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## **Ozone Treatment of Defrost Water for In-Plant Reuse**

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Many fruit and vegetable processing operations are examining technologies to reduce effluent volume and encourage water recovery and reuse. Implementing technologies and programs to promote in-plant reuse and recycling of discharge water is cost-effective and may improve processing efficiency. The efficacy of ozonation of defrost water has been investigated to determine the appropriate treatment level for in-plant reuse at a fruit processing plant in Clovis, California. Treatments of 0.5 ppm and 1.0 ppm of aqueous ozone achieved water quality acceptable for in-plant reuse, while an ozone treatment of 1.0 ppm was needed to significantly (P < 0.05) reduced the microbial load of flume wash effluent.

Keywords Ozone, Fruit Industry, Defrost Wastewater, Water Conservation, Water Reuse

## INTRODUCTION

The fruit and vegetable industry requires significant amounts of water for processing operations. It is estimated that California fruit and vegetable plants use between 0.5 to 3 million gallons of water per day during a processing season (Shoemaker 2004). Realizing the need for water conservation and recovery, the food industry is strategizing ways to implement new technologies and improve process efficiencies. Reconditioning process water for the purpose of reuse is a viable solution when proper consideration is given to the wastewater analysis, proposed treatment method, and processing operation designated for water reuse. Wastewater treatment methods need to be closely examined to ensure effectiveness and most importantly, product and consumer safety.

Interest in the application of ozone has been growing thus exhibiting its potential to effectively act as a broad-spectrum antimicrobial agent while leaving no residual by-products.

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Applications of ozone in the food industry have included food surface hygiene, sanitation of food plant equipment, and of significant interest, treatment of wastewater intended for reuse (Guzel-Seydim et al. 2004; Hampson 2000; Kim et al. 1999; Kim et al. 2003; Pascual et al. 2007; Waldroup et al. 1993). For example, researchers have investigated the use of ozone to recondition poultry chill water and found that using an ozone dose of 7.0–11.7 ppm, the total aerobic plate count was low, ranging from 2.10–2.95 Log CFU/mL, and no viable E. coli, or presumptive coliforms existed post ozonation; thus meeting the USDA specifications of at least a 60% reduction in total microorganisms and similar reduction in coliforms, E.coli, and Salmonella spp. for recycling poultry chill water (Waldroup et al. 1993). As demonstrated, the microbiocidal efficiency of ozone encourages future research opportunities. Ozone presents great possibilities as an effective food processing wastewater treatment method and its effectiveness is well known.

In 1997, an Expert Panel convened by the Electric Power Research Institute (EPRI), declared ozone GRAS (Generally Recognized as Safe) for use in food processing (EPRI 2001; Graham 1997). The Food and Drug Administration (FDA) later recognized ozone as a secondary direct food additive for its use "in gaseous and aqueous phases as an antimicrobial agent on food, including meat and poultry" (US FDA 2001). Finally, in 2002, the U.S. Department of Agriculture (USDA) affirmed ozone's GRAS status "in accordance with current industry standards of good manufacturing practices" (USDA 2002). With these regulations in place, the use of ozone in food processing operations began to gain popularity as novel applications were further investigated.

Although treatment of wastewater intended for reuse has not been the most common use of ozone in the food industry, ozone has been an effective wastewater treatment method in treating effluents in other industries such as pulp and paper production, Shale oil processing, production and use of pesticides and textile dyeing (Gogate and Pandit 2004). Understanding that treatment methods are product and process-specific, ozone applications exhibit potential for reconditioning process water in the food industry.

In this study, defrost wastewater was treated with ozone in a fruit processing plant located in Clovis, CA, to evaluate the applicability of the treatment method for water intended for reuse. Prior to this study, the defrost water was not analyzed for chemical, physical or microbiological quality. This water was part of the effluent leaving the plant and was sent directly to the municipal wastewater treatment plant. The objective was to analyze the chemical, physical, and microbial quality and determine the level of ozone needed for treating the defrost water intended for reuse as the primary wash for incoming fruit, in the same food processing plant.

