

Oxidation-Reduction Potential of Chlorine Solutions and Their Toxicity to *Erwinia carotovora* subsp. *carotovora* and *Geotrichum candidum*

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ABSTRACT

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The toxicity of chlorine solutions to cells of *Erwinia carotovora* subsp. *carotovora* and conidia of *Geotrichum candidum* suspended in water at pH 6.0, 7.0, or 8.0 was correlated with the free chlorine concentration and oxidation-reduction potential of the solutions. The oxidation-reduction potential was directly correlated with the Log_{10} of the chlorine concentration at each pH. Cells of *E. c. carotovora* were 50 times more sensitive to chlorine than were conidia of *G. candidum*, with populations of 1×10^7 cfu/ml and 1×10^7 conidia per milliliter, respectively. Populations of *E. c. carotovora* were reduced below detectable levels ($<10^2$ cfu/ml) by approximately 0.5, 0.5, or 0.75 mg of free chlorine per liter at pH 6.0, 7.0, or 8.0, respectively. In contrast, with conidia of *G. candidum*, 25, 25, and greater than 30 mg/L, respectively, were required to produce a similar level of efficacy. With both organisms, population reductions were associated with higher initial oxidation-reduction potentials at pH 6.0 than at pH 8.0.

Additional keywords: hypochlorite ion, hypochlorous acid, postharvest diseases

Chlorine is the principal biocide used to sanitize flumes, dump tanks, and hydrocoolers in fruit and vegetable packinghouses (3,12). Three different chemicals are available commercially to chlorinate water: liquid elemental chlorine, aqueous solutions of hypochlorite ions, or calcium hypochlorite, a dry powder (2). When added to water at recommended concentrations, these chemicals are converted to hypochlorous acid and hypochlorite ions, with the ratio controlled by the pH of the solution. Both chemicals are strong oxidants with listed oxidation-reduction potentials (ORPs) of +1.5 and +0.9 mV, respectively (6). The free or unreacted chlorine content in water is the sum of the concentrations of both chemicals, and most tests for free chlorine do not distinguish between these chemicals (2). However, certain bacteria were reported to be 80 times more sensitive to the acid than to the ion (2,4,8), and the concentration of acid was concluded to be important and of ions unimportant in the efficacy of chlorine solutions against bacterial spores (8). With fungal spores, the potency of solutions decreased in toxicity as the pH increased (and the OCl^- concentration increased) (2,11).

When used to sanitize wash water or the water in dump tanks and flumes of packinghouses, chlorine solutions are not very stable (3,5,12). The strong oxidation potential of free chlorine leads to contact oxidation of organic matter and certain inorganic chemicals that become suspended or dissolved in the water (3,9). Electrophilic reactions with amino groups or ammonium ions occur relatively quickly and result in the substitution of chlorine for hydrogen on the nitrogen atom (2,9). Most of the products of these reactions have little or no toxicity to bacteria or fungi. Therefore, for the proper sanitation of circulating water systems at packinghouses, such as dump tanks, flumes, or hydrocoolers, a free or unreacted chlorine residual must be maintained in the water, usually by the periodic or continual addition of a product that generates chlorine (3,5,12).

Various practices have been used in packinghouses to maintain effective chlorine concentrations in water systems. In early recommendations, assumptions were made that measurements of free chlorine would be infrequent, whereas the addition of chlorine to the water would be periodic or continuous but not always based on the amount needed to replace the chlorine lost to various reactions (5,12). Consequently, the recommended concentration range represented an overdose, so that adequate chlorine would be present over time. These water chlorination practices likely led to peaks and valleys of free chlorine in the water, which could mean excessive and insufficient concentrations, respectively. Insufficient levels would allow produce to

become inoculated, whereas excessive levels would produce undesirable odors and increased corrosion of equipment.

