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Microbial Destruction by Low Concentrations of Hypochlorite and Iodophor Germicides in Alkaline and Acidified Water[1]

HELEN HAYS, P. R. ELLIKER, AND W. E. SANDINE
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

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Hypochlorite and iodophor germicides were evaluated for their ability to destroy a variety of organisms at levels approximating those used for final sanitizing rinse for dairy and food equipment and beverage bottles (3 to 50 ppm). Test organisms included *Escherichia coli*, *Streptococcus lactis*, *Lactobacillus plantarum*, *Pediococcus cerevisiae*, and *Saccharomyces cerevisiae*. The hypochlorites and iodophors demonstrated approximate rates of destruction at equivalent concentrations for the bacterial species tested, except where the hypochlorite contained excess alkalinity. The hypochlorite responded more readily to a downward shift to a *p*H of 5.0 than did the iodophor. Excess alkalinity of the hypochlorite significantly affected its bactericidal activity. The iodophor exhibited a consistently greater rate of destruction of yeast cells than the hypochlorite. Successive treatment with low levels of iodophor (6 ppm) followed by a hypochlorite (12 to 25 ppm) resulted in a high level of destruction of all test organisms. Possibilities for employing these measures in a sanitizing rinse of bottles for maximal destruction of organisms were discussed. Among the test organisms, *S. lactis* showed a comparatively high resistance and was a useful organism for comparing the halogen preparations.

Development of improved procedures for continuous pasteurization of milk, beer, and other beverages by heat and filtration and improved sanitary condition of equipment after use of the pasteurizer have placed greater emphasis on the microbial condition of postpasteurization equipment and of bottles or other containers at time of filling. Bottle-washing procedures usually involve a water rinse, hot caustic detergent action, further rinsing with water, and a final sanitizing rinse with low concentrations of a germicide such as sodium or calcium hypochlorite or iodophor. After the germicide rinse, the bottle, after a varying period of draining, is transported to the filler with no further sterilizing or sanitizing treatment before receiving the final product. The sanitizing rinse for postpasteurization equipment, as for dairy operations, usually involves higher concentrations of germicide than for bottle sanitizing, and usually is applied just prior to use of equipment.

The following study was designed to evaluate the effectiveness of low concentrations of hypochlorite and iodophor germicides that might be employed in the final sanitizing rinse of bottles or equipment or in periodic rinsing of equipment, such as fillers or vats, to remove condensate or product film accumulating during long periods of operation. The procedures were common laboratory methods of comparing germicidal activity in water solutions. The test organisms were species often used for germicidal studies as well as types encountered under plant conditions, i.e., postprocessing contaminants of milk, beer, and other beverages.

MATERIALS AND METHODS

Escherichia coli 198, *Streptococcus lactis* E, *Lactobacillus plantarum* 17-5, *Pediococcus cerevisiae* P-60, and *Saccharomyces cerevisiae* were used. *E. coli* represented the postprocessing contaminants found in a variety of food or beverage operations. *S. lactis* is a typical dairy contaminant and is useful because of its exceptional resistance to common germicides. The *Pediococcus*, *Lactobacillus*, and yeast (*S. cerevisiae*) strains are common brewery contaminants of concern in postprocessing development. The organisms were incubated 24 hr at 30 C on bottle slants of the following agar medium: *E. coli*, on tryptone-glucose-yeast extract agar (2); *S. lactis*, *L. plantarum*, and *P. cerevisiae*, on lactic agar (4); and *S. cerevisiae* on

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TABLE 1. *Destruction of Escherichia coli by iodophor and hypochlorite germicides*

Germicide	Concn (ppm)	Test water	*p*H of germicide solution	Avg no. of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor	3	USDA[b]	7.2	—[c]	—[c]	10	0	0
	6		6.9	—[c]	7	0	0	0
Hypochlorite	3	USDA	8.5	11	0	0	0	0
	6		8.6	2	0	1	0	0
	12		8.5	0	0	0	0	0
	3	Buffer (*p*H 5.0)	5.1	0	0	0	0	0
	6		5.1	0	0	0	0	0
	12		5.2	0	0	0	0	0

[a] Initial number of cells, 3.7×10^6 per ml.
[b] U.S. Department of Agriculture.
[c] Too numerous to count.

potato-dextrose agar (2). The growth was washed off with sterile phosphate-buffered water adjusted to *p*H 7.2 (2) and filtered through sterile Whatman no. 2 filter paper.