## MATERIALS AND METHODS

#### Warehouse Defrost Water

The studied wastewater was collected from the refrigeration coils during defrosting at a frozen foods warehouse freezer, in Clovis, CA. A series of sprayers (above the coils), use a combination of potable water and recycled defrost water to defrost the coils. This cycle occurs nightly. The defrost water is collected in a tray that sits directly below the coils, is pumped to the sump and finally to one of the two designated holding tanks. Prior to experimentation, the defrost water was sent directly to the city's municipal wastewater treatment plant (approximately 39,000 gallons per day).

#### **Ozone Treatment**

An in-line ozonator (Model #SGC21, Pacific Ozone Technology, Benicia, CA) was used to treat the defrost water held in the stainless steel holding tanks, with aqueous ozone at concentrations of 0.5 ppm and 1.0 ppm. Ozone was introduced continuously into the water stream in a single pass as it was flowing in and out of the ozonator at 1-1.5 gallons per minute. The water flow rate was controlled to obtain the desired ozone concentration in the output water. An ozone concentration measuring port, located at the outlet of the ozone generator, continuously measured the concentration of the ozonated water leaving the machine.

After treatment, the water was continuously pumped through PVC pipes to a 15-ft long flume (340-gallon capacity) and used as the primary wash for incoming fresh peaches. Washing fruit with ozonated water is desirable because the reaction with residual ozone, if any, will not affect the fruit quality as the skin is later removed during the peeling operation. During experimentation, the treated water was also collected in a stainless steel storage tank for analysis.

#### Sample Collection and Preparation

Defrost water was sampled from seven different locations; the warehouse defrost water collection pan, the warehouse defrost water sump, the warehouse defrost water holding tank, the water inlet pipe before entering the ozonator, the water outlet pipe exiting the ozonator, the ozonated water storage tank, and the flume located inside the manufacturing plant (Figure 1). Flume samples taken from the middle and both ends were composited and tested. The flume was sampled at 8 AM, 10 AM, 12 PM and 2 PM to ensure that results were representative of a typical day of production. All samples were collected in sterile bags (Whirl-Pak, Nasco, Modesto, CA) and stored at refrigerated temperatures until analysis. Each sample was analyzed to determine the chemical and physical quality, which was defined by the following parameters: 1) chemical oxygen demand (COD), 2) total suspended solids (TSS),



3) total dissolved solids (TDS), 4) pH, 5) electrical conductivity (EC), and 6) turbidity; the microbial load of the water was determined by: 1) aerobic plate count (APC), 2) coliform, 3) *E. coli*, and 4) yeast and mold counts.

## **Chemical and Physical Quality of Water**

#### Chemical Oxygen Demand

COD analysis of water samples were measured according to standard method 5220 D (APHA/AWWA/WPCF 2005). A calibration curve was generated using stock solutions of 0.0, 1,000, 2,500, 5,000, 10,000, 15,000 and 25,000 ppm Potassium Phthalate (Crystal AR<sup>®</sup> (ACS), Primary Standard, Mallinckrodt Laboratory Chemicals, Phillipsburg, NJ). The COD reactor (Bioscience, Inc., Allentown, PA) was preheated to 150 °C. Triplicate samples of 0.2 mL from each sample site were added to pre-measured reagent vials (174–318 accu-TEST<sup>TM</sup> Standard Range Twist Cap Chemical Oxygen Demand Vials, Bioscience Inc., Allentown, PA) and placed into the digester block for a total of 2 h. During incubation, samples were inverted after 15 min of digestion and again after 2 h to ensure complete homogenous mixtures. After cooling in a dark cabinet for 15 min, absorbance was measured with a spectrophotometer (DR/2000, Hach Company, Loveland, CO) set at 600 nm. The samples COD (mg/L) was determined using the calibration curve.

## **Total Dissolved Solids and Total Suspended Solids**

Standard methods 2540 C and 2540 D (APHA/AWWA/ WPCF 2005) were used to measure TDS and TSS of each sample, respectively. Briefly, a preweighed 47-mm glass fiber filter was used for each procedure to complete the necessary filtration process. Results for TDS are expressed as mg of dissolved solids per mL of sample. Results for TSS are expressed as mg of suspended solids per mL of sample.

