CHLORINE DIOXIDE INJECTION THROUGH DRIP IRRIGATION REDUCES *E. COLI*

Stuart R. Reitz, Malheur County Extension, Oregon State University, Ontario, OR, 2015

Clinton C. Shock, Malheur Experiment Station, Oregon State University, Ontario, OR

Harry Kreeft, Western Laboratories, Inc., Parma, ID

Jim Klauzer, Clearwater Supply, Ontario, OR

Abstract

The delivery of bacteria through irrigation water could contaminate produce destined for fresh consumption. To mitigate the potential for this risk, growers would need systems to remediate irrigation water quality. Ideally, such a system would not require additional infrastructure and could be implemented with minimal disruption of current production practices. Chlorine dioxide currently is used by growers to maintain drip irrigation systems by inhibiting the growth of algae and biofilms, which clog drip irrigation lines. We tested chlorine dioxide at 1 and 3 ppm, the current standard maintenance treatment concentration, through drip irrigation to reduce generic *Escherichia coli* in water delivered to a commercial onion field. Generic *E. coli* is used as an indicator species for fecal contamination. Water was sampled for *E. coli* at its canal source, after the filter station, and progressively at sites across the field. The most probable number of *E. coli* in water samples was determined using IDEXX Colilert® +Quanti-Tray/2000®. Chlorine dioxide substantially reduced *E. coli* counts at all sampled locations and concentrations. Therefore, the use of chlorine dioxide could be adapted as a means to remediate microbial contamination of irrigation water, if needed for compliance with water quality standards.

Introduction

The Food Safety Modernization Act (FSMA), which was signed into law in January 2011, charges the U.S. Food and Drug Administration (FDA) with ensuring the safety of the U.S. food supply by acting preventively rather than reactively to foodborne illness outbreaks. Of particular concern to Treasure Valley onion producers is that the final Produce Safety Rules, published on November 27, 2015, place stringent testing requirements on agricultural water quality (U.S. FDA 2015). Furthermore, the rules require some type of remediation to bring agricultural water into compliance with standards for produce covered by the rules. Agricultural water is defined in the proposed rules as "water that is intended to, or likely to, contact the harvestable portion of covered produce or food-contact surfaces." The quality of agricultural water intended for irrigation will have to be consistent with quality standards for microbial contamination of recreational water in order to be used (U.S. FDA 2015). The FDA has adopted generic *E. coli* as

the indicator for determining agricultural water quality although not all strains of the bacterium are pathogenic (Edberg et al. 2000; U.S. FDA 2013, 2014).

Growers will be responsible for assuring that their irrigation water complies with FSMA standards. The burdens of the water quality rules for onion growers in the Treasure Valley will consist of the labor for frequent sampling of water and record keeping, the cost of laboratory analyses, and any potential remediation methods.

Historically, onions in the Treasure Valley have been grown under furrow irrigation. Today, over half of the onions are grown with drip irrigation (Shock et al. 2013). Drip irrigation systems provide many benefits for onion production, but they are prone to fouling and clogging from algal growth and biofilm formation within the drip tape (Shock et al. 2013). Consequently, most systems use some sort of chemical injection to inhibit and remove growth (Raudales et al. 2014). Chlorine dioxide (ClO_2) is an effective chemical that many farmers use to keep irrigation lines clean; it is a strong oxidizing agent that is a more potent bactericide than chlorine (Bernarde et al. 1965, Lazarova et al. 1999). It is also more effective as a disinfectant than chlorine when high concentrations of organic compounds or ammonia are present in water (Parish et al. 2003).

Our objective was to determine if currently available treatments for reducing clogging of drip irrigation systems could be adapted to reduce microbial levels to comply with FSMA standards, if necessary. Specifically, we wished to determine if the small concentrations of chlorine dioxide used to keep bacteria and algae from clogging drip lines would significantly reduce *E. coli* contamination in the water used to irrigate onions.

Materials and Methods

The trial was conducted at a 60-acre commercial onion field located in Payette County, Idaho during July and August of the 2015 growing season when onion crop water needs are the greatest and biological activity in drip lines is the greatest (Shock et al. 2013). For the drip irrigation system, the field was divided into 4 equally sized irrigation zones of 15 acres each. We used the zones along the west side of the field for the trial.

