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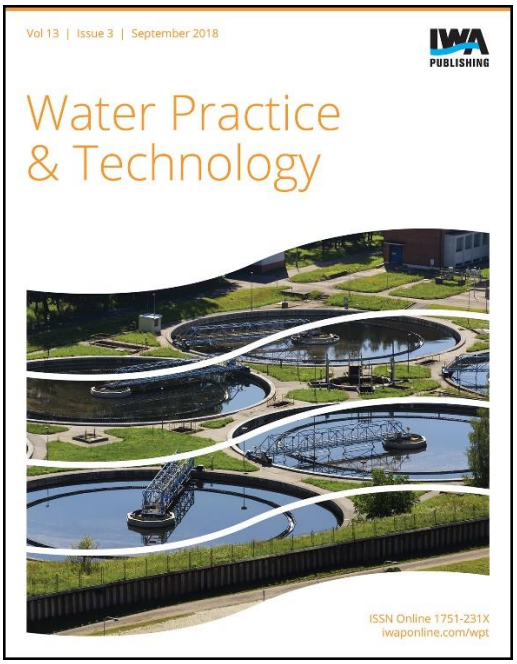

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A novel system for the treatment of wastewater from a tomato processing plant with UV light

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Abstract

In tomato processing plants, the production of paste involves the use of heat to evaporate water to concentrate the tomato juice. The hot water isolated from the juice is then passed through cooling towers to cool it sufficiently before discharge. Recovery of excess and blowdown water from the cooling towers would decrease the net water demand of the plant and improve water efficiency. However, since this water has been exposed to the open air, it must be disinfected before reuse. This research investigated the use of a novel ultraviolet (UV) system to disinfect water from the cooling tower basins located at an industrial tomato processing facility. The objective was to assess, *in situ*, the disinfection system's performance with regards to its utility, and its ability and to treat wastewater generated in an operational, industrial-scale setting. Beyond typical wastewater microbial assays, 16S rRNA gene sequencing was employed to understand the bacterial communities present in the wastewater, and to screen for microorganisms that may pose a risk for water reuse in food processing facilities.

Key words: 16S rRNA sequencing, cooling tower, tomato processing plant, UV disinfection

INTRODUCTION

The urgent need for efficient methods for the treatment of industrially-generated wastewater to an acceptable standard to permit its reuse is best illustrated with reference to the conditions and prevailing practices in the State of California. In that state, 85% of the population depends on groundwater for some part of their drinking water supply while about 34 million-acre feet of water (42 billion cubic meters), much of which is groundwater, is extracted annually to irrigate approximately 9.6 million acres of land for agricultural use (Megdal *et al.* 2009; ACWA 2011). Unsurprisingly, groundwater reservoirs are regularly overdrafted to meet these demands. Areas of overdraft occur mainly in the Central Valley, along the coast, and in some parts of southern California (Lund & Harter 2013). In the Tulare Lake Basin, one of the most productive agricultural regions in the Central Valley with an estimated value of $17 billion per year, overdraft accounts for about 10% of total yearly water use (Croyle *et al.* 2014). In addition, from 2007 to 2013, groundwater levels in this region declined by almost 60 feet (18.2 m) while, from 2007 to 2011, the land subsided by 3.9 ft (1.2 m) (Borchers & Carpenter 2014; Croyle *et al.* 2014). One way to decrease water use and help meet overall water demand is to treat industrial wastewater onsite and reuse it in the facility.

Tomato paste production in California accounts for 90% of its production in the United States, equivalent to 35% of global production (Amon *et al.* 2013). Tomato processing relies heavily on the use of water (Barrios-Masias & Jackson 2014), with roughly 890 gallons (3.37 m^3) of water

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used per ton of raw material (Mannapperuma *et al.* 1993). Reduction of these flows improves water efficiency and reduces draws from surface and groundwater sources.