The methods of preparing the cultures and evaluating the germicidal activity were described by Chambers (3). Germicide trials were carried out at 25 C. The procedure was modified by transferring a 1-ml sample of the germicide-organism mixture at the end of the exposure period directly into a petri dish containing 2 ml of thiosulfate neutralizer (0.0004 M sodium thiosulfate plus 0.01 M phosphate buffer adjusted to *p*H 7.2). The contents then were mixed immediately by swirling, and agar was added. The germicides used were an iodophor consisting of a nonylphenoxpoly (ethyleneoxy) ethyl alcohol-iodine complex with 25% phosphoric acid (also referred to as nonyl phenol type) and sodium hypochlorite (NaClO). The available iodine in the iodophor was determined by titration to a colorless end point with standard thiosulfate; the standard thiosulfate titration (1) was used for determining available chlorine in the sodium hypochlorite. Germicide solutions were prepared in distilled water, U.S. Department of Agriculture (USDA) buffered synthetic hard water (Federal Register; 500 ppm of hardness unless otherwise indicated), and in 0.01 M acetate-acetic acid buffer at *p*H 5.0. The iodophor was used in concentrations of 3 and 6 ppm, and the hypochlorite, in concentrations of 3, 6, 12, and 25 ppm. In some trials, the germicides were tested at higher levels. Concentrations of organisms were approximately 10^6 cells per ml, except when otherwise indicated.

It was observed that some organisms were more readily destroyed by iodophor and others by hypochlorite, and it was considered possible that, to obtain consistent sterility, consecutive treatments with the two germicides might combine the desirable features of both. Another series of trials was, therefore, conducted to determine the effect of consecutive treatments with iodophor and hypochlorite germicides. In the tests, 1-ml suspensions of organisms were added to 3 or 6 ppm of iodophor, the mixture was agitated, and, after 15 sec of exposure, sufficient hypochlorite was added to provide concentrations of 3, 6, 12, and 25 ppm of this compound. The medication flask was agitated again by swirling, and samples were transferred directly into the thiosulfate inactivator in the plating dish, after exposures to the germidice for 30, 60, 120, and 300 sec.

RESULTS

Data in Table 1 show that 3 ppm of iodophor in USDA water caused complete destruction of *E. coli* in 2 min; with 6 ppm, the time was reduced to 1 min. When sodium hypochlorite was used in USDA buffered water, the destruction time was 30, 30, and 15 sec with 3, 6, and 12 ppm, respectively. However, when the hypochlorite was buffered at *p*H 5.0, there was complete destruction with all concentrations in less than 15 sec.

S. lactis E, as shown in Table 2, was destroyed by 3 ppm in 5 min and by 6 ppm in 2 min by iodophor in USDA water. When the iodophor was buffered at *p*H 5.0, the killing time with 3 ppm was the same but was reduced to 60 sec with 6 ppm. Destruction was comparable with sodium hypochlorite when USDA water was used, because 5 min was required for 3 ppm, 1 min for 6 ppm, and less than 15 sec for 12 ppm. When the sodium hypochlorite was buffered at *p*H 5.0, complete destruction was achieved in less than 15 sec with 3, 6, and 12 ppm.