Irrigation water was pumped from an open canal by a diesel-powered pump. The water was filtered through a 345 sand media filter (Fresno Flow-Gard, Fresno Valves and Casting, Selma, CA), using crushed garnet as the medium. Irrigation flow was monitored by a 10-cm mechanical propeller type flow meter (McCrometer, Hemet, CA) to determine system health and hygiene. The system was pressurized by a 47.1-kW diesel engine, connected to a centrifugal pump (Cornell 3RB, 26.7 cm impeller, Cornell Company, Portland, OR) that was capable of delivering a minimum of the 852 L/min of water at a minimum of 33.5 m of total dynamic head.

The drip irrigation tape (Toro Aqua-Traxx EA5060817-1000, Toro Co., El Cajon, CA) was 6 mil thick, 5/8 inches wide and had emitters spaced 8 inches apart. The emitter flow rate was 0.066 gal/hour. Tapes were laid at a 2-inch depth at planting. Three tapes were installed on an 88-inch bed in a single tractor pass, 22 inches between tapes, and with the center of the double rows of onions planted 6 inches from the drip tape.

This irrigation system was designed to deliver a maximum of 0.41-acre inches of water daily, when operating on a 24 hour basis, or 0.069 acre-inches/hour. This calculation is based on rotating automatically among zones at a 6 hour interval when in continuous use. An AG Tech

controller (Clearwater Supply, Othello, WA) was used to automatically switch among zones.

Chlorine dioxide was generated with an AgriSystem 2.3 in-field generator (CH2O, Olympia, WA), which was placed before the sand media filter station of the drip irrigation system. The generator utilized two metering pumps (Model EWC21Y1VC, Walchem, Holliston, MA) to mix 15% sulfuric acid (H_2SO_4) plus proprietary additives (Sure Flow F, CH2O, Olympia, WA) with 15% sodium chlorite ($NaClO_2$) plus proprietary additives (Clean Finish, CH2O) at a 1:1 ratio in an AgriSystem 2.3 (CH2O, Olympia, WA) to produce the chlorine dioxide. The metering pumps allowed chlorine dioxide concentrations to be adjusted to the appropriate test levels. At each sample time, the chlorine dioxide concentration was set to the appropriate level, and the irrigation system was allowed to run for 45 min before any sampling was performed to allow the test concentration of chlorine dioxide to be distributed throughout the drip lines.

Water was sampled for *E. coli* progressively along the irrigation path. Because constituents in irrigation water are in constant flux, the canal source water was always sampled to obtain a representative initial concentration of *E. coli* in the source water at the time of sampling. The next sample took place at the filter station, after the chlorine had been injected and the water had passed through the sand media filters. These samples were collected from a release valve at the filter station. Samples also were collected from the layflat hose at the top of the field, the midpoint of drip tape lines, and the end of the drip tape lines from each of two onion beds, for a total of eight water samples at each sampling time. The samples at the top and mid-points of the lines were collected from tubing that had been inserted into the layflat or drip tape lines. These tubes were closed between samplings. Samples from the ends of the drip tape lines were collected by opening the release valves at the end of the lines. Samples were collected over about a 2-hour period on each sample date, from 10:00 a.m. to 4:00 p.m.

The water sampling procedure consisted of letting the intended spot flush for 1-5 min (with longer flush times further along the irrigation line because of sediments in the lines). Sample bottles were filled once, never flushed, and immediately placed in a cooler. A new pair of nitrile rubber gloves was worn for each sample. Collected samples were stored in a travel cooler with two ice packs covered by a cardboard barrier to keep them cool during storage and transportation. Before collecting a sample for *E. coli*, an oxidant test was performed on the water using a testing kit (TEI-1, CH2O Inc., Olympia, WA) to estimate the concentration of chlorine dioxide, in ppm, at that particular location. The intensity of the colorimetric reaction is an indicator of the chlorine dioxide levels.