Although treatment and reuse of tomato processing wastewater may be an avenue to increase water efficiency, effective treatment of such wastewater is highly dependent on the quality of the wastewater generated from the various operations in the facility. When all effluent streams are consolidated, the wastewater from tomato paste production is high in suspended solids, chemical oxygen demand (COD), and color (Iaquinta *et al.* 2009). Much of this is due to carryover of dirt from the field that's removed during washing, and the presence of juice from damaged fruit. Often, a combination of biological treatment, nano-filtration, and some type of oxidative process is used to treat this type of waste stream (Sun *et al.* 2013; Alghooneh *et al.* 2015). However, these types of intensive treatments are not necessarily needed for all individual unit operation effluent streams within the facility. For example, the evaporation step to remove water from tomato juice, to concentrate the solids into paste, yields a condensate that is relatively low in dissolved solids and organic compounds. This condensate is then routed to cooling towers to reduce its temperature to a manageable level. Thus, isolating and treating cooling tower wastewater separately may avoid the need for rigorous treatment and yield water suitable for reuse in the facility, such as in the flumes that transport unloaded fruit into the facility.

A physical treatment method, such as disinfection with ultraviolet (UV) light, is a promising method for treating water from cooling tower effluent, because it avoids generating toxic by-products caused from the use of oxidizing chemical disinfectants, such as chlorine. Similarly, there is no additional smell or taste added to the water, no danger of overdosing the disinfectant, and no need to store hazardous materials onsite (Crittenden *et al.* 2012). A physical treatment process is also desirable because of the seasonal nature of tomato paste production; no biological membrane has to be reestablished at the start of the season or maintained during the off-season. Ultraviolet light primarily works as a disinfectant by exciting the nucleic acids in DNA and RNA. This excitation results in the dimerization of adjacent nucleic acids and prevents the further transcription of the DNA or RNA and inhibits replication (Crittenden *et al.* 2012; Metcalfe & Eddy, 2014). Because organisms are inactivated and not removed, evaluation of disinfection efficiency depends on cultivation-based approaches. However, since the fraction of cultivable species in wastewater is typically 15% to 20% of counted cells, there is a significant cultivation bias when using these methods.

Conventional UV reactors involve flowing water through a bank of UV lights in a closed conduit or an open channel. Such systems often demand high capital and operating costs, inhibiting the wider adoption of the technology. This is due to the fact that the UV-emitting lamps (more specifically, their outer quartz tubes) come into direct contact with the untreated water and become fouled due to the accumulation of organic and inorganic material. To prevent this, mechanical cleaning systems are sometimes used, with periodic acid cleaning to further remove any residual fouling (Metcalfe & Eddy, 2014). These systems are costly to manufacture and operate, and can involve significant periods of downtime. Hence the availability of a UV system in which fouling does not occur would improve the prospects of adaptation of UV treatment in practice. Another unsatisfactory feature of the conventional design is that the channel through which water flows is lined with concrete which also easily fouls, causing frequent closures for cleaning. Thus, to maintain continuous operation while cleaning occurs, several redundant channels are employed at increased cost to the user (Metcalfe & Eddy, 2014).

The purpose of this paper is twofold. First, it is to establish the efficacy of using UV disinfection to treat cooling tower effluent from a tomato processing facility for water reuse. Secondly, it is to assess the performance of a novel UV system which is free from many of the drawbacks present in most commercially-available systems (Younis, 2016). The assessment is performed, *in situ*, at a large-scale processing facility, at the height of the tomato-paste production season.

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MATERIALS AND METHODS

The UV system

The UV system is described in Younis (2016). Figure 1(a) shows a three-dimensional rendering of the system with its major components labelled. Figure 1(b) is an engineering drawing showing relevant