#### pH and Electrical Conductivity

Samples were brought to room temperature (approximately 25 °C) for pH and conductivity measurements. A pH meter (UltraBasic-10, Denver Instrument, Bohemia, NY) was used to measure the pH of each sample following the manufacture's standard operating procedures. Using a conductivity meter (Accumet Basic AB30, Fisher Scientific, Pittsburgh, PA) the EC of each sample was measured following standard procedure 2510 B (APHA/AWWA 2005). To ensure that the EC readings were within 2–5% of the initial reading, the EC standard solution was remeasured after 5–20 determinations of conductivity for samples. If the reading was not within the criteria, the EC meter was restandardized and samples were remeasured. Results are expressed as microsiemens per meter ( $\mu$ S/m), where 1  $\mu$ S/m is equivalent to 1 EC.

#### Turbidity

A portable turbidimeter (Model 996, Orbeco Analytical Systems, Inc., Farmingdale, NY) was used to measure

the turbidity of each sample following standard 2130 B, the Nephelometric Method (APHA/AWWA/WPCF 2005). Briefly, samples were inserted into the meter well and the intensity of light scattered by the sample (under defined conditions) is reported as compared to the intensity of light scattered by a standard reference. Results were expressed in Nephelometric Turbidity Units (NTU).

## **Microbiological Quality of Water**

#### APC, E. coli/Coliform, Yeast, and Mold

The microbiological analyses included Aerobic Plate Count (APC), *E. coli*/Coliform (Ec/C) count and yeast and mold (Y&M) counts using the  $3M^{TM}$  Petrifilm<sup>TM</sup> plates ( $3M^{TM}$  Petrifilm,<sup>TM</sup> 3M, St. Paul, MN). The plates were incubated at 30 °C for APC, 35 °C for Ec/C and 20 °C for Y&M. Samples were plated and enumerated as per  $3M^{TM}$  Petrifilm<sup>TM</sup> standard procedures.

#### **Statistical Analysis**

The averages and standard errors of the chemical parameters of water sampled from the water outlet pipe exiting the ozonator and the ozonated water holding tanks were evaluated and compared to the samples taken from the warehouse water holding tank (before treatment). Analysis of variance (oneway ANOVA) and comparison of means was used to analyze the effect of ozone on the chemical and physical quality and microbial load of samples taken from the warehouse water holding tank, ozonated water holding tank and flume wash effluent. The difference of the means was considered statistically significant if P < 0.05. This analysis was performed using SAS (JMP Pro 9.0, SAS Institute, Inc., Cary, NC).

#### **RESULTS AND DISCUSSION**

#### Defrost Water Quality

The baseline water quality is represented by samples taken from the warehouse water holding tank (defrost water) and is used to compare the effectiveness of the two ozone treatments (0.5 ppm and 1.0 ppm). According to the Draft Guidelines for the Hygienic Reuse of Processing Water in Food Plants (Codex Alimentarius Commission 1999), reuse water should be safe for its intended use and should not jeopardize the safety of the product through the introduction of chemical, microbiological or physical contaminants in amounts that represent a health risk to the consumer. The reuse water should be reconditioned to obtain a microbiological level that meets the specifications for drinking water. The quality of the defrost and treated water is thus compared to the National Primary Drinking Water Regulations (Table 1; note that only the post-ozonation samples are used in this comparison, because the treated water bypasses the ozonated water holding tanks during production). Untreated defrost water meets all regulated parameters except APC (Table 1). The APC was 3.37 Log CFU/mL, which is above the regulation

**TABLE 1.** Summary of Defrost Water Quality and Treated (0.5 ppm and 1.0 ppm Ozone) Water Quality Compared to the National Primary Drinking Water Regulations<sup>a</sup>

Parameter	National Primary Drinking Water Regulations (EPA, 2009)	Defrost Water <sup>c</sup>	Post Ozonation 0.5 ppm	Post Ozonation 1.0 ppm
Chemical				
$TDS^{b} (mg/mL)$	1	0.214	0.219	0.203
Electrical conductivity <sup>b</sup> ( $\mu$ S/m)	1,600	276	275	275
Turbidity (NTU)	$< 5^{d}$	1.26	1.20	1.09
pH <sup>e</sup>	6.5-8.5	7.29	7.30	7.29
Microbial				
E. coli (Log CFU/mL)	Zero <sup>f</sup>	$< 1^{h}$	$< 1^{h}$	$< 1^{h}$
Coliform (Log CFU/mL)	Zero <sup>f,g</sup>	<1 <sup>h</sup>	$< 1^{h}$	$< 1^{h}$
APC <sup>j</sup> (Log CFU/mL)	≤2.7	3.37	$0.95^{h,i}$	0.95 <sup>h, i</sup>

<sup>a</sup>Data displayed only for EPA regulated parameters unless indicated otherwise.

