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Efficiency of Chlorine Dioxide as a Bactericide[1]

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ABSTRACT

BENARDE, MELVIN A. (Rutgers, The State University, New Brunswick, N.J.), BERNARD M. ISRAEL, VINCENT P. OLIVIERI, AND MARVIN L. GRANSTROM. Efficiency of chlorine dioxide as a bactericide. Appl. Microbiol. **13**:776–780. 1965—We found chlorine dioxide to be a more effective disinfectant than chlorine in sewage effluent at *p*H 8.5. Chlorine dioxide was also found to be a more stable bactericide in relation to *p*H in the range studied.

Chlorine dioxide, prepared early in the 19th century, was used in the treatment of water supplies in Europe after 1850. It was not until the 1940's, however, that experimental data on its bactericidal efficiency became available. Although chlorine and chlorine dioxide are similar in many respects, including the fact that both are powerful oxidizing agents, ClO_2 has 2.5 times the oxidation capacity of Cl_2. It was this feature that recommended ClO_2 for the control of odors and tastes in water supplies.

McCarthy (1944) reported ClO_2 to be an effective germicide in water with low organic content. However, he found it to be less effective than chlorine at equal concentrations when the organic content was high.

Shortly thereafter, Ridenour and Ingols (1947) reported ClO_2 to be at least as effective as chlorine. Their conclusions were based upon the absence of growth after 30 min of contact with the disinfectant. Efficiency was based on residual values, and these did not represent equal initial dosages.

Trakhtman (1946) and Bedulevich, Svetlakova, and Trakhtman (1953) found ClO_2 to exceed or at least equal chlorine in bactericidal efficiency. They reported decreased effectiveness of ClO_2 under alkaline conditions.

Because physicochemical characteristics and kinetics of ClO_2 were unavailable at the time these studies were performed, the data obtained are of questionable value.

Preparation of chlorine dioxide from chlorine, or by the action of acid on $NaClO_2$, as is the general procedure, would of necessity introduce interfering substances which would not only hasten the decomposition of ClO_2, but would also yield erroneously high values on iodometric analysis. This would suggest that the initial concentrations of ClO_2 in the studies noted were probably lower than the reported values; the bactericidal efficiency of ClO_2, when compared with chlorine, would suffer accordingly. Preparation of ClO_2 by addition of alkali results in major losses of ClO_2 almost instantaneously. Granstrom and Lee (*unpublished data*) have shown the disproportionation that occurs above *p*H 10. Additionally, these older methods probably did not account for the high degree of volatility exhibited by ClO_2. Depending upon the concentration and length of exposure, 7 to 30% loss can occur within 1 hr. This means that dilutions prepared from stock solutions cannot be assumed to be quantitative. Spectrophotometric analysis of each dilution immediately prior to use is required. Such a procedure has only recently become available.

The general analytical procedure for detection of ClO_2 residuals employed *o*-tolidine in a colorimetric determination. This procedure is subject to similar interferences, as noted for the iodometric titration. Post and Moore (1959) reported that *o*-tolidine and *o*-tolidine arsenite methods made no distinction between Cl_2 and ClO_2. Consequently, it is nonspecific and allows only a range of values for any given concentration. Thus, critical comparison of chlorine versus chlorine dioxide was impossible. As a result, ClO_2 is sparingly used in water and waste water treatment.

The study reported herein used as its point of departure the detailed physicochemical findings of Granstrom and Lee (1958) and Granstrom et

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al. (*unpublished data*) to avoid the pitfalls of the past and to obtain more reliable and specific data.

MATERIALS AND METHODS

Organic-free distilled water. Organic-free water was prepared by distilling distilled water in the presence of an acid-permanganate solution (1% concentrated H_2SO_4 and 1% $KMnO_4$). The distillate traveled through a vertical column 6 ft (183 cm) long. It was necessary to use a heating tape at the top of the column to break the film of water from the distillation flask; otherwise, creeping of the permanganate occurred and would appear in the distillate. Extremely small amounts of permanganate can interfere in the *o*-tolidine determination of chlorine by giving itself a positive test (yellow color). This, however, provides a means of checking the quality of the distillate (Israel, 1961).

Organic-free water was used in preparing the phosphate buffers needed for the various trials.

Glassware. To obtain chlorine demand-free glassware, tubes, pipettes, reaction vessels, and syringes were soaked overnight in chlorine water of approximately 5,000 mg per liter.

Prior to use, these were rinsed several times in distilled water and heat-sterilized when necessary.

Neutralization of disinfectant. Tubes (16 by 150 mm) containing several crystals of $Na_2S_2O_3$ were sterilized at 121 C for 15 min. These were used for quenching further disinfection action of both chlorine and chlorine dioxide solutions.