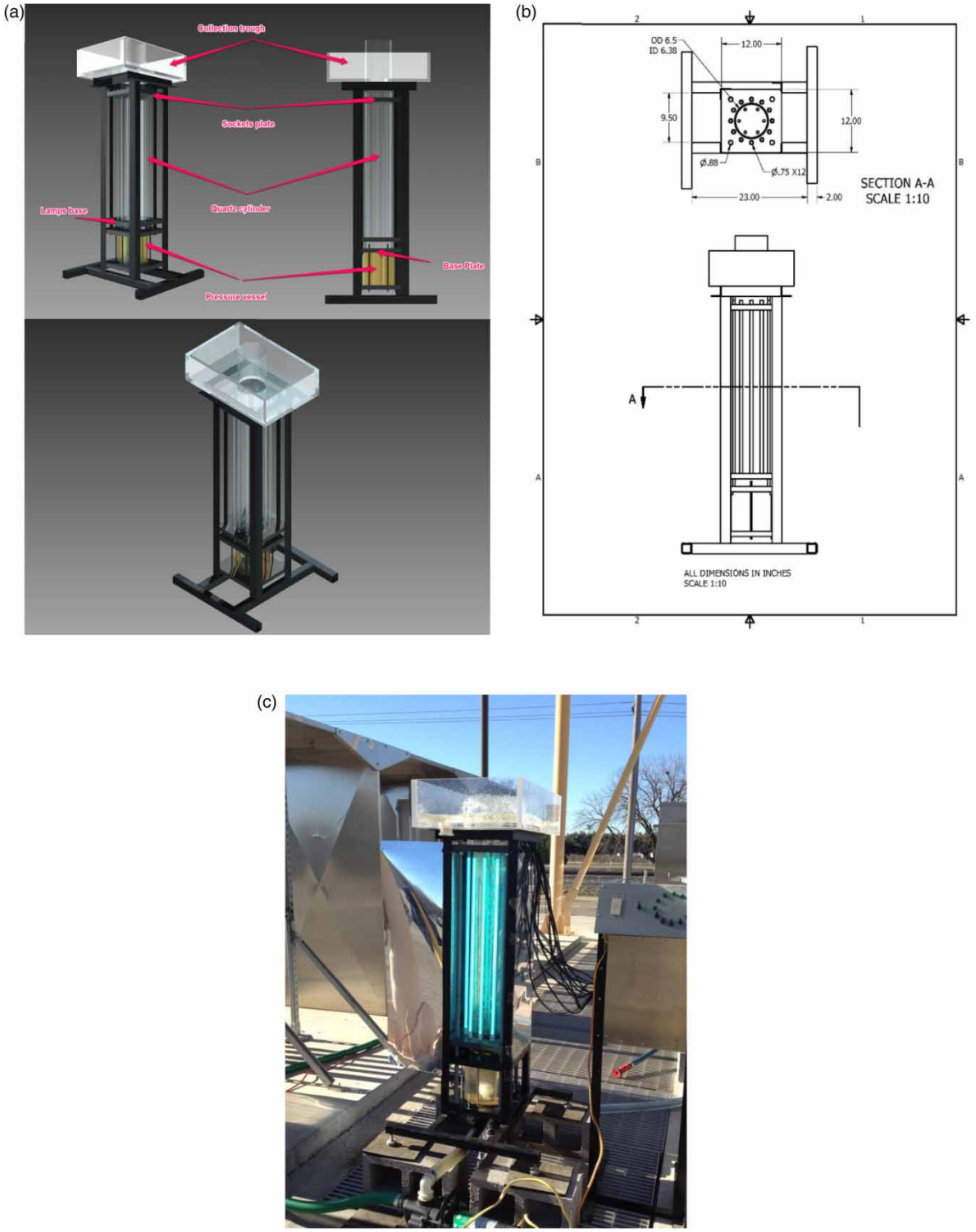

Figure 1 | (a) Three-dimensional rendering of the UV system, (b) schematic giving system dimensions, (c) photograph of system in operation.

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dimensions. Figure 1(c) is a photograph of the system in operation. Untreated water is introduced into a pressure vessel at the base of the system from which it flows through nozzles into a vertically-mounted quartz tube. The nozzles are arranged around the circumference of the quartz tube and hence the water rises within it in the form of a strong vortex. Through adaptation to the base of this tube, a central air core is formed in the shape of an elongated cone. This confines the water between the air column and the inner wall of the tube. Lamps emitting UV light are placed outside of the quartz tube and thus remain free of contact with the water. The number, length and power output of the lamps depends on the design flowrate and on the required fluence. In the design shown, 12 low-pressure Hg lamps, each 40 inches (101 cm) in length, were used. Since quartz allows for the transmission of UV light, the untreated water is exposed to light at the germicidal wave length without contacting the lamps. This altogether eliminates the lamp fouling problem. Further, the presence of the strong vortex leads to the generation of high levels of shear stress at the tube walls. These stresses form a mechanism for self-cleansing in that they prevent the accumulation on the surface of material that can eventually cause fouling on the inside of the tube. Finally, and since the lamps are located outside the quartz tube, they become easily accessible for the purpose of replacement, thereby eliminating the need for prolonged down-times for maintenance and for the infrastructure required to remove the banks of immersed UV tubes used in most commercial systems. After passing through the quartz tube, the treated water flows from the top into a collection trough from where it is conveyed away through tubes attached to the base of the trough. The top of the tube acts in the same way as a circular weir such that the water flowing over it becomes well aerated through the entrainment of ambient air (Figure 1(c)). Further details can be found in Younis (2016).