<sup>b</sup>Parameters not regulated by EPA; secondary maximum contaminant level (MCL) set by City of Clovis Water Division to protect the odor, taste and appearance of drinking water (Public Utilities Department 2011).

Defrost water represents the baseline measurements sampled from the warehouse water holding tank prior to any treatment.

<sup>d</sup>For systems that use filtration other than conventional or direct filtration; no filtration system is included in this experiment.

<sup>e</sup>National Secondary Drinking Water Regulation; recommended by EPA but is a nonenforceable guideline.

<sup>f</sup>Public health goal (mg/L).

<sup>g</sup>Maximum contaminant level (MCL) indicates that no more than 5.0 percent samples total coliform-positive in a month.

<sup>h</sup>None detected; detection limit of 10 CFU/mL.

<sup>i</sup>Assuming worst case scenario of 9 CFU/mL.

<sup>j</sup> Heterotrophic place count (HPC) used interchangeably with APC; HPC reported on the National Primary Drinking Water Regulations.

of  $\leq$ 2.7 Log CFU/mL. With this in consideration, the defrost water is an excellent candidate for reuse and the initial evaluation suggests that only a small quantity of ozone will be necessary to bring the defrost water to regulatory levels. The analysis of ozone treated defrost water indicated that it is safe for reuse (Table 1) in processing unit operations such as washing incoming fruit.

## Effect of Ozone on Chemical Quality of Defrost Water

Ozonation of the defrost water significantly (P <0.05) changed the chemical quality of the water in terms of COD, TDS, and turbidity. TSS, electrical conductivity and pH were not affected regardless of the treatment. Both ozone treatments significantly (P < 0.05) reduced the COD post ozonation (Figure 2a). Chemical oxygen demand indicates the amount of a specified oxidant that reacts with the sample under controlled conditions. Inorganic and organic components are subject to oxidation and the COD is often used as a measurement of pollutants in the water. Thus, a decrease in COD suggests an increase in the water quality. Treatment of 1.0 ppm ozone showed a greater reduction post ozonation and in the ozonated water holding tank when compared to treatment of 0.5 ppm ozone. The most significant reduction was observed with 1.0 ppm ozone post ozonation. The COD increase in the ozonated water holding tanks may be due to the breakdown of large molecules or the destruction of a biofilm present in the lines or the tank by the residual ozone. This increase may also be attributed to the decrease in residual ozone as the water travels to and resides in the tanks for a period of time.

Ozone treatment of 1.0 ppm significantly (P < 0.05) decreased the TDS post ozonation and in the ozonated water holding tank (Figure 2b). TDS increased though from 0.214 mg/mL in the warehouse water holding tank to 0.219 mg/mL immediately after the 0.5 ppm ozone treatment to 0.223 mg/mL in the ozonated water holding tank (0.5 ppm) (Figure 2b). This may be attributed to the oxidation of suspended solids (TSS). As a result, TSS decreased slightly from 0.339 mg/mL in the warehouse water holding tank to 0.323 mg/mL directly after each ozone treatment (Figure 2c). The observed change in TSS, however, was not significant (P > 0.05).

The ozonated water holding tanks also showed decreased TSS measurements when compared to the warehouse water holding tank (prior to treatment). TSS measurements were 0.0327 mg/mL and 0.033 mg/mL in the ozonated water holding tank with water treated with 0.5 ppm and 1 ppm of ozone, respectively. These measurements, however, were not significantly different (P > 0.05). Similarly, EC (Figure 2e), and pH (Figure 2f) measurements were not significantly (P > 0.05) different after treatment with ozone. Treatment of 1.0 ppm ozone however, significantly (P < 0.05) decreased the turbidity of the defrost water (Figure 2d) post-ozonation. Turbidity and pH measurements taken from each location though are in compliance with the National Primary Drinking Water Regulation (Table 1). TDS and EC results also meet the







secondary MCL requirements set by the city of Clovis, CA (Table 1).