In buffered USDA water, 3 ppm of iodophor did not cause complete destruction of *L. plantarum* within 5 min, whereas 6 ppm in 1 min did (Table 3). Results in buffer of *p*H 5.0 were slightly better since 3 ppm caused complete destruction after 5 min. At the higher *p*H, sodium hypochlorite was no more active than iodophor,

TABLE 2. *Destruction of Streptococcus lactis by iodophor and hypochlorite germicides*

Germicide	Concn (ppm)	Test water	*p*H of germicide solution	Avg no. of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor	3	USDA[b]	7.1	—[c]	—[c]	2,200	287	0
	6		6.8	—[c]	720	14	0	0
	3	Buffer (*p*H 5.0)	5.0	—[c]	—[c]	520	2	0
	6		5.0	—[c]	412	0	0	0
	12		4.8	180	1	0	0	0
Hypochlorite	3	USDA	8.4	—[c]	—[c]	—[c]	—[c]	—[c]
	6		8.4	38	5	0	0	0
	12		8.5	0	0	0	0	0
	3	Buffer (*p*H 5.0)	5.0	0	0	0	0	0
	6		5.0	0	0	0	0	0
	12		5.1	0	0	0	0	0

[a] Initial number of cells, 6.8×10^5 per ml.
[b] U.S. Department of Agriculture.
[c] Too numerous to count.

TABLE 3. *Destruction of Lactobacillus plantarum by iodophor and hypochlorite germicides*

Germicide	Concn (ppm)	Test water	*p*H of germicide solution	Avg no. of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor	3	USDA[b]	7.0	—[c]	—	—	—	—
	6		6.7	—	—	0	0	0
	3	Buffer (*p*H 5.0)	5.1	—	—	—	143	0
	6		5.0	—	—	328	0	0
	12		4.8	—	940	0	0	0
Hypochlorite	3	USDA	8.4	—	—	—	—	—
	6		8.4	—	—	—	—	—
	12		8.4	0	0	0	0	0
	3	Buffer (*p*H 5.0)	5.0	0	0	0	0	0
	6		5.0	10	0	0	0	0
	12		5.1	0	0	0	0	0

[a] Initial number of cells, 2.8×10^5 per ml.
[b] U.S. Department of Agriculture.
[c] Dashes represent results too numerous to count.

but at *p*H 5.0 there was complete destruction in 15 sec by 3 ppm of hypochlorite.

In USDA water, 3 ppm of iodophor did not cause complete destruction of *P. cerevisiae* in 5 min, but 6 ppm in 2 min did (Table 4). At *p*H 5.0, there was complete destruction in 5 min with 3 ppm and in 2 min with 6 ppm. Sodium hypochlorite in USDA water failed to sterilize in 5 min with 3 and 6 ppm, but did so in 30 sec with 12 ppm. However, 3, 6, and 12 ppm of sodium hypochlorite buffered at *p*H 5.0 killed in 15 sec.

S. cerevisiae was destroyed in 30 sec by 3 ppm of iodophor in buffered USDA water and in less than 15 sec by 6 ppm (Table 5). For complete destruction, the hypochlorite in buffered USDA water required 5 min with 3 ppm, 2 min with 6 and 12 ppm, and 30 sec with 25 ppm. When the hypochlorite was buffered at *p*H 5.0, the killing time was 1 min with 3 and 6 ppm and was less than 15 sec with 12 and 25 ppm.

In trials with a combination of iodophor and sodium hypochlorite on *P. cerevisiae* in buffered USDA water, 2 min were required for complete

TABLE 4. *Destruction of Pediococcus cerevisiae by iodophor and hypochlorite germicides*

Germicide	Concn (ppm)	Test water	*p*H of germicide solution	Avg no. of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor	3	USDA[b]	7.2	—[c]	—	—	—	—
	6		6.9	—	980	4	0	0
	3	Buffer (*p*H 5.0)	5.1	—	—	—	315	0
	6		5.0	—	—	700	0	0
	12		4.9	—	770	5	0	0
Hypochlorite	3	USDA	8.4	—	—	—	—	—
	6		8.5	—	496	19	127	20
	12		8.6	4	0	0	0	0
	3	Buffer (*p*H 5.0)	5.1	0	0	0	0	0
	6		5.1	0	0	0	0	0
	12		5.1	0	0	0	0	0

[a] Initial number of cells, 8.5×10^6 per ml.
[b] U.S. Department of Agriculture.
[c] Dashes represent results too numerous to count.

