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Inactivation of *Salmonella* and *Escherichia coli* in Surface Agricultural Water Using a Commercial UV Processing Unit

ABSTRACT

Treatment of agricultural water aids in the prevention of foodborne disease outbreaks linked to contaminated fresh produce. UV light is a suitable alternative for treating drinking water but is not always effective for surface irrigation water due to interference caused by turbidity and high microbial loads. The effectiveness of UV treatment for reducing *Escherichia coli* and *Salmonella* in surface water used in agriculture was evaluated. Six pond water samples were collected on each of 16 sampling dates over a 3-year period. On each corresponding testing date, three samples were inoculated with *Salmonella enterica* serovars Hartford, Montevideo, and Gaminara and the other three samples were inoculated with *E. coli* ATCC 25922, targeting a concentration of 7 log CFU/mL. Inoculated water was UV treated with a commercially available juice processing UV device at a constant UV dose of 14.2 mJ/cm^2 and a turbulent flow regime. The effects of date, initial bacterial counts, and water pH and turbidity on log reductions of both microorganisms were determined. Initial bacterial counts and test date significantly predicted microbial reduction (multivariate $P < 0.001$), but neither pH nor turbidity influenced microbial reductions ($P > 0.05$). UV treatment reduced both *Salmonella* and *E. coli* by a mean of >6 log CFU/mL.

INTRODUCTION

Several foodborne disease outbreaks due to consumption of contaminated fresh produce have been reported in past years worldwide *(3, 4, 10)*. Many of these cases have been linked to untreated contaminated irrigation water and include outbreaks due to the consumption of jalapeno and serrano peppers, alfalfa sprouts, tomato, lettuce, and cauliflower contaminated with *Salmonella* or *Escherichia coli* O157:H7 *(14)*. Agricultural water is one of the main vehicles by which pathogenic microorganisms reach fresh produce *(3)*, and the risk of contamination increases when untreated surface water is used for irrigation *(8, 11)*. About 52% of the water used for irrigation in the United States is surface water *(5)*.

Improved food safety practices, including water treatment and microbial water quality monitoring, are some of the currently applied preventive and mitigation strategies for reduc-

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ing consumer exposure to foodborne pathogens. Current regulations such as the Food Safety Modernization Act Produce Safety Rule for the United States *(19)*, private standards such as GLOBALG.A.P. *(7)*, and guidelines from the World Health Organization *(3)* require that all agricultural water must be safe and of adequate sanitary quality for its intended use. The U.S. Food and Drug Administration (FDA) *(19)* has clarified that when agricultural water does not comply with this criterion, treatment is only one of the options available to comply with the regulation and avoid safety issues. For safety assurance, water may be disinfected with traditional approaches, including chlorine, ozone, UV radiation, and filtration. These methods are effective for drinking water but are not always suitable for the treatment of agricultural surface water due to the effects of factors such as pH, turbidity, dissolved solids, and high microbial loads on the efficacy of the treatments *(9, 10)*.

UV light inactivates microorganisms by damaging their nucleic acids and therefore preventing replication *(10)*. UV light can eliminate human pathogens, including bacteria, protozoa and most viruses, in drinking water, in water from nursery settings where recycling is a common method of water and nutrient conservation, and in certain liquid foods and beverages including unfiltered fruit juices such as apple cider *(3, 9, 15)*. Unlike surface water, drinking water is characterized by low turbidity and low microbial populations *(18)*. UV light is generally not recommended for disinfection of surface water with turbidity levels >1.0 nephelometric turbidity units (NTU) because UV light blocking or absorption may shield pathogens of concern. However, the commercial UV unit used in the present study was designed to overcome turbidity issues even in highly turbid beverages such as unfiltered apple cider, with a turbidity of >1,000 NTU *(9, 10)*. In a previous study, Jones et al. *(9)* evaluated the efficacy of the same commercial UV processing unit for decontamination of unfiltered surface water (stream) samples inoculated with bacterial and oomycete pathogens. The authors reported ≥ 99.9% inactivation rates for all of the inoculated microorganisms. However, profiles of agricultural waters, and particularly surface irrigation waters, are highly variable and may change over time due to weather events or human activities *(9)*.

The present study was focused on pond surface irrigation water samples collected over an extended period and included samples with higher turbidity levels than previously evaluated and reported. The specific study objective was to evaluate the effectiveness of UV radiation for reducing levels of *E. coli* and *Salmonella* in longitudinally collected pond water samples. A commercially available UV juice processing reactor that can also be used for the industrial treatment of irrigation water was used to treat the samples. Specific suggestions for measures that growers can take to reduce microbiological contamination from agricultural water are still lacking *(3)*. Therefore, this research was conducted to provide tangible recommendations regarding the application of UV light as an antimicrobial treatment for surface agricultural water.