Systems have been developed for the addition of chlorine products to water based on continuous on-line measurement of free chlorine concentrations (Stranco, Inc., Bradley, IL). These systems were originally developed for municipal and industrial water treatment, where chlorine concentrations and reaction times are lower and longer, respectively, than would occur in a typical packinghouse dump tank. However, the systems have been adapted to and are being used in tomato packinghouses and other fresh produce handling systems. In one system, an electrode of highly purified platinum is immersed in the water at the downstream end of the dump tank to measure the ORP of the water. Based on this measurement, a microprocessor controls pumps or gas valves so that chlorine is added to the water as needed to maintain desired concentrations. The pH of the solution is measured by a second sensor, and solutions of acids or bases are added as needed to maintain a desired pH. The ORP and pH set points for the system are usually based on the old recommendations for water treatment in packinghouses. Before updated recommendations for ORP and pH can be developed for this new system, information is needed on the correlation of the solution ORP with toxicity against postharvest pathogens. Moreover, the influence of pH on the correlation between ORP and toxicity is not clear. Lund (7) reported that the rate of inactivation of poliovirus by chloramine, a combined chlorine compound, was directly correlated with the ORP at pH 5.0 and 7.0, whereas at pH 8.4 the rate for any particular chloramine concentration was lower than at 7 but somewhat higher than expected given the functional relationship between ORP and inactivation rate. In a review of water disinfection, Carlson (1) noted that the killing of microorganisms was not based on a defined chlorine reaction and that higher ORPs were required for complete kill of *Escherichia coli* (the population decreased by more than three Log_{10} units) within a specified time when solution pH levels were 8.0, compared with 7.0 or 9.0.

Below, we describe tests with *Erwinia carotovora* subsp. *carotovora* (Jones)

Bergey et al and *Geotrichum candidum* Lk. ex Pers. emend. Carmichael, causes of bacterial soft rot and sour rot of tomatoes, respectively, to find what ORP levels might be necessary for the sanitation of water containing typical bacterial and fungal postharvest pathogens and to learn if the solution pH must be considered.

MATERIALS AND METHODS

Pathogens. *E. c. carotovora* (Florida strain SR38) was grown for 24 hr in nutrient broth at 25 C. The cells were pelleted by centrifugation, and the pellets were resuspended in sterile distilled water; the process was repeated three times. The final suspension was diluted in sterilized distilled water, based on optical density at 600 nm, to 5×10^8 cfu/ml. Conidia of *G. candidum* (Florida strain GC1) were prepared from cultures grown on potato-dextrose agar plates for 3–5 days. The plates were flooded with sterile distilled water and brushed with a bent glass spreader to suspend the conidia. The suspension was filtered through coarse filter paper to remove spore aggregates and centrifuged to concentrate and wash the conidia. The final pellet was resuspended in sterile distilled water and diluted to 5×10^8 conidia per milliliter based on the optical density of a 1:10 dilution of the suspension at 600 nm. The correlation between optical density and spore or bacterial concentrations (spore counts in a hemacytometer or dilution plate analysis of bacterial populations) was determined previously.

Chlorine solutions. The chlorine concentration of a standard commercial laundry bleach was measured by titration with 2.00 N sodium thiosulfate according to instructions provided with the titrator by the vendor (HACH Company, Loveland, CO). The bleach was diluted in distilled water to stock solutions containing 100, 1,000, or 10,000 mg of available chlorine per liter. These solutions were then added to a stock solution of sodium phosphate buffer at pH 6.0, 7.0, or 8.0; 1 N HCl was added as necessary to obtain the desired pH; and water or cell suspensions of the test organisms were added, depending on the test, to obtain the desired chlorine concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 5, 10, 15, 20, 25, or 30 mg/L with a buffer concentration of 0.05 M. The free chlorine concentration (unreacted HOCl plus OCl⁻) of a diluted sample was determined by the diethyl-*p*-phenylene diamine (DPD) method (10), as per instructions provided by the vendor (HACH Company), to confirm the accuracy of the dilution sequence. A sample (40–45 ml) of each chlorine solution was added to a 50-ml Erlenmeyer flask. The flasks were stoppered, placed in a refrigerator, and protected from light. These solutions were used in tests of toxicity to the test

organisms within 8 hr of their preparation. A second sample of each solution, approximately 1 L, was added to a 4-L beaker containing a stirring bar. The ORP of this solution was measured with a Strantrol Model 753 high-resolution oxidation reduction detector (Stranco, Inc.). The detector was set to register 750 mV with a 1 mg/L solution of free chlorine buffered to pH 7.0 at 25 C. Four separate tests were conducted with each set of chlorine solutions, 0–1.0 mg/L and 5–30 mg/L.

