

Microbiological Control of Cucumber Hydrocooling Water with Chlorine Dioxide†

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ABSTRACT

The time required to cool size 2B (3.43 to 3.75-cm-diameter) pickling cucumbers by a commercial spray-type hydrocooler to less than 9°C was about 18 min at typical initial fruit temperatures of 25 to 29°C. During this period, the fruit was exposed to the recycled water, which reached relatively high populations of bacteria (10^6 to 10^7 colony forming unites [CFU]/g total aerobes and 10^5 to 10^6 CFU/g total Enterobacteriaceae) during a typical day's operation. These numbers exceeded those present on the unwashed fruit, depending upon the volume of fruit previously cooled. Residual chlorine dioxide at 1.3 ppm was found to optimally control (2 to 6 log-cycles reduction) the numbers of bacteria. At 0.95 ppm chlorine dioxide, the numbers of bacteria in the water were relatively static, while at 2.8 and 5.1 ppm the odor of chlorine dioxide became excessive. The bacterial populations in/on the cucumbers were not greatly influenced by chlorine dioxide, even at 5.1 ppm. Apparently, microorganisms on or in the fruit were protected from the chlorine dioxide. Thus, the use of chlorine dioxide in hydrocooling water of cucumbers seems to be an effective means of controlling microbial build-up in the water, but has little effect upon microorganisms on or in the fruit.

Key words: Chlorine dioxide, cucumber, hydrocooling, microbiology

Pickling cucumbers are perishable fruit which should be cooled immediately after harvest if they are to be shipped or held for extended periods before processing. Cooling removes field heat and reduces microbial activity, thereby preserving quality (20). Hydrocooling is relatively inexpensive and convenient compared to air cooling, because water is more efficient than air in transferring heat (23). In most instances, the water of hydrocooling, washing, and other post-harvest treatments is recycled, perhaps for several days, and becomes contaminated with spoilage microorganisms. These microorganisms contaminate subsequently cooled produce through natural openings (lenticels, stomata) and mechanical injuries created during harvesting (5, 13). The more

concentrated the microbes in the water, the more likely that wounds or natural openings will become contaminated (5). Microorganisms located in openings are not affected by desiccation and other harsh environments, so that they are more likely to survive. Adams et al. (1) reported that failure of conventional water and hypochlorite washing to remove more of the microflora present in lettuce was due to the presence of hydrophobic pockets or folds in the leaf surface where microorganisms were protected.

In order to avoid microbial build-up during hydrocooling, it is necessary to treat water with antifungal and antibacterial agents, thus reducing the possibility of contamination of the fruit during cooling. A major concern is the accumulation of pathogenic microorganisms such as *Listeria monocytogenes*, a psychrotrophic microorganism that has been implicated in food-borne human listeriosis (18). Al-Ghazali and Al-Azawi (3) reported that soils treated with sewage sludge cake (agricultural fertilizer used in Iraq) were contaminated with *L. monocytogenes* and also that the crops grown on those soils became contaminated. The first reported incidence of food poisoning was from cabbage grown on soil previously fertilized with sheep manure contaminated with *L. monocytogenes* (27). Heisick et al. (19) reported the presence of *Listeria* spp. in potatoes, radishes, cabbage, cucumbers, mushrooms, and lettuce obtained from the supermarket. They recommended that fresh produce be carefully handled and washed to reduce, if not eliminate, pathogenic bacteria such as *L. monocytogenes*. Other pathogens, such as *Salmonella*, have been shown to be associated with various fruits (17).

Chlorine as sodium, potassium, or calcium hypochlorite has been used to eliminate microorganisms from water. Chlorine is a potent antimicrobial agent and is particularly effective in killing microbes in solutions or on surfaces of clean equipment. However, several researchers have found that it is not effective in removing microorganisms from fruit and vegetables (7, 9). A chlorine dip of 200 ppm (often used to sanitize vegetables) only reduced the population of *L. monocytogenes* by about $2 \log_{10}$ CFU/g, whereas dipping in water alone reduced the population by about $1 \log_{10}$ CFU/g on Brussels sprouts (7). Similar concentrations of chlorine were ineffective in inhibiting the growth of *L. monocytogenes* in lettuce (8).