MATERIALS AND METHODS

Water samples

To determine efficacy of UV light against *E. coli* and pathogenic *Salmonella*, six water samples (800 mL each; three for each of the two microorganisms tested) were collected on each of 16 sampling dates over a 3-year period (2016 to 2018) at the Texas A&M AgriLife Research and Extension Center (Weslaco, TX). These samples were tested on the corresponding testing dates: 16 dates of testing × 2 microorganisms × 3 replicates = 96 water samples collected and processed. The irrigation water was collected from an open pond (used for irrigation of produce crops) fed by canals from the Rio Grande River and filtered with sand filters. The average electrical 100% conductivity was 0.13 S/m. Water was pumped from the pond into holding tanks, and samples were aseptically collected and delivered to Cornell University within 24 h. The water was mixed thoroughly, and the pH (HI 2211 pH/ORP meter, Hanna, Woonsocket, RI) and turbidity (2100P portable turbidimeter, Hach, Loveland, CO) were measured.

Sample inoculation

Each of the 96 collected water samples was independently inoculated immediately before conducting the experiments with 8 mL of either (i) a three-strain cocktail of *Salmonella enterica* (serovars Hartford H0778, Montevideo, and Gaminara) or (ii) a single strain of *E. coli* ATCC 25922 (a nonpathogenic surrogate with UV sensitivity similar to that of *E. coli* O157:H7) *(13)*, to reach an initial level of 10^7 to 10^8 CFU/mL. For inoculum preparation, a single isolated colony of each pathogen strain (*E. coli* and *Salmonella*) was grown overnight in Trypticase soy broth (Difco, BD, Sparks, MD) at 35 ± 2°C for 20 ± 2 h to stationary phase in an rotary platform shaker (Innova 2300, New Brunswick Scientific, Edison, NJ) at 250 rpm. The *Salmonella* cocktail was prepared by mixing equal amounts of each strain previously grown to stationary phase. The inoculum was added with the growth medium without a previous wash but the volume represents 1% of the total volume so the intrinsic physicochemical properties of the water samples would not be compromised *(2)*.

UV treatment

A 750 mL volume of inoculated water was immediately treated at room temperature (25°C) with the UV treatment unit (CiderSure 3500, FPE, Rochester, NY). A thorough description of the processing device was previously published *(15, 16)*. Based on information from the UV sensors, the flow rate in the UV unit was automatically adjusted to overcome differences in water quality parameters (i.e., solid contents, turbidity, and color) *(9)*. For water samples with high absorption, the pump flow rate was automatically reduced so that a constant UV dose of 14.2 mJ/cm^2 at a wavelength of 254 nm was consistently delivered to all samples while ensuring a turbulent flow regime (Re > 2200). The maximum flow rate of the UV unit was 378 L/h.

Microbial enumeration

E. coli and *Salmonella* in water samples were enumerated before and after UV treatment using the methodology reported by Usaga et al. *(15)* and Jones et al. *(9)* for UV-treated liquid foods and unfiltered surface irrigation water, respectively. Appropriate serial dilutions in sterile 0.1% peptone water were aseptically plated in duplicate in petri dishes, and 15 mL of Trypticase soy agar (Difco, BD) was pour plated. After solidification, petri dishes were incubated at 35 ± 2°C for 20 ± 2 h. The differences between the log-transformed microbial counts before and after UV exposure were calculated. Although high microbial loads were expected due to background microbiota in surface water, a nonselective growth medium was used, as described previously *(9)*. Because UV exposure may sublethally damage bacterial cells and affect their growth in selective media, use of a nonselective nutrient medium prevents overestimation of the log reductions, which represents a safety concern. The inoculated microorganisms (*Salmonella* and *E. coli*) were not differentiated from the background microbiota during enumeration on nonselective medium, but due to the high inoculum level, the target microorganism levels significantly surpassed the background microbial populations. The total log reduction was calculated.

Statistical analysis

Effects of testing dates, initial bacterial counts, and water pH and turbidity on the difference in log-transformed microbial counts before and after treatment were explored with the Wilcoxon rank sum test (for categorical variables) and an analysis of variance (for numerical variables). Multivariate regression analyses were conducted including variables with a univariate $P < 0.20$, using a backward stepwise elimination procedure.