Site description

The Campbell Soup Company tomato processing plant in Dixon, CA is located in a rural area and obtains fresh water through onsite wells. The facility operates for about 90 days per year, typically July through September, depending on the tomato harvesting season. The site has five induced draft cooling towers that are used to cool the water from 160°F to 78°F (71°C to 25°C) at a loading rate of 31,111 gallons/hr (117 m^3/hr) (Amon *et al.* 2013).

Analysis of UV system at NSF

The tests at the National Sanitation Foundation (NSF) facility in Ann Arbor, MI were conducted to determine the system's ability to meet the requirements for the NSF/ANSI 50 and 55 standards for residential drinking water treatment and recreational water treatment, respectively. The system exceeded the requirements for 3 log reduction of *Pseudomonas aeruginosa* and *Enterococcus faecium*. A single pass test sample showed a log reduction of 6.4 of *Pseudomonas aeruginosa* and a 6.7 log reduction of *Enterococcus faecium*. When tested with the bacteriophage MS2, a single pass test sample showed a log reduction of 4.9. Comparison of the single pass log reduction to the results of a collimated beam test performed on the MS2 indicates that the test sample provided an equivalent UV dose (fluence) of greater than 60 mJ/cm^2 (assuming linearity of dose response curve, the UV dose would be 104 mJ/cm^2) for the observed test conditions.

UV treatment of cooling tower basin water

Onsite testing took place on 8/28/2015. The system was placed downstream of the cooling towers and drew from the water collecting in the cooling tower basins. The system was allowed to warm-up for 10 min before testing. The UV system was tested under two different flow rates: 10 gpm and 15 gpm (38 and 57 liters/min, respectively). For each experiment, the system used 6 low-pressure Hg lamps, each of 60 W power output.

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Microbial analyses

A standard water reuse system in California must follow California's water reuse policy, Title 22, which requires a demonstration of 5-log removal of the virus MS-2, an analog to the polio virus, to ensure a minimum level of safety to the public (NWRI 2012). However, these regulations were developed to ensure the safety of the public and not the safety of the manufactured product at the facility. Therefore, to promote the safety of the tomato product, both metagenomics and traditional cultivation techniques were also used to investigate disinfection efficacy and pathogenic risk.

Samples of basin water were analyzed for total and fecal coliform counts using Colilert Quanti-Tray^{TM} system (IDEXX Laboratories, Westbrook, ME). Samples were incubated at 37°C for 24 hours. Fungal counts were assessed using Potato Dextrose Agar (PDA) plates. Plates were incubated for 5 days at 25°C in the dark according to standard methods (APHA 2012).

UV radiation treats water by causing linkages in the DNA and RNA of microorganisms, thereby preventing further replication. Therefore, the organism remains viable, but can no longer replicate following treatment (Crittenden *et al.* 2012). Because of this, samples of the UV treatment system influent and effluent were enriched for viable microorganisms capable of replication using Luria Broth (LB). For the enrichment, 5 mL of sample was added to 45 mL of LB media and grown for 24 hrs at 35 $\pm$ 2°C at 300 rpm. This was performed in triplicate to identify statistically significant shifts in community composition in response to the UV treatment.