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In recent years, chlorine dioxide has received attention due to some advantages over chlorine, e.g., bactericidal efficacy is not affected by pH, greater sporicidal activity, and lack of reaction with ammonia (2, 12), so that it does not form dangerous chloramine compounds. Lillard (22) compared the effectiveness of chlorine and chlorine dioxide in reducing the number of bacteria present in poultry-processing water. She found that 5 ppm chlorine dioxide was as effective as 34 ppm chlorine. Costilow et al. (10) studied the effects of chlorine dioxide on preventing the build-up of microorganisms in water used for handling cucumbers and on the microorganisms present in fresh cucumbers. The studies, done under laboratory conditions, showed that chlorine dioxide (2.5 ppm) was very effective in the destruction of microorganisms present in water used for handling and washing cucumbers, but it failed to reduce the population of microorganisms present in/on fresh cucumbers, even at higher concentrations (105 ppm). It was concluded that many microorganisms were so intimately associated with the cucumber fruit that they were protected from labile compounds such as chlorine or chlorine dioxide.

The present study was conducted at a cucumber-processing company under commercial conditions to determine the antimicrobial effectiveness and optimum concentration of chlorine dioxide in the water used to hydrocool pickling cucumbers. The chlorine dioxide was generated by mixing sodium chlorite, sodium hypochlorite, and hydrochloric acid in a commercially available system.

MATERIALS AND METHODS

Hydrocooling system

A tunnel hydrocooler with overhead, spray-type action installed at Mount Olive Pickle Company, Mount Olive, NC, was used in these studies (Fig. 1). Cucumbers contained in 20-bu field crates were placed on a 15.24 m (50 ft) conveyor section, where they typically were conveyed at 1.01 cm/s (0.033 ft/s). Retention time of cucumbers in the hydrocooler could be varied according to incoming fruit temperature from 18 to 24 min. Internal cucumber temperature was measured by inserting the stem of a metal thermometer (Baxter Scientific Products Div., Chicago, IL) into the fruit center and holding for about 1 min. The water temperature was held between 4 and 7°C.

Cucumbers

Cucumber sizes 2A (2.65 to 3.43-cm diameter) or 2B (3.43 to 3.75-cm diameter) of unknown variety and part of the normal intake of the company were used in this study. The fruit was in good condition and free of serious mechanical damage. Samples of cucumbers (approximately 200 g total) consisted of four fruits. Each sample was blended with approximately 400 ml of saline solution (0.85%; i.e. 2:1, ml saline:g cucumbers) in a Waring™ blender for 2 min at high speed for microbiological analyses.

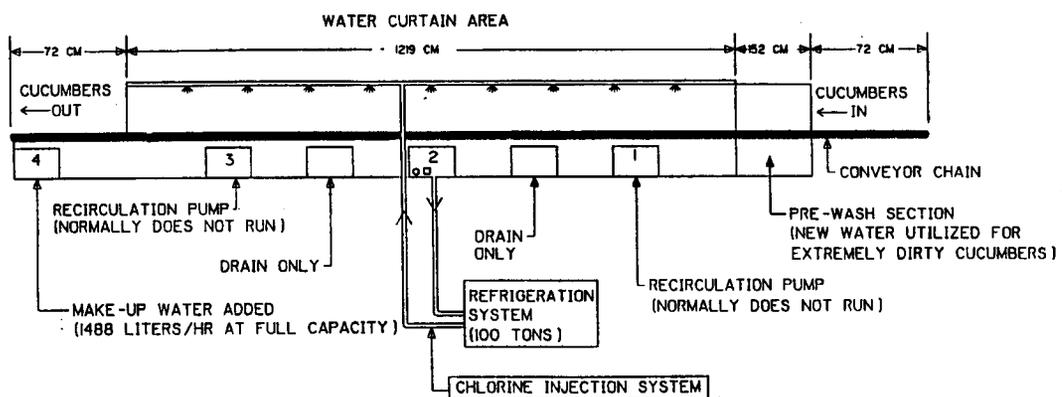
Water

Water samples were obtained from the refrigerated tank of the hydrocooler system (location 2, Fig. 1). Samples were taken from the water after its return from cooling the cucumbers and within 50 cm of the chlorine dioxide sensor.