TABLE 5. *Destruction of Saccharomyces cerevisiae by iodophor and hypochlorite germicides*

Germicide	Concn (ppm)	Test water	*p*H of germicide solution	Avg no. of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor	3	USDA[b]	7.1	571	0	0	0	0
	6		6.8	0	0	0	0	0
Hypochlorite	3	USDA	8.6	—[c]	—	—	61	0
	6		8.5	—	—	14	0	0
	12		8.5	—	117	1	0	0
	25		8.6	75	0	0	0	0
	3	Buffer (*p*H 5.0)	5.2	—	66	0	0	0
	6		5.3	81	2	0	0	0
	12		5.3	0	0	0	0	0
	25		5.3	0	0	0	0	0

[a] Initial number of cells, 4.7×10^5 per ml.
[b] U.S. Department of Agriculture.
[c] Dashes represent results too numerous to count.

destruction for 3 ppm of each germicide (Table 6). When the concentrations of both germicides were increased to 6 ppm, the destruction was complete after 1 min. When the germicides were diluted in *p*H 5.0 buffer, there was complete destruction in 30 sec with 3 ppm of both germicides.

Against *S. cerevisiae*, the results (Table 7) were essentially the same as with *P. cerevisiae*, except that at *p*H 5.0 a level of 3 ppm of the germicides required 1 min of exposure for complete destruction.

Results of additional trials with *S. lactis* in concentrations of hypochlorite and iodophor ranging from 12.5 to 50 ppm indicated first of all that hypochlorites in a buffered hard water (Table 8) had a lower initial *p*H than distilled water (Table 9), which would be comparable to a very soft water supply. With the iodophor, the opposite final *p*H effects occurred (Tables 8 and 9). Germicidal activity of the two types of compounds was comparable at equivalent concentrations.

Results in Table 10 illustrate quite strikingly the critical nature of *p*H in bactericidal activity of hypochlorites in use dilutions and particularly

TABLE 6. *Destruction of Pediococcus cerevisiae by 15 sec of exposure to iodophor followed by varying exposures to hypochlorite*

Concn of germicide (ppm)		Test water	pH of germicide solution	Avg no. of surviving organisms[a]			
Iodophor	Hypochlorite			30 sec	60 sec	120 sec	300 sec
3	3	USDA[b]	7.3	274	3	0	0
6	6		6.9	45	0	0	0
6	12		6.8	1	0	0	0
6	25		6.9	0	0	0	0
3	3	Buffer (*p*H 5.0)	5.0	0	0	0	0
6	6		5.0	0	0	0	0
6	12		5.0	0	0	0	0
6	25		5.0	0	0	0	0

[a] Initial number of exposed cells, 6.0×10^6 per ml.
[b] U.S. Department of Agriculture.

TABLE 7. *Destruction of Saccharomyces cerevisiae by 15 sec of exposure to iodophor followed by varying exposures to hypochlorite*

Concn of germicide (ppm)		Test water	pH of germicide solution	Avg no. of surviving organisms[a]			
Iodophor	Hypochlorite			30 sec	60 sec	120 sec	300 sec
3	3	USDA[b]	7.2	274	20	0	0
6	6		6.9	1	0	0	0
6	12		6.9	0	0	0	0
6	25		7.0	0	0	0	0
3	3	Buffer (*p*H 5.0)	5.0	1	0	0	0
6	6		4.9	0	0	0	0
6	12		4.4	0	0	0	0
6	25		4.9	0	0	0	0

[a] Initial number of cells, 7.1×10^5 per ml.
[b] U.S. Department of Agriculture.