Preparation of chlorine dioxide. A 4.0-g amount of $NaClO_2$ was dissolved in 50 ml of distilled water in a reaction vessel. To this was added a solution of 2.0 g of $K_2S_2O_8$ in 100 ml of distilled water. High-purity nitrogen gas (Linde Division, Union Carbide Corp., New York, N.Y.) was used to sweep out the ClO_2 formed; this N_2-ClO_2 gas mixture was passed through a dry $NaClO_2$ column to remove any traces of HOCl that might have been formed. The gases were then passed into a trap to remove any $NaClO_2$ dust that might have been carried over. ClO_2 was then collected in cool (0 to 10 C) organic-free water.

Preparation of chlorine. Aqueous chlorine stock solutions were prepared by bubbling chlorine gas into distilled water for 15 to 20 min. This yielded a 10,000 mg per liter concentration.

Concentrations of ClO_2 and Cl_2 were prepared on an equivalent chlorine basis: 1 mg per liter of chlorine is equal to 1.4×10^{-5} moles per liter; 1 mg per liter of ClO_2 is equal to 1.5×10^{-5} moles per liter. For this study, ClO_2 was prepared to equal 1.4×10^{-5} moles per liter. Thus, although equal in molar concentration, ClO_2 was low in per cent concentration. Since the high molar absorbtivity of ClO_2 lends itself to spectral analysis, we were able to measure the initial and residual concentrations of ClO_2 directly at 357 mμ. This was not the case with chlorine, whose molar absorbtivity is approximately one-tenth that of ClO_2. Although we were able to prepare stock solutions of chlorine of 20 ppm, using the spectrophotometer (238 mμ), the very low initial concentrations used in this study could not be so measured. For this reason, dilutions of chlorine were prepared in organic-free water, since it had been shown in our laboratory that quantitative dilutions of Cl_2 could be made.

For this study, initial dosages of both chlorine and chlorine dioxide of 0.25, 0.5, 0.75, 2.0, and 5.0 mg per liter were used.

Suspensions of washed cells (freashly isolated fecal strain of *Escherichia coli*) were mixed by magnetic stirrer in a reaction vessel with an equal volume of disinfectant solution to yield the final desired concentrations of Cl_2 or ClO_2, or both, and a bacterial density of approximately 15,000 cells per milliliter. The density of washed cells had been standardized spectrophotometrically by optical-density measurement at 525 mμ. Confirmation of this was obtained by spot-plate counts on nutrient agar, using a Unopette (Becton, Dickinson and Co., Rutherford, N.J.) for delivery of 0.025 ml. We found this technique to be more accurate and reproducible, and a great deal easier, than standard plate counts. Figure 1 shows the type of spot-plate counts obtained.

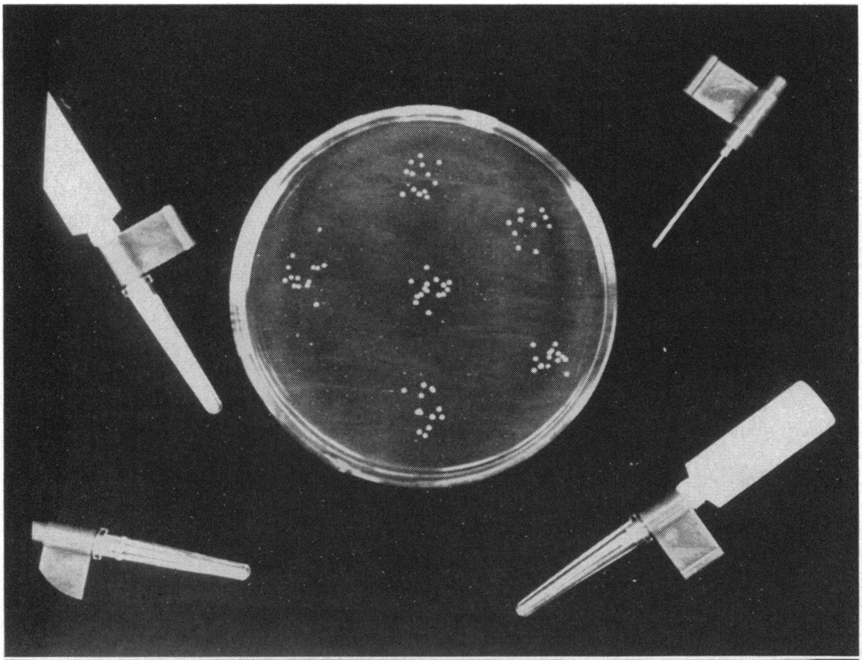
The sampling apparatus consisted of two automatic hypodermic syringes, each with a two-way flap valve assembly, permitting rapid drawing and dispensing of the solution from the reaction vessel. Figure 2 shows the apparatus ready for use. At 15-sec intervals, samples were removed for estimation of remaining population. Sterile thiosulfate crystals were used to quench further disinfectant effects. In the case of chlorine, residuals were determined by the *o*-tolidine method. Chlorine dioxide residuals, however, were determined spectrophotometrically. A 30-ml sample was withdrawn from the reaction vessel and placed in a 10-cm cuvette. Absorbance values at 357 mμ were obtained at intervals, and were used to calculate concentrations.