RESULTS

The overall median difference in bacterial counts before and after the UV treatment was 6.3 log CFU/mL *(Table 1)*. When *E. coli* and *Salmonella* were evaluated separately, the median difference before and after UV treatment was slightly lower for *E. coli* (6.2 log CFU/mL) than for *Salmonella* (6.4 log CFU/mL) (multivariate $P < 0.001$). The date of testing (six samples per date) was a significant predictor of the reductions obtained (multivariate $P < 0.001$). Within the ranges tested, neither water pH (6.35 to 8.19) nor turbidity (11.9 to 58.9 NTU) *(Table 2)* were significantly associated with the reduction in the multivariate models ($P > 0.05$). However, inoculation levels (initial counts) also differed by testing date and were slightly higher for *Salmonella* (median, 7.8 log CFU/mL) than for *E. coli* (median, 7.7 log CFU/mL), which could explain the difference in log reductions obtained. Although a nonselective medium was used for microbial enumeration, the results indicated significant inactivation of the total microbial load. Differences in initial microbial populations may be influenced by the presence of uneven background microbiota in the water samples because with the selected approach the total microbial load was enumerated.

DISCUSSION

UV light is a nonthermal and environmentally friendly approach for inactivation of pathogens in surface agricultural water. In the present study, UV light treatment effectively reduced a high load of two inoculated vegetative microorganisms of food safety relevance, regardless of the normal and expected variability of surface water properties over time. That variability, over the period tested, may explain why testing date significantly influenced the microbial reductions obtained. These results are in agreement with previously reported findings for unfiltered surface irrigation water from different geographic locations with lower turbidity ranges (≤ 20 NTU) collected over a shorter sampling period *(9)*.

Because UV systems are most effective when water is clear and free of suspended particles, most technical recommendations for surface water indicate the need to couple filtration with UV to guarantee treatment efficacy *(3)*. However, this suggestion may need to be revised based on recent findings. Jones et al. *(9)* described 3-log microbial inactivation in UV-treated irrigation water at relatively high turbidity (20 NTU), and in the present investigation the inactivation was > 6 log CFU/mL with the same technology and water samples with more than two times higher turbidity values of up to 58.9 NTU. These results should be replicated with other commercially available thin-film UV devices that ensure exposure to a constant UV radiation dose using a turbulent flow regime and an adjusted flow rate based on the sample absorptivity profile. Nevertheless, microbial validation of each UV device is necessary before use, and the performance of UV units must be monitored periodically to confirm the efficacy of the technology as required by the U.S. Environmental Protection Agency for the UV treatment of drinking water *(17)*.

Water disinfection by UV radiation is dependent on multiple variables in addition to water properties, such as UV treatment duration and intensity. The relationship between the required UV dose and the UV intensity (measured by UV sensors), flow rate, and transmittance must be established and monitored to ensure sufficient disinfection of microbial pathogens *(18)*. For example, UV water sterilization systems for greenhouse irrigation water are designed for exposures of 80 to 250 mJ/cm^2 *(12)*. The considerably lower UV dose (14 mJ/cm^2) used in the present study, albeit with a turbulent flow regime, may represent a treatment alternative for turbid water sources.

Because any material that absorbs or reflects UV light, such as dissolved solids in water (e.g., iron), can decrease UV transmittance and therefore reduce the germicidal effect, a detailed physicochemical characterization of each water source is necessary before implementing UV

TABLE 1. Bacterial counts in water samples before and after UV treatment

Inoculated water	Bacterial counts (log CFU/mL)				
	Median	Minimum	25th percentile	75th percentile	Maximum
Before UV	7.7	7.3	7.6	7.8	8.1
After UV	1.4	1.0	1.3	1.5	2.4
Difference	6.3	5.6	6.2	6.4	6.7

TABLE 2. Testing date, pH, and turbidity of water irrigation samples, initial *Salmonella* and *E. coli* inoculum levels, and log reductions after UV treatment

Sample set ID	Testing date (day/mo/yr)	Mean pH[a]	Mean turbidity (NTU)[a]	Mean ± SD initial count (log CFU/mL)[b]		Mean ± SD reduction (log CFU/mL)[b]	
				Salmonella	*E. coli*	*Salmonella*	*E. coli*
1	13/05/2016	6.84	21.00	7.4 ± 0.2	7.55 ± 0.03	6.1 ± 0.1	6.1 ± 0.1
2	20/05/2016	6.86	17.90	7.50 ± 0.06	7.54 ± 0.06	6.1 ± 0.3	6.3 ± 0.1
3	26/05/2016	7.23	24.00	7.48 ± 0.03	7.54 ± 0.03	6.1 ± 0.1	6.3 ± 0.6
4	30/11/2016	6.98	11.90	8.00 ± 0.03	7.9 ± 0.1	6.2 ± 0.1	5.9 ± 0.3
5	07/12/2016	7.43	21.95	7.72 ± 0.04	7.68 ± 0.01	6.28 ± 0.04	6.24 ± 0.05
6	13/1/2017	7.50	39.23	7.89 ± 0.02	7.9 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
7	15/02/2017	7.47	13.00	8.09 ± 0.00	7.68 ± 0.04	6.62 ± 0.07	6.4 ± 0.2
8	07/04/2017	7.80	13.20	7.72 ± 0.04	7.68 ± 0.01	6.3 ± 0.1	6.37 ± 0.04
9	26/4/2017	7.79	30.00	7.74 ± 0.09	7.64 ± 0.01	6.21 ± 0.04	6.1 ± 0.1
10	05/05/2017	7.80	25.40	7.8 ± 0.1	7.8 ± 0.1	6.41 ± 0.07	6.4 ± 0.1
11	01/06/2017	6.91	19.30	7.65 ± 0.09	7.77 ± 0.08	6.2 ± 0.1	6.4 ± 0.3
12	17/11/2017	7.39	27.30	7.78 ± 0.03	7.7 ± 0.1	6.5 ± 0.1	6.2 ± 0.2
13	06/12/2017	7.29	12.60	7.70 ± 0.05	7.62 ± 0.03	6.51 ± 0.09	6.32 ± 0.03
14	19/12/2017	6.35	21.50	7.9 ± 0.1	7.7 ± 0.1	6.41 ± 0.03	6.19 ± 0.07
15	25/01/2018	6.70	33.20	7.59 ± 0.06	7.38 ± 0.08	6.3 ± 0.1	6.0 ± 0.1
16	15/02/2018	8.19	58.90	7.73 ± 0.03	7.6 ± 0.2	6.50 ± 0.07	6.2 ± 0.2