Genomic DNA was extracted from the samples using a Powersoil DNA Isolation Kit (Mo-Bio, Carlsbad, CA). This genomic DNA was used for obtaining count data for ITS and V4/V5 sequencing using the MiSeq platform. The samples were amplified for sequencing using a two-step process. The forward primer was constructed with the (5′-3′) Illumina i5 sequencing primer, a barcode (8–10 bp), a primer pad, and the 515 f primer (GTGCCAGCMGCCGCGGTAA) for bacteria and the ITS9 primer (GAACGCAGCRAAIIGYGA) for fungi. The reverse primer was constructed with the (5′-3′) Illumina i7 primer, a barcode (8–10 bp), a primer pad, and the 926r primer (CCGYCAATTYMTTTRAGTTT) for bacteria and the ITS4 primer (TCCTCCGCTTATTGATATGC) for fungi. The Qiagen HotStar Taq master mix was used to preform 25 µl reactions, with 1 µl of each 5 µM primer, and 1 µl of template (Qiagen Inc, Valencia, CA). ABI Veriti thermocyclers were used to perform the reactions using the following thermal profile: 95°C for 5 min, then 25 cycles for 94°C for 30 s, 54°C for 40 s, 72°C for 1 min, ending with one cycle of 72°C and a 4°C hold.

Products from the first stage of amplification were then added to a second PCR based on qualitatively determined concentrations. This second stage PCR used the following Nextera PCR primers: Forward – AATGATACGGCGACCACCGAGATCTACAC [i5index] TCGTCGGCAGCGTC and Reverse – CAAGCAGAAGACGGCATACGAGAT [i7index] GTCTCGTGGGCTCGG. The amplification had the same thermal profile as the first stage and was run for 10 cycles.

The amplification products were visualized using eGels (Life Technologies, Grand Island, NY). The products were then pooled equimolar and each pool was size selected in two rounds using Agencourt AMPure XP (Beckman Coulter, Indianapolis, IN) in a 0.7 ratio for both rounds. A fragment Analyzer (Advanced Analytical) and a Qubit 2.0 fluorometer (Life Technologies), was used to assess the size and quantify the selected pools, respectively. Then the pools were loaded on an Illumina MiSeq (Illumnia, Inc., San Diego, CA) 2 X 300 flow cell at 10 pM and sequenced according to the manufacturer's standard protocol.

The sequences were then assessed for quality to remove failed sequence reads, sequences with low tags, and sequences that were less than half the expected amplicon length. To account for paired sequences, PEAR Illumina paired-end read merger was used, these sequences were then processed using a trimming algorithm (Zhang *et al.* 2014). The USEARCH clustering algorithm was used to cluster the sequences into operational taxonomic units (OTUs) at a 4% divergence (Edgar 2010). Once the OTUs were selected using UPARSE OTU selection algorithm (Edgar 2013), UCHIME chimera

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detection software was used to locate chimeras (Edgar *et al.* 2011). Finally, to assess the final quality of the data, the OTU sequences were then compared to a database of high quality sequences derived from the NCBI database using a combination of the USEARCH global search algorithm and an internally developed python program that assigns taxonomic information to each sequence and then computes and writes the final analysis files.

Data processing

All statistically analyses were conducted in Microsoft Excel, R using the vegan package (Oksanen *et al.* 2007), and Past 3.x (HAMMER 2014). The R code is available in Appendix B.

RESULTS AND DISCUSSION

Water quality analysis

A summary of the water quality characteristics from the blowdown and overflow from the facility's cooling towers is given in Table 1. The high concentration of coliforms in this water makes it unsuitable for reuse under NSF and Title 22 standards. These standards would limit the allowed concentration of total coliform to 2.2 or 23 MPN/100 mL, depending on how the water is used. The turbidity of this water is also slightly too high for Title 22 standards. Title 22 requires a turbidity of less than 5 NTU to help prevent the spread of pathogens that are embedded in the particles in the water. This encasement can shield the pathogens from receiving the required disinfection dose by physically blocking the UV light or by contributing more organic matter in the water, necessitating of a higher UV dose (Crittenden *et al.* 2012).

Table 1 | Initial water quality of the cooling tower runoff at the Campbell tomato processing facility in Dixon, CA

Analysis	Cooling Tower Runoff
Turbidity, NTU	7.24 ± 1.2
Total coliform, MPN/100 ml	36,500 ± 7,354
Total fecal coliform, MPN/100 ml	0
Total fungal counts, CFU/ml	67.33 ± 11.02
UVT at 254 nm	95.9% ± 0.04%
Absorbance at 254 nm, 1/cm	0.0181 ± 0.0002
ORP, mV	214.7 ± 3.51
COD, mg/L	8.33 ± 2.52
Conductivity, μS	506.6 ± 3.50
TDS, ppm	351.9 ± 3.00
pH	7.42 ± 0.141

Nevertheless, these initial water quality characteristics have some qualities that are advantageous for the use of UV treatment. Namely, the UV transmittance (UVT) of this water is very high. This clarity means that UV light will easily be able to penetrate through the water column and inactivate any pathogens in the water.