Plate counts were made on nutrient agar, after 18 and 48 hr of incubation at 35.5 C. To prevent clumping and spreading of colonies, it was necessary to add the sample to a layer of melted agar and swirl. Between trials, the sampling apparatus was sterilized by drawing a strong chlorine solution into it and allowing it to remain in contact for 10 min. Rinsing with sterile organic-free distilled water was repeated until negative *o*-tolidine tests were obtained. Sterility was determined by plate counts of the rinse water. This procedure additionally provided a chlorine demand-free system.

RESULTS AND DISCUSSION

It is generally understood that the germicidal activity of chlorine results from its hydrolysis in aqueous solutions to form hypochlorous acid, the disinfectant constituent:

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+Cl^- \qquad (1)$$



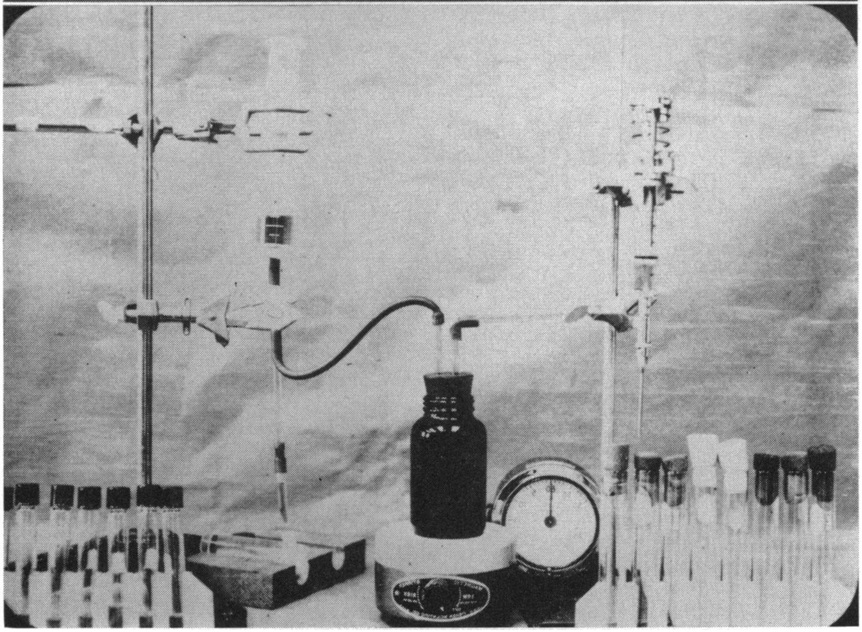


FIG. 1 (top). *Spot-plate counts obtained with Unopette.*
FIG. 2 (bottom). *Sampling apparatus.*

Optimal activity occurs in acidic solutions where HOCl, the initial hydrolysis product, exists in undissociated form. As the pH of solutions increases, hypochlorous acid ionizes increasingly; the resulting hypochlorite ion, OCl^-, has little disinfectant value. Table 1 indicates the degree of ionization at various pH levels.

$$HOCl \rightleftarrows OCl^- + H^+ \qquad (2)$$

Chlorine dioxide, on the other hand, does not

Table 1. *Ionization of hypochlorous acid at various pH levels*

*p*H	Per cent HOCl (20 C)*	Per cent OCl^-*
4.0	100.0	0.0
5.0	99.7	0.3
6.0	96.8	3.2
7.0	75.2	24.8
8.0	23.2	76.8
9.0	2.9	97.1
10.0	0.3	99.7
11.0	0.03	99.97

* Values calculated from the *K* values of equations 1 and 2 (Moore, 1951).

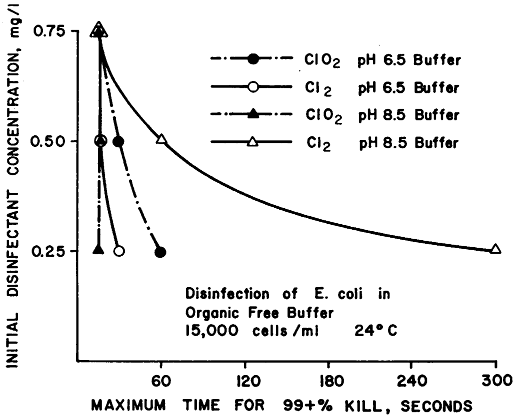


Fig. 3. *Effect of pH on kill.*

hydrolyze in aqueous solutions and, therefore, is not subject to dissociation in the manner of HOCl (Mellor, 1922). A stock solution of 10.2 mg per liter of ClO_2 was diluted and its absorbance at 357 mμ was measured. At *p*H 4.0, 6.45, and 8.42, the ClO_2 was unaltered (1.39 mg per liter, 100%). Thus, the intact ClO_2 molecule appears to be the bactericidal compound.

