coli* population in chiller wastewater. Ngadi *et al.* (2003) reported more than 5 log reduction of *E. coli* cells using UV irradiation for liquid foods such as apple juice. The turbidity of chiller water apparently limited the efficacy of UV treatment. *E. coli* inactivation increased with increasing UV intensity.

There was a significant (at the 5% level) difference of 0.8 log between non-injured and viable (combined injured and non-injured) cell populations after ozone treatment as enumerated on the VRBA and TSA plates, respectively. However, there was no significant difference between the non-injured and viable cell populations after UV and PEF treatments. This implied that significant number of cells in the chiller water were injured but not totally inactivated with ozone treatment whereas UV and PEF treatments resulted in no injured cells. Previous studies (Ngadi *et al.*, 2003) also reported that UV treatments resulted in complete inactivation with no injured cell population.

Combining ozone treatment with UV irradiation at the 117 and 234 mW/cm² intensities resulted in 2.5 and 3.4 log reductions, respectively. There was a significant (at the 5% level) difference between the cell population reductions obtained using only UV irradiation at 117 mW/cm² (UV1) and using combined UV1 and ozone (O₃-UV1). However no significant (at the 5% level) difference was obtained between reductions obtained using only 234 mW/cm² UV irradiation (UV2) and using combined UV2 and ozone (O₃-UV2). The result indicates some

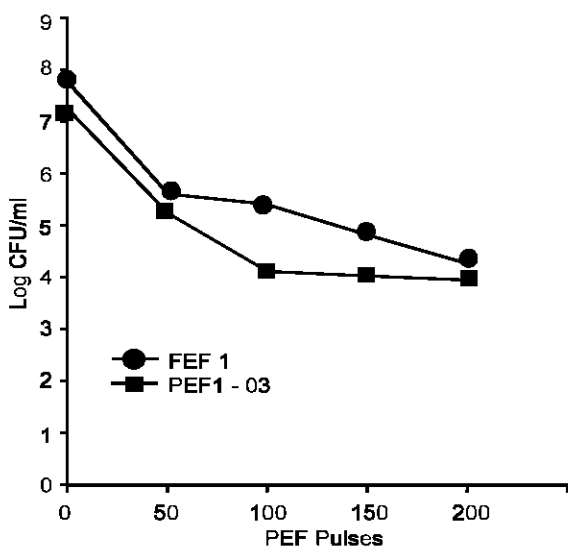


Fig. 3: Inactivation of *E.coli* O157:H7 in poultry chiller water using combined ozone (O_3 , at 1 $\mu\text{g}/\text{ml}$) and pulsed electric fields (PEF, at 23 kV/cm)
In the legend: O_3 represents O_3 ; PEF1 - PEF at 23 kV/cm ; PEF1- O_3 - combine PEF1 and O_3 .

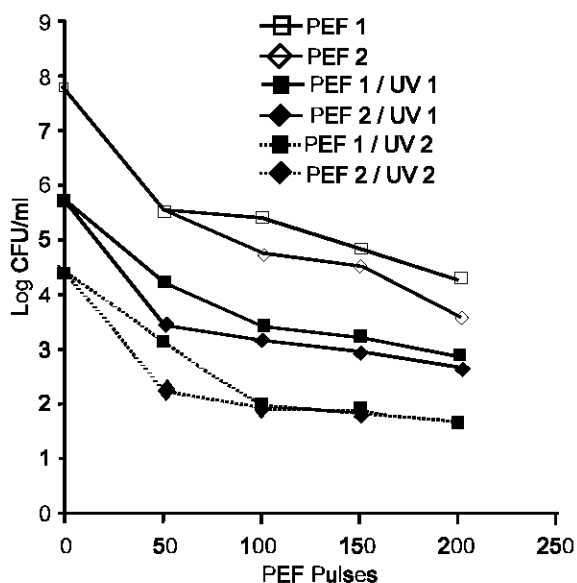


Fig. 4: Inactivation of *E.coli* O157:H7 in poultry chiller water using combined pulsed electric fields (PEF, at 23 and 30 kV/cm) and ultraviolet light (UV, at 117 and UV2 - 234 $\mu\text{W}/\text{cm}^2$)
In the legend: PEF1 represents PEF at 23 kV/cm ; PEF2 represents PEF at 30 kV/cm ; UV1 - UV at 117 $\mu\text{W}/\text{cm}^2$; UV2 - UV at 234 $\mu\text{W}/\text{cm}^2$; PEF1/UV1 - combine PEF1 and UV1; Same indications for other combined treatments

slight synergy between ozone and UV treatments at the lower UV intensity of 117 mW/cm^2 . Increasing UV

intensity, do not offer any ozone enhancement benefit.

Fig. 3 shows cell inactivation using combined ozone and PEF treatments. Electric field intensity was 23 kV/cm (PEF1) and 0, 50, 100, 150 and 200 pulses were applied following ozone treatment. Microbial reduction increased with increasing PEF pulses consistent with other results obtained for PEF treatment (Hulsheger and Nieman, 1980; Jeyamkondan *et al.*, 1999; Barbosa-Canovas *et al.*, 2000). The rate of microbial inactivation was higher at lower pulse numbers whereas tailing phenomena occurred at higher pulse numbers (Amiali *et al.*, 2003). As pulses increased from 100 to 200, reduction of *E.coli* O1575:H7 cells was very nominal. The reduction recorded at 0 pulse for the combine O_3 and PEF (O_3 -PEF1) treatment corresponded to cell inactivation using ozone alone. Comparison of the difference between cell reductions obtained using PEF1 and O_3 -PEF1 treatments at the various pulse numbers showed no significant (at the 5% level) difference at the 0, 50 and 200 pulse numbers. However, combined ozone and PEF treatment resulted in significantly higher reduction at 100 and 150 pulse numbers. Again the result suggests slight synergistic relationship between the ozone and PEF hurdles at the 100 and 150 pulses. The synergy may be attributed to complete inactivation of the injured cells after ozone treatment.

Combined treatments using PEF and UV are shown in Fig. 4. Inactivation increased with increasing PEF treatment intensity as well as with increasing UV intensity. The data recorded at 0 pulse corresponded to application of UV alone. More than 6 log reduction was obtained using combined UV and PEF treatments. There was apparently synergy between PEF and UV treatments at 50 pulses. However, this synergy was lost at higher pulse numbers. Again, the synergy between the 2 hurdles is attributed to the complete inactivation of the injured cells generated after UV treatment. Apparently effect of combined treatment became largely additive when the injured cells were inactivated.

Conclusions: Inactivation of *E. coli* O157:H7 in poultry chiller water using treatments with ozone, UV and PEF alone and in various combinations has been investigated. About 0.7 log reduction was obtained after 30 s application of ozone at the dose of 1 mg/ml of sample. UV treatments at 117 and 234 mW/cm^2 for 1 min resulted in 2.1 and 3.4 log reduction, respectively. PEF treatment of 200 pulses at 23 and 30 kV/cm resulted in 3.5 and 4.1 log reductions, respectively. Cell population decreased with increase in intensity of the treatments. Additional and synergistic effects were obtained when the treatments were combined in tandem. Although ozone is normally highly effective against microorganisms, in pure cell suspensions, it is not likely to be effective for poultry chiller water due to its organic and solute content. The results indicate high

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potential for combined UV and PEF treatment in developing new strategy of decontamination of poultry chiller water.

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