For the toxicity tests, 4.9 ml of each chlorine solution was added to a threaded test tube, the cap was attached, and the tube was warmed to 25 C in a water bath. Next, 0.1 ml of an aqueous cell or conidial suspension was added and the tube was vortexed (final population for both organisms was 1×10^7 propagules per milliliter). The tube was vortexed again after 1 min. Two minutes after the test began, a drop of 0.02 N sodium thiosulfate was added to the mixture to neutralize unreacted chlorine. Decimal dilutions of the tube contents were made, and 0.1-ml portions of at least three dilutions were plated on nutrient agar or potato-dextrose agar for *E. c. carotovora* and *G. candidum*, respectively, with duplicate plates per dilution. The plates were incubated at 30 C for 24 or 36–48 hr, respectively. Viable microbe counts were calculated from the plates that contained between 30 and 300 colonies. Four separate sets of chlorine solutions containing 0.1–1.0 mg/L were used with the cell suspensions of *E. c. carotovora*, and four sets with 5–30 mg/L were used with the conidia of *G. candidum*. A 0-chlorine concentration was included as a control with each microbe. One additional test was

conducted with *E. c. carotovora* and the more dilute chlorine concentrations.

Statistical analyses. The relationship between the independent and dependent variables, such as chlorine concentration, solution pH, and ORP or ORP and Log₁₀, respectively, was explored through analyses of variance and regressions by the PROC GLM program of the Statistical Analysis System (SAS Institute, Cary, NC). The coefficient of determination or *R*² value, defined as the ratio of the sum of squares for the effects of the independent variables and their interactions to the total sum of squares in the documentation for the SAS programs, was used as a measure of how much of the variation in the dependent variable was explained by changes in the independent variables or their interactions. For the analyses, colony counts on duplicate plates were averaged to provide a single value and the different tests were used as replicates. The regression of the Log₁₀ (*N*/*N*₀) (*N* = number recovered, *N*₀ = original population) over chlorine concentration or ORP was analyzed similarly. Since a clear threshold concentration of chlorine must be present in the water before measurable numbers of microbes are killed, the 0-chlorine concentration samples were not included in the regressions. Also, the first “not detected” population recovered from each chlorine concentration series was arbitrarily assigned a value of 1 cfu/ml to avoid problems with the Log₁₀ transformation.

RESULTS

ORP. Changes in the chlorine concentration and pH of the solution plus an interaction between chlorine concentration and pH explained more than 99%

Table 1. Analysis of variance of solution oxidation-reduction potential (ORP) for populations of *Erwinia carotovora* subsp. *carotovora* or *Geotrichum candidum* (Log₁₀ viable propagules per milliliter) as influenced by chlorine concentration and pH^a

Source of variation	df	Mean square	F value	Probability
For ORP (mV)				
Chlorine conc. (C)	12	89,947.24	624.32	0.0001
pH (P)	2	262,878.76	1,824.64	0.0001
C × P	24	84.72	2.67	0.0003
Error	93	144.07		
	<i>R</i> ² = 0.99 ^b			
For <i>E. c. carotovora</i>				
C	7	18.01	213.22	0.0001
P	2	12.34	22.30	0.0001
C × P	14	1.63	2.95	0.0009
Error	93	0.55		
	<i>R</i> ² = 0.94			
For <i>G. candidum</i>				
C	6	63.95	488.63	0.0001
P	2	0.49	3.71	0.0301
C × P	12	11.05	84.43	0.0001
Error	63	0.13		
	<i>R</i> ² = 0.98			

^aChlorine concentrations = 0.1–30 mg/L, pH = 6.0, 7.0, or 8.0, and initial pathogen population approximately 1×10^7 propagules per milliliter. *E. c. carotovora* or *G. candidum* were recovered after a 2-min exposure to chlorine on nutrient agar or potato-dextrose agar, respectively.