Microbial analyses

Results from operation of the UV system at flow rates of 10 and 15 gallons/minute (2.27 and 3.41 m^3/hr) revealed complete disinfection of fungal species, but incomplete disinfection of total

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coliforms found in the water (Table 2). For total coliforms, there was a 2 and 3.8 log reduction at flow rates of 10 and 15 gallons/minute, respectively. However, this system previously achieved log reductions of 6.4 and 6.7 of *Pseudomonas aeruginosa* and *Enterococcus faecium* during NSF testing using synthetic wastewater. Thus, the lower level of treatment with cooling tower water at the processing facility is likely due to either the turbidity in the water or the species of bacteria present in the water. This inefficiency highlights the roles that water properties and the microbial community plays in determining disinfection performance and how performance against indicator species is not always a reliable parameter to measure disinfection performance.

Table 2 | Results for the biological testing under flow each condition of the UV system

Sample	Total fecal coliform, MPN/100 mL	Total coliform, MPN/100 mL	Total fungal counts, CFU/mL
Influent (cooling tower runoff)	0	36,500 ± 7,354	67.33 ± 11.02
UV treatment at 15 gpm	0	321.75 ± 32.60	0
UV treatment at 10 gpm	0	5.75 ± 0.78	0

Genomic analysis

The sequencing data was used to better understand the microbial community present in this water source and the respective sensitivity of the species within it.

Relative abundance

Rarefaction curves generated from OTUs showed a clear asymptote for both communities, this indicates that there was sufficient sampling to identify most OTUs within the microbial communities (Appendix A). This analysis revealed that microbial communities from the enriched samples lacked diversity; the culturing method favored *Pseudomonas*, *Clostridium*, and *Bacillus* species (Figure 2).

NMDS plots

As seen in Figure 3, the ordination results indicate a divergence in the culturable microbial communities after UV treatment. The 10 gpm (38 liters/min) samples are more dissimilar to the influent samples, since the lower flow rate allows for a greater UV contact time through the system and a greater level of disinfection. The cultured samples from the UV treatment at 15 gpm (57 liters/min) are more similar to the influent samples. However, the lack of overlap for the ellipses around the samples, indicating the 95% confidence interval, confirms that the samples are separate populations.

Species of interest

Table 3 presents a list of species isolated from spoiled canned tomatoes. Of these 12 species associated with spoilage, 8 of the species were found in the cooling tower runoff. Of these spoilage organisms identified, 6 were harmful bacteria and 2 were harmful fungi.

These microorganisms were not culturable on the PDA media used for plating and, as a result, viable counts could not be obtained for the treated samples. As shown in Table 3, a variety of media are necessary to culture all of the listed spoilage species. This highlights the diversity in microhabitats available in the cooling tower and the difficulty in assessing the treatment level for a range of pathogens.