HYDROCOOLER SCHEMATIC DIAGRAM

OVERALL LENGTH - 1525 CM

CROSS-SECTION - 119 CM WIDE X 94 CM HIGH



TYPICAL CONDITIONS:

CUCUMBERS IN AT TEMPS $>32^{\circ}\text{C}$
 CUCUMBERS OUT AT TARGET TEMP OF 8°C
 TOTAL WATER CAPACITY - 7,560 L
 CIRCULATION CAPACITY - 3850 L/MIN
 WATER TEMP - 5°C
 CHAIN SPEED IS VARIABLE - 2.0 - 2.5 CM/S

OTHER INFORMATION:

SAMPLE LOCATIONS FOR ClO_2 1,2,3,4
 (NO. 2 IS THE MAIN SAMPLE PORT AND
 LOCATION OF ClO_2 PROBE)
 MAIN SAMPLING LOCATION
 o ClO_2 PROBE

Figure 1. Schematic diagram of the flood-type hydrocooling system.

Chlorine dioxide

Chlorine dioxide was generated by a Rio Linda generator system (Rio Linda Chemical Company, Inc., Sacramento, CA). Its concentration in the water was measured with a Hach test kit (Hach Company, Ames, IA; 0 to 3.5 mg/ml CN 66). The final colorimetric readings were made in terms of chlorine, which were multiplied by 1.9, as established by amperometric method I to convert to chlorine dioxide concentration (4).

Enumeration of microorganisms

Bacteria, molds, and yeasts were enumerated by general procedures as previously described (16). Media for the various microorganisms included: standard methods agar (plate count agar [PCA], BBL Microbiology Systems, Cockeysville, MD) for total aerobes; violet red bile agar (BBL) + 1% glucose (VRBG) for total Enterobacteriaceae; (MRS) broth (Difco Laboratories, Inc., Detroit, MI) + 1.5% agar + 0.02% sodium azide (MMRS) for lactic acid bacteria; standard methods agar (BBL) + 0.1 mg/ml of chlortetracycline HCl + 0.1 mg/ml of chloramphenicol for molds and yeasts. All pour plates were duplicated and incubated at 30°C. Plates for yeasts and molds (YM) were incubated at room temperature (20 to 25°C). VRBG plates were read after 24 h and PCA and MMRS plates after 48 h. yeast and mold plates were read after 72 h and confirmed after 120 h since both yeasts and molds were enumerated on the same plates, and molds frequently overgrew the plates after 72 h (21). Although yeast colonies were smaller at 72 h, the numbers did not change appreciably after 120 h, when they could be counted.

Statistical analysis

The data in the first two experiments (Table 1) were statistically analyzed by an analysis of variance. Statistical calculations on the data of the last four experiments (Tables 2, 3, 4, and 5) were carried out by the General Linear Model procedure of Statistical Analysis Systems (SAS) software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

A series of experiments was conducted to determine typical loads of microorganisms in water and fruit of the commercially hydrocooled cucumbers and the influence of chlorine dioxide and exposure time on microbial loads.

Microbial loads with untreated water

The first two experiments were done in situ without

previous notice at the commercial firm where these studies occurred. These experiments were intended to determine microbial loads of cucumber fruit and hydrocooling water under typical commercial conditions without the use of chlorine dioxide. The water for hydrocooling was being recycled over an 8-h period of operation, and our sampling occurred about 3 h after the day's operations had begun. Results of the two experiments, done on separate days, are summarized in Table 1.