It is important to note the scale used for each figure; this accounts for what may appear as large standard errors. With all chemical parameters considered, both treatments (0.5 ppm and 1.0 ppm) of ozone produced water that is of equal or greater quality compared to the water sampled from the warehouse water holding tank. Both treatments yield acceptable results for in plant reuse based on the National Primary Drinking Water Regulations (Table 1).

## Effect of Ozone on Microbial Quality of Defrost Water

No coliforms, E. coli, yeast or mold were detected (detection limit of 10 CFU/mL) in the microbial analysis of the warehouse water holding tank, the water exiting the ozonator or the ozonated water holding tanks regardless the treatment (Table 1, data for yeast and mold not shown). Post ozonation APC results for both treatments indicate that no aerobic bacteria were detected (Figure 3). APC results increased when held in the ozonated water holding tanks; however, results





indicate a significant (P < 0.05) log reduction from the warehouse water holding tank to the ozonated water holding tanks (0.85 and 0.89 log reductions for 1.0 ppm and 0.5 ppm ozone treatments, respectively). As aforementioned, the treated water is used directly on incoming fruit as the primary wash. The significant microbial reduction in combination with further processing (pitting, halving and peeling) reaffirms that treatment with ozone minimizes the potential risk for the product and/or consumer.

Based on APC, results indicate that treatment with 1 ppm ozone is not significantly different from treatment with 0.5 ppm ozone (P = 0.383). Both treatments significantly (P < 0.05) reduced the microbial load and successfully meet the EPA standards (Table 1) (see US EPA 2009). As a result, either treatment will yield desirable results in regards to the microbial quality of water. In a different study, ozone dose rates of 7.0 to 11.7 ppm applied to poultry chill water (for a total contact time of 30 min) achieved reductions in





excess of 99% for total aerobes, *E. coli*, and presumptive coliforms (Waldroup et al. 1993). Our study also showed significant reductions in the microbial load of water intended for recycling in a food processing plant.

In addition, several studies have investigated the efficacy of ozone on different food processing plant surfaces. Various studies show that low doses of ozone between 0.5 ppm and 3.5 ppm are effective in achieving significant



microbial reductions on various surfaces (Pascual et al. 2007). For example, an application of ozonted water at 0.5 ppm for 10 min produced a 5.6 and 4.4 log reduction in *Pseudomonas fluorescens* and *Alcaligenes faecalis*, respectively, when applied to dairy biofilms on a stainless steel surface (Greene et al. 1993). Also, an application of ozonated water at 2 ppm for 1 min on a stainless steel kettle, table, and shroud reduced microbial counts by 63.1–99.9%, depending on the surface (Hampson 2000). This same application reduced microbial counts by 67.0–95.6% in "high-traffic" and "low-traffic" floor areas located in a food processing pilot plant.

## Effect of Ozone-Treated Water on Flume Wash Effluent

#### Chemical

Compared to flume wash effluent that used untreated (potable city) water, the chemical quality of the flume wash effluent did not significantly (P > 0.05) change TDS, turbidity or EC when using water treated with 0.5 ppm ozone (Table 2). There was however a significant (P < 0.05) decrease in COD and TSS measurements when using water treated with 1.0 ppm ozone (Table 2). The decreases in COD and TSS are a desired change as it correlates with an increase in water quality. Although samples were taken at 2-h intervals to ensure

that results were representative of a typical day of production, the variability of incoming fruit accounts for the inconsistency of the range of detection for each parameter when considering the ozone treatment (Table 2). These chemical quality results indicate that either treatment of ozone yields flume wash effluent that is of equal quality to the flume wash effluent that is produced from the single-use of potable city water. Water treated with 1.0 ppm ozone may be preferred to see a greater reduction in COD and TSS.