^bCoefficient of determination.

of the variation (R^2 value) in the ORP of the solution (Table 1). The measured ORP was highly consistent with the free chlorine concentration and pH of the solution. The interaction occurred because the millivolts increased more rapidly with chlorine concentrations of 0.1–1.0 mg/L at pH 6.0 than at pH 8.0. At low chlorine concentrations (0.1–1.0 mg/L), the ORP increased rapidly with small additions of chlorine, whereas at somewhat higher concentrations (15–30 mg/L), the ORP did not respond as rapidly. This is consistent with a linear relationship between the ORP and the Log_{10} of the chlorine concentration (Fig. 1). The average increase in ORP associated with the initial 0.1 mg/L of free chlorine was 320, 251, or 180 mV for pH 6.0, 7.0, or 8.0, respectively; whereas the average increase in ORP associated with a 10-mg/L increase from 10 to 20 mg/L was 16, 10, and 20 mV, respectively.

Effect of initial concentration and oxidation-reduction potential of chlorine solutions on viability of test organisms. The decrease in pathogen populations in the chlorine solutions was related mostly

to solution pH, chlorine concentration, and an interaction between the two (Table 1). The interaction provided only a minor contribution to the population decrease and was associated with a larger decrease per unit increase of chlorine concentration at pH 6 or 7 compared with at pH 8 (Figs. 2 and 3). Overall, the chlorine concentration was not well correlated with population decrease unless the pH level was considered. The R^2 for the influence of initial chlorine concentration on decrease in populations of *E. c. carotovora* and *G. candidum* improved from 64 and 47%, respectively, when the solution pH was ignored, to 77–89% and 70–99%, respectively, when analyses were conducted at each pH. The lowest R^2 values occurred at pH 6.0 for both organisms and the highest at pH 7.0 for *E. c. carotovora* and pH 8.0 for *G. candidum*. A lethal dose of chlorine (reduced population below detectable limits) for *E. c. carotovora* was between 0.4 and 0.5 mg/L at pH 6 and 7, and between 0.5 and 0.75 mg/L at pH 8. By contrast, lethal doses for *G. candidum* were between 20 and 25 mg/L at pH 6 and 7, and greater than 30 mg/L at pH 8.

The initial ORP of the chlorine solutions was not directly correlated with toxicity (pathogens killed) unless the pH was held constant (Figs. 4 and 5). For example, with *E. c. carotovora* and *G. candidum*, the R^2 values for ORP vs. population were 42–28%, respectively, when the pH was ignored, and 54–82% and 46–89%, respectively, when analyses were conducted at each pH. The lowest correlations occurred at pH 6 and the highest at pH 7.0. When the 0-chlorine concentration and all but the lowest chlorine concentration needed for complete kill were deleted from the analyses, most of the regressions of populations recovered vs. solution ORP were linear.

DISCUSSION

Hypochlorous acid and hypochlorite ions are strong oxidants, but their toxicity to bacteria and fungi is not strictly related to their ability to oxidize (1,2). For example, the acid, which has an ORP less than twice that of the ion (6), is up to 80 times more toxic to microbes than the ion (2,4,8). Consequently, the relationship of the ORP of a chlorine solution with the solution's effect on a single influx of postharvest pathogens or against a continuous influx of pathogens, as would occur in the dump tanks and flumes in packinghouses, is not clear. In the tests reported here, the initial ORP of aqueous solutions of chlorine predicted the toxicity of solutions at pH 6.0 or 7.0, but not at pH 8.0. For example, when cells of *E. c. carotovora* were treated in chlorine solutions with an ORP of 600 mV, the population decreased by 1.0, 1.5, or 3.5 Log_{10} cfu/ml at pH 6.0, 7.0, and 8.0, respectively. This observation appears consistent with the reports by Carlson (1) and Lund (7), where the ORP was not functionally related to kill of *E. coli* and poliovirus, respectively, independently of pH level or at pH 7.0 vs. pH 8.4, respectively. However, we measured the ORP of solutions before the bacteria or conidia were added. Since the ORP decreases as free chlorine is lost to oxidation reactions with microbes (1,2,7), the ORP would be expected to decrease during the microbe treatments, and solutions with mostly HOCl are not as stable as those with mostly OCl^- . Therefore, a larger and likely more rapid ORP decrease would be expected with solutions at pH 6.0 vs. pH 8.0. Therefore, the correlation between ORP and reduction in microbe populations for solutions at pH 6.0 might be somewhat closer to that of solutions at pH 8.0 than is apparent in the tests reported here.