In both experiments the initial load of total aerobes and total Enterobacteriaceae in the water was quite high. These numbers are similar to those reported earlier in cucumbers for total aerobes, 10^6 to 10^7 CFU/ml (11), and total Enterobacteriaceae, 10^5 to 10^6 CFU/ml (15, 25, 26). Similar numbers were present in the fruit, expressed as log CFU/g. Samples of fruit taken after hydrocooling gave inconsistent indications of the influence of hydrocooling on microbial loads. In experiment 1, there was an increase of 25% in total aerobes in the fruit and in experiment 2, there was an 83% decrease. An analysis of variance indicated that hydrocooling had no significant ($P > 0.05$) effect on the microbial load of cucumbers. It has been reported that contaminated cooling water can increase the microbial load of fruits and vegetables (5, 7, 20, 24). During the hydrocooling period of 18 to 20 min, the fruit temperature was reduced from 21 to 26°C to 9 to 10°C, which was considered commercially acceptable for holding or shipping purposes.

Effect of 0.95 ppm chlorine dioxide on microbial loads

A similar experiment (Table 2) was conducted in the presence of 0.95 ppm chlorine dioxide in the hydrocooling water. This concentration was recommended by the manufacturer of the chlorine dioxide generator, based on experience with flume water for tomatoes. The flume water for tomatoes typically is at ambient temperature; however, whereas the hydrocooling water in these studies was 4 to 7°C. In this experiment the water had a much lower (3 to 4 log cycles) microbial load than that in the previous experiments (Table 1). In this experiment the hydrocooler had just been put into operation for the day and had not been contaminated with previously cooled cucumbers. After hydrocooling, total aerobes

TABLE 1. Effect of hydrocooling (HC) in the absence of chlorine dioxide on microbial populations of size 2B cucumbers and recycled cooling water.

Experiment	Before HC			After HC			% Reduction	
	Temperature °C	Total Aerobes	Total Enterobac- teriaceae	Temperature °C	Total Aerobes	Total Enterobac- teriaceae	Total Aerobes	Total Enterobac- teriaceae
No. 1: air 36°C Cucumbers (2 samples)								
Mean, log CFU/g	26	5.68	5.23	10	5.87	4.89	-25	41
SD		0.42	0.07		0.10	0.43		
Water (1 sample)								
log CFU/ml	7	6.00	5.32	7	-	-	-	-
No. 2: air 27°C Cucumbers (2 samples)								
Mean, log CFU/g	21	6.10	5.26	9	5.84	4.69	83	58
SD		0.46	0.63		0.06	0.06		
Water (1 sample)								
CFU/ml	6	6.15	5.40	6	-	-	-	-

TABLE 2. Effect of hydrocooling in the presence of 0.95 ppm chlorine dioxide on microbial populations of size 2B cucumbers and fresh cooling water.

Samples	Before HC			After HC (17 min)			% Reduction	
	Temperature °C	Total Aerobes	Total Enterobac- teriaceae	Temperature °C	Total Aerobes	Total Enterobac- teriaceae	Total Aerobes	Total Enterobac- teriaceae
Cucumbers (2 samples)								
Mean, log CFU/g	16	7.90	5.49	7	6.25	4.25	98	94
SD		0.06	0.03		0.08	0.13		
Water (1 sample)								
log CFU/ml	4	2.85 ^a	1.50 ^a	4	2.53 ^b	<1.00 ^b	52	>69

^a Water before addition of chlorine dioxide.

^b Water 30 min after turning on 0.95 ppm chlorine dioxide.

had been reduced in the cucumbers by 98% and in the water by 52%. Reduction in microbial loads of the cucumbers may not have been due entirely to chlorine dioxide. Etchells et al. (14) found that 63% of the total aerobes on cucumbers was removed by rinsing the cucumbers in sterile water. The relatively clean cooling water could have removed many of the superficial microorganisms from the fruit. However, the reduction of total aerobes and Enterobacteriaceae in the cooling water indicated that chlorine dioxide may have had a bactericidal effect.

A second experiment was done to determine the effect of exposure time of cucumbers to 0.95 ppm chlorine dioxide (Table 3). Again, the experiment was begun with fresh water. Cucumbers were held submerged in the cooling water for up to 2 h with sampling every 30 min. There was a small, and consistent, but statistically insignificant ($P > 0.05$) trend toward decline in the total aerobe and Enterobacteriaceae counts, but no apparent trend occurred with lactic acid bacteria, yeasts, or molds. During this period, a total of 45, 20-bu boxes of cucumbers (about 4,090 kg) was sent through the hydrocooler. Over a period of 3.5 h, total aerobes in the cooling water increased over 1,000-fold. Total Enterobacteriaceae and lactic acid bacteria increased over 100-fold.