Water sample bottles contained sodium thiosulfate ($Na_2S_2O_3$), which reacts with chlorine dioxide in water to deactivate it and prevent any further degradation of the bacteria by the chlorine dioxide during storage. Therefore, *E. coli* levels reflected amounts present at the time of collection.

Water analysis for generic *E. coli*

Water samples were kept refrigerated until analysis. We used the IDEXX Colilert® +Quanti-Tray/2000® system (IDEXX Laboratories, Westbrook, ME) to quantify total coliform bacteria and generic *E. coli* concentrations in the samples. The Colilert system has been approved as a water quality testing system by the U.S. Environmental Protection Agency (1999) and we followed the manufacturer's directions in conducting assays. Briefly, a reagent pack that contains two enzyme substrates and a nutrient broth was added to 100 ml of a water sample. One substrate reacts with galactosidase enzyme found in coliform bacteria. The second substrate

reacts with the glucuronidase, which is only present in *E. coli*. Aliquots of each sample are then placed in wells of Quanti-Tray/2000 trays. The presence of coliform bacteria is indicated by a yellow color and the presence of *E. coli* is indicated by fluorescence. The most probable number (MPN) of either total coliforms of *E. coli* is determined from the number of positive wells for each sample (Edberg et al. 1988, 1990). If all sample wells are positive, MPN values are reported as greater than 2,420. Quantification would then require serial dilutions of the original sample to be tested.

Results

We sampled water 4 times with the system delivering 1 ppm chlorine dioxide and 4 times with the system delivering 3 ppm chlorine dioxide and 4 times with no chlorine dioxide being injected into the system. This last treatment provided a control to determine if *E. coli* levels decline within drip lines in the absence of chlorine dioxide. The levels of *E. coli* in the canal source water were low throughout the season, ranging from 7.5 to 24.6 MPN/100 ml water.

When no chlorine dioxide was injected into the lines, *E. coli* levels remained relatively constant (Fig. 1).

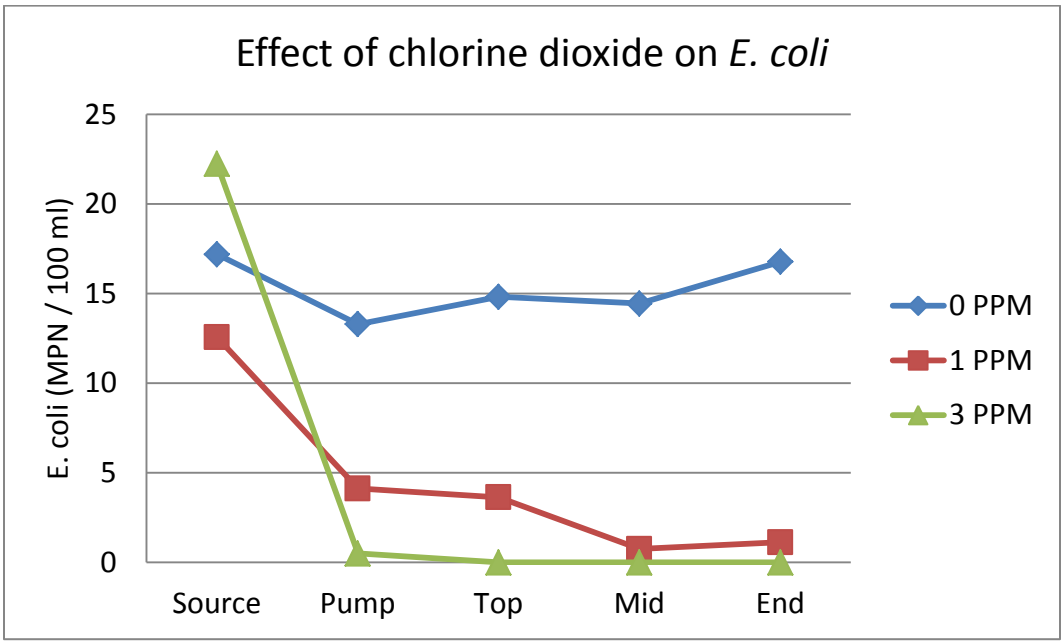

Figure 1. E. coli levels in response to chlorine dioxide injected into drip irrigation system after the source. Chlorine dioxide was injected at either 1 ppm or 3 ppm. E. coli *levels were measured at the water source, pumping station, top, middle, and bottom of the field. Data are the means of two samples collected at each site on four sample periods for each chlorine dioxide treatment.*

E. coli levels in the water were substantially lower at all points along the field than at the canal source when 3 ppm of chlorine dioxide was injected (Fig. 1). Chlorine dioxide exposure appeared to be almost instantaneously lethal to *E. coli* as levels dropped to 0-1 MPN at the filter station and *E. coli* was completely eliminated from the water by the time the water reached the lay-flat hose at the top of the field.