Alternatively, during the 2-min exposure used here, at pH 8.0, significant concentrations of OCl^- participated in the killing of the test organisms through conversion to HOCl (9). In most reports, a large proportion of microbe populations is killed immediately after they have

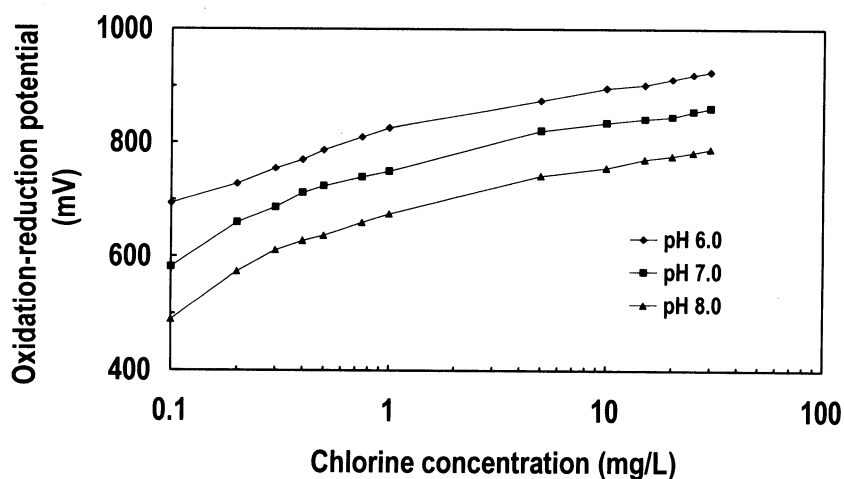


Fig. 1. Oxidation-reduction potential of chlorine solutions at concentrations of 0.1–30 mg of free chlorine per liter (Log_{10} scale) at pH 6.0, 7.0, or 8.0.

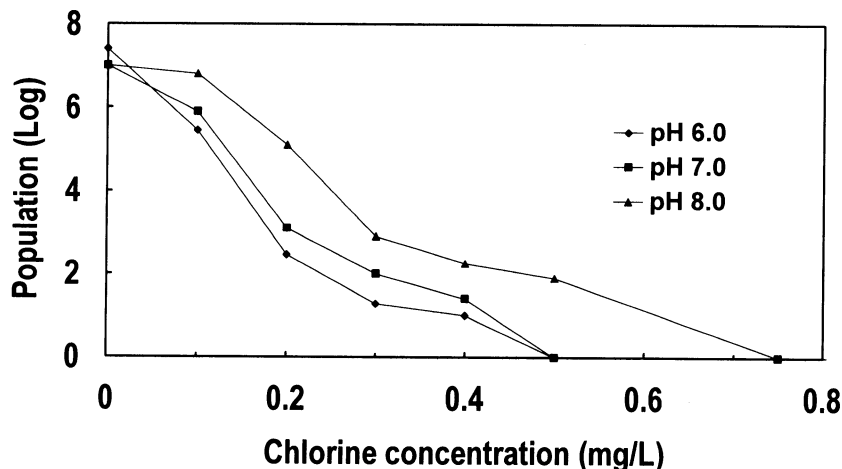


Fig. 2. Viable population (Log_{10}) of *Erwinia carotovora* subsp. *carotovora* after 1×10^7 cfu/ml was suspended for 2 min in an aqueous chlorine solution (0–1 mg of free chlorine per liter) buffered (0.05 M phosphate) to pH 6.0, 7.0, or 8.0.

been mixed with chlorine (=primary count drop), followed by slower secondary reductions (1). However, it seems likely that the primary count drop would decrease in proportion to the secondary count drop as the solution pH increased, so that the HOCl concentration would become limiting for a rapid, direct effect on the microbes. At an equal ORP, solutions at pH 6.0 compared with those at pH 8.0 have much lower free chlorine concentrations and are much less stable. The potency of a chlorine solution reflects the amount of HOCl absorbed by the target microbes during an exposure period (1,4,7,8). If the initial concentration of HOCl is not sufficient to kill microbes on contact, then HOCl formed by conversion from OCl^- should become involved, provided the exposure period is long enough and the pH is not high enough to inhibit that conversion. With the 2-min exposure used here and the pH level of 8.0, OCl^- , by conversion to HOCl, would likely have contributed to the potency of the solutions. If the contribution of the ions to the potency exceeded their contribution to the ORP, an effective solution at pH 8.0 might have a lower initial ORP than would an effective one at pH 6.0.