Data from the experiments summarized in Tables 2 and 3 indicated that 0.95 ppm chlorine dioxide was insufficient for

consistent reductions in the numbers of microorganisms in the cucumber cooling water. We suspected that the concentration of chlorine dioxide was marginal for antimicrobial activity at the low temperature of the cooling water, in contrast to previous reports for tomato flume water at ambient temperature.

Effect of chlorine dioxide concentration on antimicrobial activity in cooling water

After obtaining the results reported in Tables 2 and 3, another experiment was carried out to determine the concentration of chlorine dioxide necessary to cause definite microbial destruction in the cooling water (Table 4). For this experiment the water was allowed to become highly contaminated before starting chlorine dioxide generation. A water sample was taken before starting chlorine dioxide addition. Then the chlorine dioxide concentration was adjusted incrementally as shown in Table 4. Approximately 30 min was required to obtain each chlorine dioxide concentration indicated, and then the cucumbers were submerged and the chlorine dioxide was held at that concentration for 30 min.

There was about a 10-fold or greater reduction in total aerobes between each of the increments of chlorine dioxide concentrations of 0, 0.85, 1.33, and 2.85 ppm (Table 4). Only a relatively slight decrease in total aerobes occurred, however, when the chlorine dioxide concentration was increased from

TABLE 3. Effect of exposure time of size 2A cucumbers to 0.95 ppm chlorine dioxide in fresh hydrocooling (HC) water on microbial populations; (HC Time, 16 min).

Sample	Time of Day	Total Time (min)	Total Aerobes	Total Enterobacteriaceae	Lactic Acid Bacteria	Total Yeasts	Total Molds	No. Boxes HC ^a
Cucumbers ^b	0945	0	6.90	5.84	3.23	2.50	3.27	0
(log CFU/g)	1015	30	6.78	5.52	3.22	2.22	3.05	7
	1045	60	6.69	5.40	3.14	2.28	2.92	5
	1115	90	6.66	5.25	3.30	2.32	2.28	23
	1145	120	6.60	5.05	3.35	2.18	3.32	10
Water ^c	0830	0	3.11	3.80	1.78	2.60	3.00	0
(log CFU/ml)	1200	210	6.87	5.90	4.13	2.48	3.18	45

^a Number of 20-bu boxes of cucumbers transferred through the cooler during the period of time indicated. The number of boxes added during specific time intervals are indicated.

^b Cucumber temperature before HC, 24°C; after HC, 9°C.

^c Water temperature, 6°C.

Table 4. Effect of chlorine dioxide concentration on microbial populations (log CFU/ml) in recycled hydrocooling water at 6°C.

Chlorine Dioxide (ppm) ^a	Total Aerobes	Total Enterobacteriaceae	Lactic Acid Bacteria	Total Yeasts	Total Molds
0.00	7.00	6.95	3.92	3.48	3.92
0.85	6.00	6.08	<1.00	2.30	3.48
1.33	4.80	<1.00	<1.00	<1.00	2.72
2.85	3.78	<1.00	<1.00	<1.00	2.14
5.13	3.54	<1.00	<1.00	<1.00	1.60

^a The chlorine dioxide concentration was increased incrementally as indicated, with a holding time of 30 min at each concentration.

2.85 to 5.13 ppm. Total Enterobacteriaceae decreased over 100,000-fold when the chlorine dioxide concentration was increased from 0.85 to 1.33 ppm. Even 0.85 ppm chlorine dioxide reduced lactic acid bacteria by over 3 log cycles. Yeasts were reduced to immeasurable numbers at 1.33 ppm chlorine dioxide. Mold counts persisted throughout all chlorine dioxide concentrations, although there was a consistent trend toward reduction in numbers.

Apparently some species of total aerobes and molds were greatly resistant to chlorine dioxide. This resistance may be associated with the spore forms of these microorganisms, although we did not confirm this possibility. The much greater sensitivity of Enterobacteriaceae and lactic acid bacteria to chlorine dioxide, however, is consistent with that possibility.