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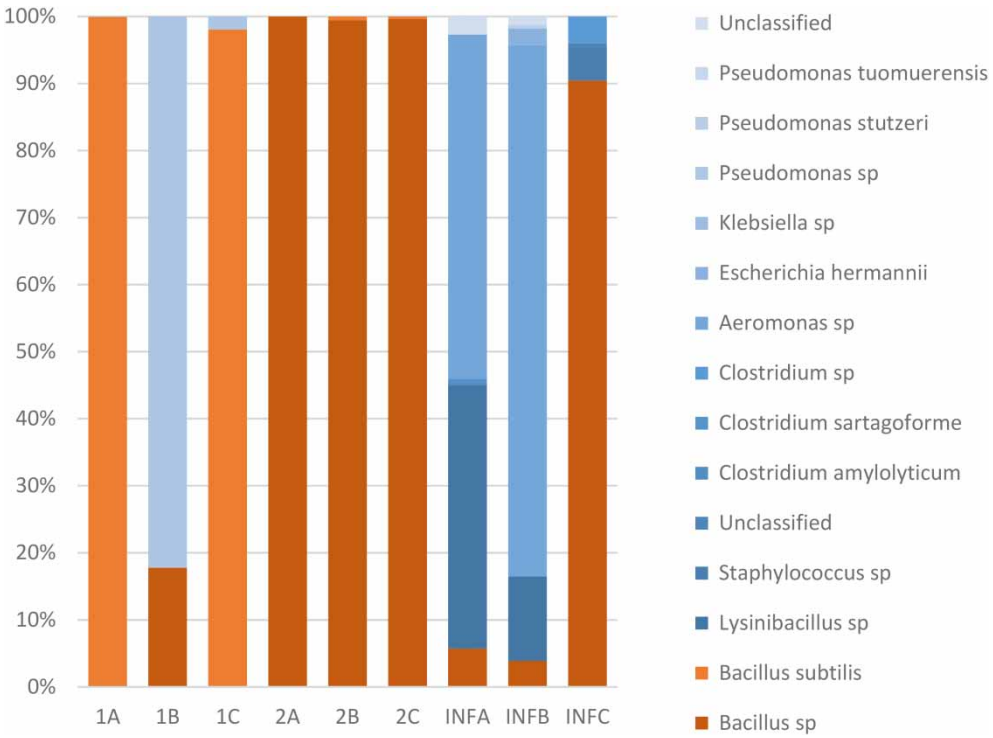

Figure 2 | Relative abundance of cultured bacterial species grouped by species, where 1A, 1B, 1C are the replicates for the effluent from the trial at 15 gpm, 2A, 2B, 2C are the replicates from the trial at 10 gpm, and INFA, INFB, and INFC are the replicates from the raw influent.

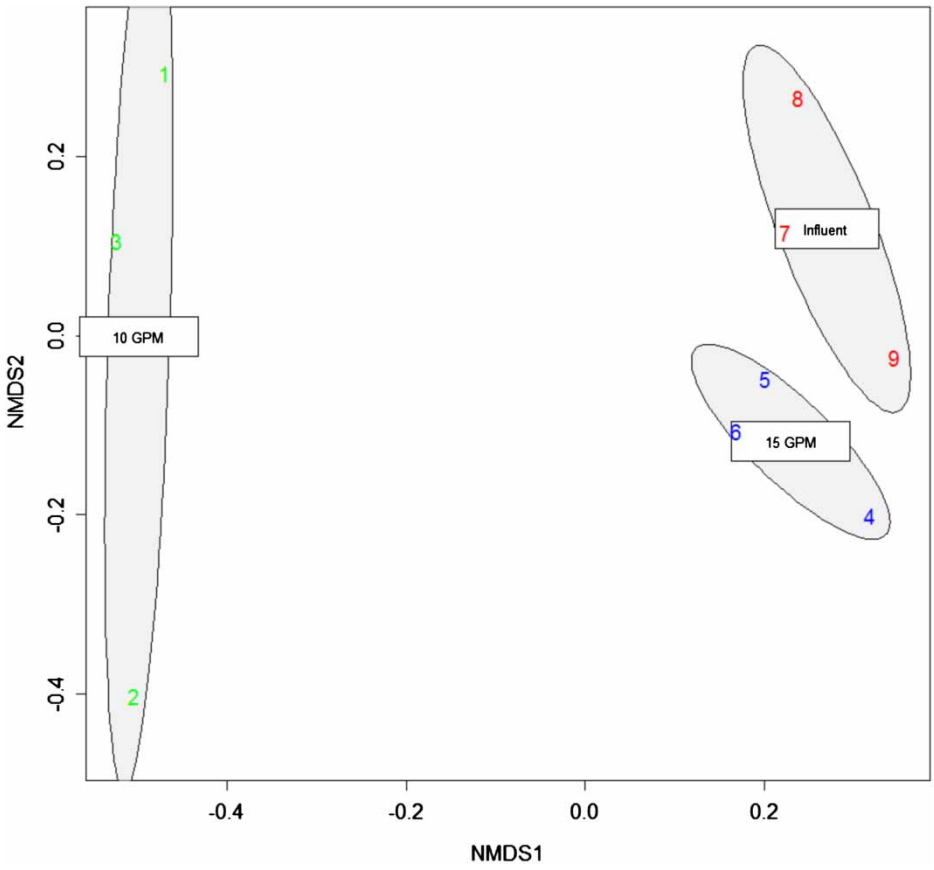

Figure 3 | Non-metric multidimensional scaling of the bacterial community data from the cultured influent and the effluent from UV treatment at 10 gpm and 15 gpm.