The relationship of the ORP with solution potency for fungal conidia appeared similar to that for the bacteria. However, about 50 times more free chlorine was required to kill the conidia than the bacteria. As with the bacteria, reductions in surviving conidia required higher ORP levels at pH 6.0 than at pH 8.0.

We are reluctant for many reasons to extrapolate our discussion to recommendations for chlorine concentrations, solution pH levels, or ORP levels for packinghouse dump tanks. First, the test organisms used in our tests were grown in the laboratory on a defined medium. Microbes produced in that manner are known to be more sensitive to chlorine than are those found in nature (1). Second, microbes in dump tank water must be selectively killed while free chlorine is reacting with many reductants. The ORP of the solution reflects the balance between all reductants and all oxidants, and not just that between chlorine and the target (1,7). The ORP of chlorinated water in a dump tank full of tomatoes is likely to be unstable, with a decreasing millivoltage at all points except where chlorine is added to the system (7). It is not clear what minimum ORP or free chlorine concentration would be required for effective sanitation of the water. Third, other fungal pathogens might be less sensitive to chlorine than was *G. candidum*. Fourth, the purpose of adding chlorine to dump tanks is to prevent tomatoes from becoming inoculated (5,12). It is not clear whether the inactivation of postharvest pathogens within a 2-min exposure

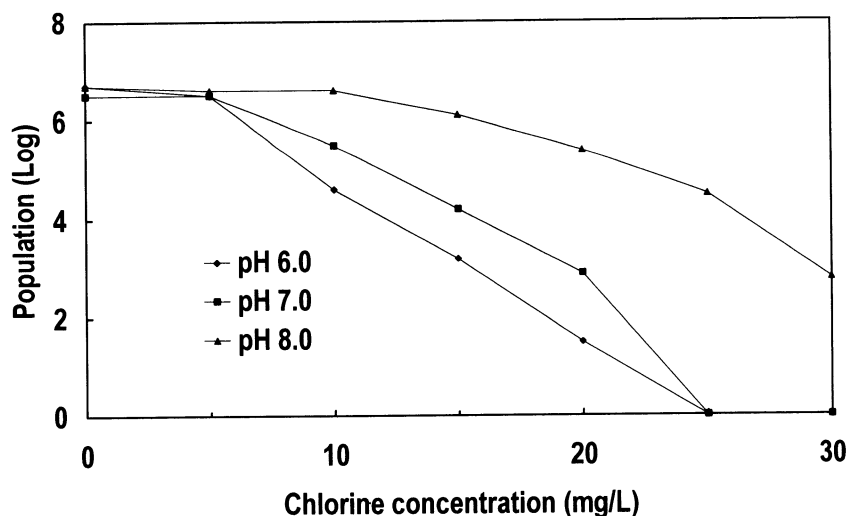


Fig. 3. Viable population (Log_{10}) of *Geotrichum candidum* after 1×10^7 conidia per milliliter was suspended for 2 min in an aqueous chlorine solution (0–30 mg of free chlorine per liter) buffered (0.05 M phosphate) to pH 6.0, 7.0, or 8.0.