The effects of chlorine dioxide concentration on the survival of microorganisms in/on cucumber fruit were determined (Table 5). This experiment was conducted after that reported in Table 4 and involved the same hydrocooling water that had been adjusted to 5.1 ppm chlorine dioxide. Cucumber fruit were held submerged in the cooling water at 5.1 ppm chlorine dioxide for 15 and 30 min. There was a relatively small reduction (about 10-fold or less) after holding the fruit in the water for 15 min, compared to those not exposed. Holding cucumbers in the water for 30 min resulted in only a slight further reduction of all groups of microorganisms (<10-fold). We concluded that most of the surviving microorganisms were in some way protected from contact with the chlorine dioxide, as earlier suggested by Costilow et al. (10).

Storage stability of chlorine dioxide-treated cucumbers

Concurrently with the previous experiment, replicate samples of approximately 2 kg each were taken from a 20-bu field crate of size 2B cucumbers and placed in open-mesh

nylon bags. Four of the samples were placed on top of a filled crate and sent through the hydrocooler before chlorine dioxide was added to the system; four other samples were similarly sent through the hydrocooler after the level of chlorine dioxide was set at 5.1 ppm. All samples were subsequently placed in refrigerated storage room, held at 10 to 12°C, and observed over a period of 6 days. Based on daily subjective evaluation of the coded samples by plant quality control personnel, no differences in storage life were evident. Both samples with and without chlorine dioxide were acceptable for processing for 5 days, but not acceptable after 6 days. These visual observations further suggest that microorganisms of the cucumber are not significantly affected by chlorine dioxide treatment of the hydrocooler water.

Optimum concentration of chlorine dioxide in cooling water

We conclude that 1.33 ppm chlorine dioxide of the concentrations tested was sufficient to acceptably reduce the microbial numbers in cucumber cooling water. The higher concentrations tested (2.85 and 5.13 ppm) resulted in an unpleasant chlorine odor that would result in an unacceptable work environment.

Suitability of chlorine dioxide to hydrocooling equipment

Chlorine and chlorine dioxide are strong oxidizing agents and are potentially corrosive to metals and hazardous, the extent depending upon concentration (28). Lillard (22) pointed out that only 1/7 as much chlorine dioxide as chlorine provided similar bactericidal effects in poultry-processing water. She suggested that the lower concentration of chlorine dioxide required is apt to be less corrosive to equipment than the higher concentration of chlorine. She found 5 ppm chlorine dioxide to be effective in poultry-processing water, whereas we found only 1.3 ppm to be effective

TABLE 5. Effect of exposure time to 5.1 ppm chlorine dioxide on microbial populations (log CFU/g) in size 2A cucumbers at 27°C starting temperature; after hydrocooling, 9°C. Recycled HC water, 6°C; air, 32°C; HC, 17 min.

Time (min)	Total Aerobes	Total Enterobacteriaceae	Lactic Acid Bacteria	Total Yeasts	Total Molds
0	7.58	6.51	3.08	3.82	4.08
15	6.20	5.99	2.53	2.56	3.08
30	6.10	5.64	2.42	2.57	2.88

in cucumber- hydrocooling water. The difference in effective chlorine dioxide concentration between poultry chill water and cucumber-cooling water could be attributed to the difference in the level and type of organic matter present in these two processing waters. The pH greatly influences corrosivity of chlorine and chlorine dioxide. At pH 2.7, stainless steel types 304 and 316 corroded rapidly when exposed to acidified chlorite solution (6). However, chlorine dioxide at pH 7.2 was noncorrosive to both types 304 and 316 stainless steel at a concentration of 100ppm during 10 days of continuous exposure (6). Thus, it seems unlikely that 1.3 ppm of chlorine dioxide in cucumber hydrocooling water near pH7 would cause significant corrosivity beyond that expected from typical residuals of free chlorine in drinking water. The pH of the hydrocooling water in this study ranged from 6.5 to 7.2, with or without the addition of chlorine dioxide.

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