Chlorine dioxide levels were 3 ppm at the filter station and ranged from 1 to 2 ppm throughout the field on each sample date, indicating that sufficient concentrations of chlorine dioxide remained in the system and could have controlled higher levels of *E. coli*.

As with the 3 ppm treatment, *E. coli* levels in the water were substantially lower at all points along the field than the canal source when 1 ppm of chlorine dioxide was injected (Fig. 1). Although *E. coli* was not completely eliminated with 1 ppm chlorine dioxide, levels were substantially reduced at the pump, with concentrations 67% lower at the pump than at the source. *E. coli* levels remained constant between the pump and the top of the field; however, by the time water reached the middle of the field, levels were less than 1 MPN/100 ml.

Chlorine dioxide levels were 1 ppm at the filter station and still detectable (~ 1 ppm) throughout the field on each sample date, again suggesting that the amount of chlorine dioxide present was not a limiting factor.

Discussion

Chlorine dioxide currently is used to maintain drip irrigation systems by inhibiting the growth of algae and biofilms that clog drip irrigation lines. Our project aimed to better understand other potential benefits of chlorine dioxide as a disinfectant to remove unwanted microbial organisms from irrigation water. If chlorine dioxide successfully controls microbes, such as generic *E. coli,* the economic burden to growers for compliance with the FSMA water quality rules could be less than if novel techniques were needed for water quality remediation.

Chlorine dioxide injected into drip irrigation systems appears to be an effective means of reducing or eliminating *E. coli* contamination. The 3-ppm treatment virtually eliminated *E. coli* in the water in the drip lines. At 1 ppm, *E. coli* levels were significantly reduced compared with levels at the water source although *E. coli* was never fully eliminated.

At 3 ppm chlorine dioxide, *E. coli* contamination was completely eliminated shortly after the injection site. In nearly every trial, *E. coli* levels were 0 by the time the water reached the filter station. Again, there was some variability in contamination levels at the end of the drip tape (Fig. 1). These results suggest that chlorine dioxide injection, while already in place to inhibit algal growth, can simultaneously bring *E. coli* contamination levels down to acceptable values.

As an algaecide for anti-clogging treatments, chlorine dioxide is normally injected into drip irrigation systems at 3 ppm with a goal of having 1 ppm available at the tail end of drip lines, thus ensuring sufficient amounts are present throughout the system. These treatments are typically done as a shock treatment at the end of an irrigation cycle. To use chlorine dioxide to remediate water for compliance, chlorine dioxide injections would need to be made on a nearly continuous basis. Therefore, we wished to determine if concentrations less than 3 ppm could satisfactorily reduce *E. coli.* This proved to be the case, as 1 ppm of chlorine dioxide essentially eliminated *E. coli* from the system.

We did not evaluate the effects of 1 ppm chlorine dioxide on algae or biofilm formation. However, if it is not sufficient to prevent clogging and fouling, automated controllers could allow higher shock levels (i.e., 3 ppm) to be delivered at the end of an irrigation cycle.

Chlorine dioxide has several advantages over other potential disinfectants. Its activity is not hindered by increasing pH as is chlorine and remains efficacious at pH 5-10 (Hoigné and Bader 1994, Ward et al. 1984). Its effectiveness at high pH is attractive for the Treasure Valley where pH in waterways ranges from 6.9 to 8.9. Most importantly, it currently is used for an important maintenance function in drip irrigation systems. Growers could be saved from substantial financial burden if they can use preexisting, established practices to treat their irrigation water to below the proposed standards for *E. coli*.

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