[a]n = 6.
[b]n = 3 for each microorganism-date combination.

treatment as a sole pathogen mitigation strategy. Selection of the most appropriate water-treatment method must take into consideration multiple factors such as technological, managerial, and sustainability criteria in addition to microbial inactivation rates.

Before this study, various turbidity causing materials (TCMs) were evaluated for their effect on the attachment of *E. coli* and *Enterococcus faecalis* in water samples with turbidities of 0 to 5 NTU *(6)*. The TCMs were representative of those that may be present in surface and ground waters. TCMs influenced inactivation of *E. coli* and *E. faecalis* due to decreasing UV transmittance with increasing TCM concentration. From 2.5- to 3.9-log reductions at a UV dose of 10 mJ/cm^2 were reported for *E. coli*. In that study, the water sam-

ples were treated statically in petri dishes, and the effect of a turbulent flow regime, which is the novelty of the commercial UV device used in the present study, was not considered. The use of this particular UV device may explain the higher inactivation rates found in our study. In another recent study, the effectiveness of UV-C radiation for reducing the microbial population in agricultural water (turbidity of 10.93 to 23.32 NTU) was evaluated *(1)*. Samples were inoculated with *E. coli* (ATCC 23716, ATCC 25922, and ATCC 11775) and treated with UV doses of 20 to 60 mJ/cm^2. In contrast to our results, UV-C treatment effectively reduced the microbial load in agricultural water, but turbidity significantly affected the disinfection efficacy. In that study, the UV chamber of the UV-C light treatment equipment (PMD 150C1/4, Aquionics, Slough, Charlotte, NC) was 0.2 m in diameter but the flow rate and Reynolds number associated with the treatments were not reported, so comparisons of results are limited. Overall, the inactivation values reported in that study were lower than those obtained in our investigation, even in water samples with a lower turbidity level treated at a higher UV dose. In our study, the liquid was pumped through the UV treatment system in a thin film, using a turbulent flow regime, which may explain the higher inactivation values and the nonsignificant effect of turbidity.

The UV device evaluated in this study could be effectively used for treatment of agricultural water, given the ease of use and its low energy requirements. This device is one of the most commonly used commercial UV machines for the nonthermal processing of apple cider in the United States *(16)*. The most important innovation with this UV treatment unit is that it senses the UV exposure every 20 ms and automatically adjusts the flow rate to ensure appropriate and consistent UV exposure. Although agricultural water is extreme variable, this unit can accommodate for variations that may be encountered. Some barriers that may hinder a broad implementation of the technology in the open fields are the required initial investment and access to an energy source in the field.

CONCLUSIONS

The present study results indicate that UV radiation can be an effective mitigation strategy for pond surface irrigation water treatment. With a commercially available juice processing unit that automatically adjusts the liquid flow rate based on the fluid UV absorbance, *E. coli* and *Salmonella* levels were reduced from approximately 8 to < 2 log CFU/mL. This method is a promising technological alternative for agricultural water treatment and is of particular relevance, considering the numerous outbreaks linked to contaminated produce and the increasingly limited supply of high-quality water for agricultural use *(12)*.

Further research is needed to confirm the efficacy of the proposed treatment against parasites of public health concern, such as *Cryptosporidium* and *Giardia*, and against viruses, considering that ≥ 4-log removal and/or inactivation is required for drinking water. The UV device evaluated in this study was previously confirmed to be effective against *Cryptosporidium parvum* in turbid and cloudy apple cider *(8)*, and a similar germicidal effect against this parasite is expected in surface agricultural water.

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