TABLE 8. *Destruction of Streptococcus lactis by various concentrations of hypochlorite and iodophor*[a]

Germicide	Germicide concn (ppm)	pH		Avg no. of surviving organisms[b]				
		Initial	Final	15 sec	30 sec	60 sec	120 sec	300 sec
NaClO	12.5	7.95	7.7	—[c]	—	—	—	—
	25	8.0	7.75	—	623	8	0	0
	50	8.2	7.7	15	12	15	0	0
Iodophor	12.5	6.65	7.25	—	—	—	—	—
	25	6.2	6.7	2,200	427	11	11	9
	50	3.55	4.5	17	3	1	0	0

[a] In U.S. Department of Agriculture test water of 200 ppm hardness.
[b] Initial number of organisms, 46×10^7 per ml.
[c] Dashes represent results too numerous to count.

the significance of excess alkalinity which was high with hypochlorite A and low with hypochlorite B. In distilled water comparable to a soft water supply with little buffering effect, hypochlorite A with its higher excess alkalinity actually was a poor bactericide. In USDA test water, a buffered hard water, hypochlorite A was greatly improved, and there was little differ-

TABLE 9. *Destruction of Streptotoccus lactis by various concentrations of hypochlorite and iodophor in distilled water*

Germicide	Germicide concn (ppm)	pH		Avg no. of surviving organisms[a]				
		Initial	Final	15 sec	30 sec	60 sec	120 sec	300 sec
NaClO	12.5	9.2	6.5	—[b]	—	—	—	—
	25	9.6	6.8	—	2,940	570	200	0
	50	10.1	6.45	2	0	0	0	0
Iodophor	12.5	2.7	3.5	—	—	293	22	55
	25	2.5	2.9	2,100	420	0	0	0
	50	2.25	2.55	0	0	0	0	0

[a] Initial concentration of organisms, 17×10^7 per ml.
[b] Dashes represent results too numerous to count.

TABLE 10. *Destruction of Streptococcus lactis by commercial hypochlorites of different excess alkalinity in distilled and USDA[a] test water*

Germicide	Germicide concn (ppm)	Dilution water	pH	Avg no. of surviving organisms[b]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Hypochlorite A	12.5	Distilled	10.5	—[c]	—	—	—	0
	25		10.8	—	—	—	10	0
	50		11.2	—	—	680	6	0
Hypochlorite B	12.5	Distilled	7.0	9	0	0	0	0
	25		7.2	0	0	0	0	0
	50		8.2	1	0	0	0	0
Hypochlorite A	12.5	USDA	9.1	333	1	0	0	0
	25		9.1	0	0	0	0	0
	50		9.6	0	0	0	0	0
Hypochlorite B	12.5	USDA	9.15	389	1	0	0	0
	25		9.2	0	1	0	0	0
	50		9.2	0	0	0	0	0

[a] U.S. Department of Agriculture.
[b] Initial number of organisms, 2.3×10^5 to 2.6×10^5 per ml.
[c] Dashes represent results too numerous to count.

ence between the two hypochlorites because the buffering effect of the water adjusted both products to an effective *p*H level.

DISCUSSION

The iodophor and hypochlorite compounds in these studies consisted of widely used commercial products and therefore were representative of types available on the market. Both showed a high rate of destruction of all species of test microorganisms, and the results indicate that they should be effective germicides for sanitizing in farm and food plant operations. Bacterial destruction by hypochlorite and iodophor was comparable in the unadjusted solutions diluted in USDA hard water. This water has been standardized to simulate a typical water of 500 ppm hardness and medium alkalinity. Trials making use of hypochlorites and iodophors in unadjusted hard waters and in distilled water at equivalent concentrations ranging up to 50 ppm have repeatedly presented a similar picture with organisms commonly encountered on dairy equipment. This suggests greater comparative bactericidal activity for the fast-acting hypochlorite of low excess alkalinity than usually has been attributed to it. However, a number of studies in which bactericide comparisons have been made and on which standards for use of the compounds have been based have included organic matter in the test solution in the form of excessive numbers of cells or culture medium constituents. Such "capacity tests" may be misleading in predicting practical effectiveness of a sanitizing