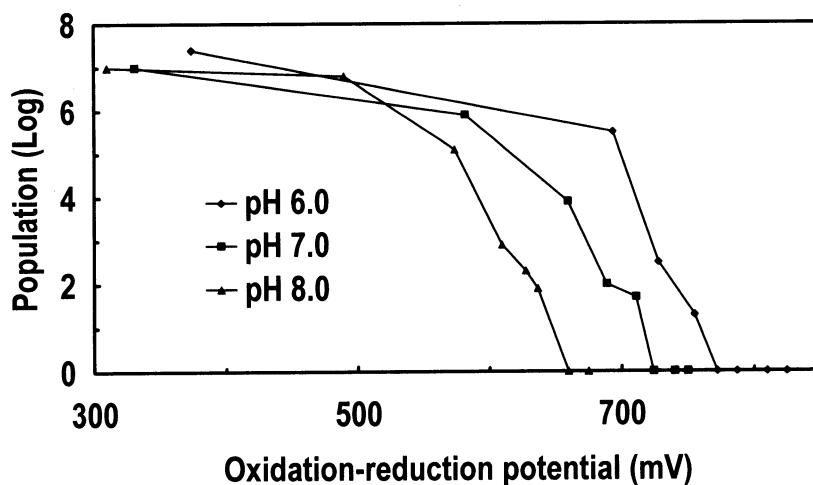


Fig. 4. Viable population (Log_{10}) of *Erwinia carotovora* subsp. *carotovora* vs. initial oxidation-reduction potential of chlorine solutions after 1×10^7 cfu/ml was suspended for 2 min in aqueous solutions buffered (0.05 M phosphate) to pH 6.0, 7.0, or 8.0.

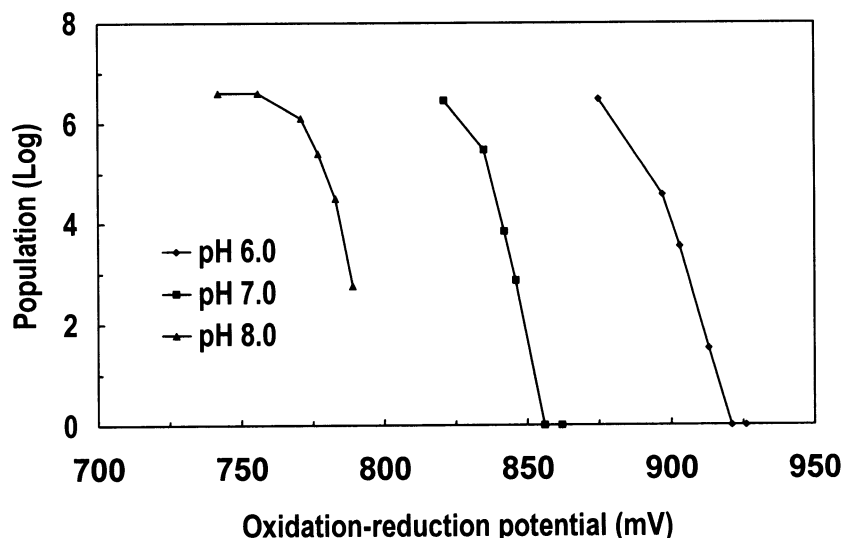


Fig. 5. Viable population (Log_{10}) of *Geotrichum candidum* vs. initial oxidation-reduction potential of chlorine solutions after 1×10^7 conidia per milliliter was suspended for 2 min in aqueous solutions buffered (0.05 M phosphate) to pH 6.0, 7.0, or 8.0.

period will accomplish this goal. Thus, it is likely that higher minimum ORPs or free chlorine concentrations than given here will be required for effective sanitation of dump tanks. However, solution ORP levels appear to be well correlated with the toxicity of the solution to two different, typical post-harvest pathogens provided that the solution pH is held constant in the range of 6.0–8.0. Thus, we conclude that continuous on-line measurement of the ORP of the water in dump tanks and flumes, coupled with additions of chlorine products as needed to maintain a desired ORP, can provide optimal sanitation with minimal input of chlorine. The extrapolation of our conclusions to pH levels much lower or higher than those used here is not recommended. At a pH level much below 6.0 and at chlorine concentrations likely to be required for packinghouse dump tanks, chlorine solutions are highly unstable (9). Even at pH 6.0, chlorine solutions may not be very stable because most of the free chlorine is in the highly reactive hypochlorous acid form (2,7). In the tests reported here, the worst correlation between initial ORP or initial free chlorine concentration and kill of the test

microbes occurred at pH 6.0. The best correlations occurred at pH 7.0 or 8.0. By contrast, at pH levels much above 8.0, little hypochlorous acid would be present in the solution and the efficacy of such solutions would depend on the acid formed by conversion from OCl^- . Given the reactivity of both the acid and the ion with various reducing groups, as well as with microbes (2,9), sufficient hypochlorous acid may not form rapidly enough at high pH levels to prevent the inoculation of produce in packinghouse dump tanks.

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