#### Microbial

Data collected from discharge water during flume washing of fruits using ozone-treated water indicated low microbial load compared to washing with city water (Table 3). Treatment with 1.0 ppm ozone showed significantly (P < 0.05) lower microbial counts in wash water for each parameter when compared to the 0.5 ppm ozone treatment. These results indicate that the 1.0 ppm ozone treatment yields flume wash effluent that is better microbial quality than the flume wash effluent that is produced from the single-use of potable city water. Treatment with 1.0 ppm ozone may result in more residual ozone which would therefore increase the antimicrobial action.

Based on the chemical and microbiological data of the flume water, an ozone treatment of 1.0 ppm level seems to

TABLE 2. Chemical Quality of Flume Wash Effluent When Using Untreated and Treated (0.5 ppm or 1.0 ppm of Ozone) Wash Water<sup>a</sup>

	No Ozone (potable city water)		0.5 ppm Ozone		1.0 ppm Ozone	
	Average	Range of Detection	Average	Range of Detection	Average	Range of Detection
COD (mg/L)	$1445\pm52_{\mathrm{A}}$	1353–1520	$1476 \pm 56_A$	1363–1553	$1327 \pm 17_{\mathrm{B}}$	1293-1351
TSS (mg/mL)	$0.761 \pm 0.012_{\rm A}$	0.744-0.782	$0.764 \pm 0.016_{\rm A}$	0.742-0.789	$0.749 \pm 0.005_{ m B}$	0.742-0.758
TDS (mg/mL)	$0.908 \pm 0.003_{\rm A}$	0.902-0.911	$0.910 \pm 0.003_{\rm A}$	0.904-0.914	$0.907 \pm 0.004_{ m B}$	0.903-0.92
Turbidity (NTU)	$259 \pm 4_{A}$	253-266	$263 \pm 7_{A}$	251-274	$259 \pm 9_{B}$	242-272
EC $(\mu S/m)$	$376 \pm 6_{A}$	369-388	$377 \pm 6_{A}$	369-386	$377 \pm 4_{\rm B}$	371-384
pH	$7.27\pm0.04_{\rm B}$	7.21–7.33	$7.31 \pm 0.03_{\rm A}$	7.26–7.35	$7.32\pm0.03_{\rm B}$	7.28-7.36

<sup>a</sup>Measurements not connected by the same letter within a row are significantly different (P < 0.05).

TABLE 3. Microbial Quality of Flume Wash Effluent Before and After Treatment with 0.5 ppm or 1.0 ppm of Ozone<sup>a</sup>

Microbial Parameter	No Ozone-Potable City Water (Log CFU/mL)	0.5 ppm Ozone (Log CFU/mL)	1.0 ppm Ozone (Log CFU/mL)
APC	$6.47\pm0.04_{\rm A}$	$6.34\pm0.28_{\rm A}$	$4.99\pm0.03_{\rm B}$
Coliform	$2.68\pm0.12_{\rm A}$	$2.67\pm0.14_{\rm A}$	$2.35\pm0.06_B$
E. coli	<1 <sup>b</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>
Yeast	$5.11\pm0.02_{ m A}$	$5.09\pm0.03_{\rm A}$	$3.92\pm0.03_{\rm B}$
Mold	$4.29\pm0.30_A$	$4.34\pm0.07_A$	$2.49\pm0.06_B$

<sup>a</sup>Measurements not connected by the same letter within a row are significantly different (P < 0.05).

<sup>b</sup>Detection limit of plate counts indicating no detectable CFUs.

be desirable. The interaction of residual ozone with the fruit peel is not a quality concern because the fruit skin is removed during a peeling operation.

## CONCLUSIONS

Defrost water treated with 0.5 ppm and 1.0 ppm ozone achieved a chemical and microbial water quality acceptable for in-plant reuse as the primary wash on incoming fruit. The application of 0.5 ppm and 1.0 ppm ozone did not significantly (P > 0.05) change TDS, turbidity or EC of the flume effluent; however, an ozone treatment of 1.0 ppm significantly (P < 0.05) reduced the microbial load of flume wash effluent. It is recommended that the fruit processing plant utilize ozone treatments of 1.0 ppm on defrost water intended for reuse. This will decrease the volume of city water needed, the volume of wastewater discharged, and contribute to a more sustainable food processing facility.

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