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Table 3 | Spoilage-causing species, media needed for culturing, and sensitivity to UV disinfection

Spoilage organisms	Type	Present in cooling tower system	Cultivability[a]	UV Sensitivity (2 log removal) mJ/cm^2
Bacillus polymyxa	Bacterium	Yes	Beef Extract Agar	11.0[b]
Bacillus coagulans	Bacterium	Yes	Beef Extract Agar	11.0[b]
Staphylococcus aureus	Bacterium	Yes	Beef Extract Agar	6.6[c]
Streptococcus lactis	Bacterium	No	Brain Heart Infusion Agar	8.8[d]
Pseudomonas sp	Bacterium	Yes	Beef Extract Agar	10.5[e]
Clostridium sporogenes	Bacterium	Yes	TSA with defibrinated sheep blood	NA
Bacillus coagulans	Bacterium	Yes	Beef Extract Agar	11.0[b]
Saccharomyces sp	Yeast	Yes	Yeast and Mold Agar	13.2[f]
Candida sp	Fungus	No	Yeast and Mold Agar	NA
Mucor sp	Fungus	No	Potato dextrose Agar	35.2[g]
Penicillium sp	Fungus	No	Sabouraud's Agar	88.0[h]
Aspergillus niger	Fungus	Yes	Potato Dextrose Agar	330.0[c]

NA, Not Available.
[a]From ATCC database.
[b]*Bacillus subtilis* (AAW 2017).
[c](AAW 2017).
[d]*Streptococcus faecalis* ATCC29212 (Chang et al. 1985).
[e]*Pseudomonas aeruginosa* (AAW 2017).
[f]*Saccharomyces cerevisiae* (AAW 2017).
[g]*Mucor racemosus A* (AAW 2017).
[h]*Penicillium digitatum* (AAW 2017).

These harmful microorganisms must be inactivated if the cooling tower water is to be recycled in any applications that could come into contact with the tomato product. Most of these microorganisms require only a low dose of UV radiation to inactivate (Table 3). The only difficult species for the UV system to treat is *Aspergillus niger*, since it requires a UV dose of 330 mJ/cm^2 for 2 log reduction. However, *A. niger* colonies did not grow on the PDA media inoculated with cooling tower basin water, suggesting that they may be present in low quantities. Because of this, it may be possible to control *A. niger* via multiple passes through the UV system or filtration upstream of the UV system.

CONCLUSIONS

Substantial amounts of water are isolated from evaporators during the production of tomato paste on the industrial scale. This condensate is often routed to cooling towers ahead of discharge (Bartz & Showalter 1981). Because of its low turbidity and total dissolved solids (TDS), this water may potentially be reused within the processing facility. However, tomato processing water can harbor dangerous microorganisms that can either spoil the product or compromise the health of the consumer. For instance, *Listeria monocytogenes* can tolerate low temperatures and can thus multiply within the product before reaching the consumer. It is argued here that disinfection with UV light, being free of chemicals, provides the safest and most convenient method for use in food processing plants. However, since most analysis of UV treatment has been performed on drinking water and on domestic wastewater, its performance on the microbial flora in tomato processing is not well characterized. In this paper, a novel UV system was introduced and was evaluated *in situ* in water runoff from the cooling towers of an industrial tomato processing plant. The system offers several advantages

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over existing designs, primarily in not being prone to the problem of lamp fouling. Tests on the treated effluent indicated that the system was able to achieve a 6.7 and 6.4 log reduction of *Enterococcus faecium* and *Pseudomonas aeruginosa*, respectively. The applied UV dose was estimated to be greater than 60 mJ/cm^2. Genomic analysis of the cultured effluent samples showed a significant shift in bacterial populations after UV treatment and evaluation of species present in non-cultured samples indicated that several species associated with food spoilage were present in the cooling tower water run-off. Of the most concern on this list was *Aspergillus niger*, because it requires the relatively high UV dose of 330 mJ/cm^2 to achieve a 2 log reduction. This result indicates that effective disinfection would require multiple passes through the system, or a number of systems that are connected in series.

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