agent, assuming that no chemical sanitizing agent will show satisfactory bactericidal activity unless an equipment or bottle surface is free of organic matter deposits. "Capacity tests" on germicides might have better application in tests for skin disinfection or mastitis sanitation procedures for which the sanitizing solution commonly encounters an appreciable organic matter load.

Consequently, for most sanitizing operations, hypochlorites and iodophors may demonstrate similar rates of destruction at equivalent concentrations unless the hypochlorite contains too great an excess alkalinity or unless a weakly buffered iodophor is used in a highly alkaline water. Consideration of the type of compound to apply under such circumstances may depend on uniformity of commercial product, relative cost, corrosive rate, flavor effect, and in some cases alkalinity of water.

The hypochlorite responded more readily in germicidal activity to a downward shift in *p*H to 5.0 than the iodophor for all microorganisms tested. Other studies (5) have demonstrated surprisingly great stability of hypochlorite solutions at such low *p*H levels; however, corrosive activity also would be increased considerably. This would need to be considered in their application in the sanitizing step of bottle-washing machines and in sanitizing metal surfaces.

These results emphasize the importance of excess alkalinity of hypochlorites and the need to control this factor to assure an active product under different water conditions. Slight differences in alkalinity of hypochlorite solutions (on the order of 1 to 2 *p*H units in use dilution) may be more significant in affecting bactericidal activity than any other factor and thus represent a problem in application of this type of sanitizer. High excess alkalinity also may render a hypochlorite less stable during extended storage.

The iodophor exhibited a consistently greater rate of destruction of yeast cells than the hypochlorite. Possibly, the larger cell mass of the yeast created a greater chlorine demand that had to be satisfied before the chlorine could destroy the yeast cells. Evidence for this was indicated by the fact that, after a certain concentration of chlorine was reached, this compound effected rapid destruction. Subsequent studies also have suggested that some yeast enzyme systems are more rapidly inactivated by iodophor than by hypochlorite germicides.

The rapid destruction of both bacteria and yeasts by the combined action of hypochlorite followed by iodophor treatment provides interesting possibilities. This combines the advantages of both germicides and appears to effect practically complete destruction of all organisms exposed under the test conditions in these studies. Two-stage "feeders" that inject two different chemicals consecutively into a water supply might be adapted for such an application.

A survey of activity of both types of germicides at various concentrations suggests that a concentration of 25 ppm might be more appropriate for iodophor sanitizing solutions than 12.5, or even 6 ppm, as has frequently been recommended for these agents. On the other hand, assuming clean equipment or bottle surfaces, the hypochlorites in these trials demonstrated a high rate of destruction at 25 and 50 ppm. These results suggest that hypochlorites of low excess alkalinity should be effective as sanitizing agents on clean surfaces at 25 to 50 ppm, which are below the concentrations generally recommended. Use of hypochlorites at such low levels should reduce corrosion of equipment which may accompany their application at higher concentrations. Hypochlorites of high excess alkalinity on the other hand would demonstrate a much lower rate of destruction and might have to be used at higher levels to provide equivalent destruction. With the hypochlorites, this raises still another problem, since increasing the concentration sometimes increases excess alkalinity which in turn decreases bactericidal activity.

Comparison of the germicidal resistance of the various bacterial species emphasizes the high resistance of the lactic acid bacteria compared with that of *E. coli*. *S. lactis* has been employed frequently as a test agent in these laboratories, particularly for hypochlorite and iodine or iodophor preparations, because of this characteristic and its ease of handling.

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