Figure 3 shows the effect of *p*H on the disinfectant efficiency of chlorine and chlorine dioxide in organic-free buffer. It can be seen that at *p*H 6.5 chlorine was somewhat more efficient than ClO_2. Based on our sampling times of 30 and 60 sec, it would appear that half the time is needed for equal kill at an initial dosage of 0.25 mg per liter. At 0.5 mg per liter, killing time was reduced, but chlorine remained more effective. Both compounds were equally efficient at an initial dose of 0.75 mg per liter.

Increasing the *p*H to 8.5 altered the comparative disinfectant properties dramatically. It is seen that at 0.25 mg per liter ClO_2 obtained 99+% kill in 15 sec. Chlorine did not achieve this until 300 sec—20 times less effective. At 0.5 mg per liter of ClO_2, kill was also completed within 15 sec, whereas chlorine requires 60 sec to achieve equal effects. Equal kill occured at 0.75 mg per liter for both compounds.

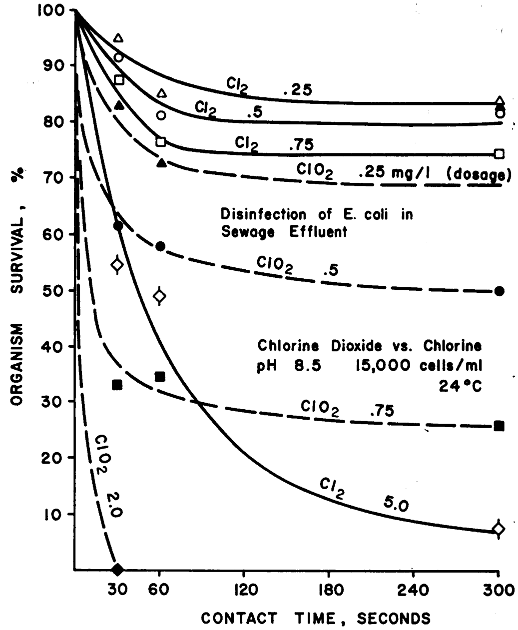


Fig. 4. *Effect of contact time on organism survival*

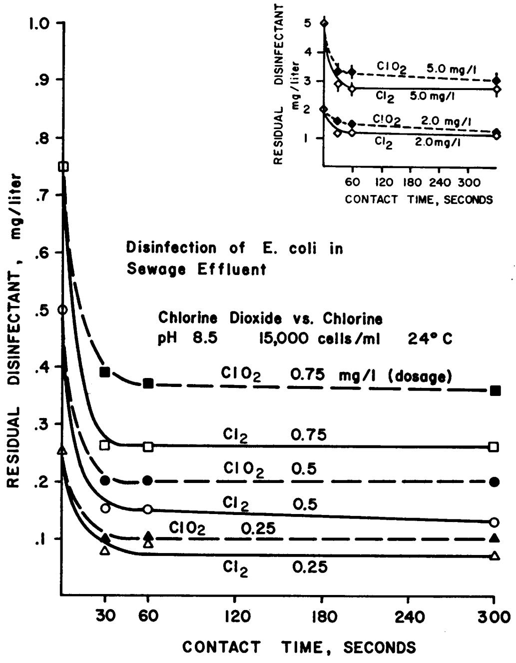


Fig. 5. *Effect of contact time on disinfectant utilization.*

From Fig. 3 it would seem that *p*H affects ClO_2 efficiency. Our data and the data of Mellor (1922) and Granstrom et al. (*unpublished data*) indicate that ClO_2 is not directly affected by *p*H in aqueous solutions; other factors are probably contributing to the loss of efficiency. Although the chemical reactions of ClO_2 with many materials are yet to be studied, it may well be that the rates of reaction of ClO_2 with substances found in our system are *p*H-dependent.

To establish the comparative bactericidal efficiencies that would more nearly resemble in-plant conditions, tests were conducted in sterile, unchlorinated sewage effluent to which a known cell density of *E. coli* was added. Since there were materials in the effluent that absorbed at 357 mμ, spectral analysis of ClO_2 residuals, in this instance, was impractical. Thus, both chlorine and ClO_2 residuals were determined by *o*-tolidine. Figure 4 presents the results of the effluent study.

It can be seen that the combination of organic matter and *p*H sharply curtails disinfectant activity. For initial Cl_2 dosages under 1 ppm, less than 30% of the initial population is removed. At the same initial dosages, chlorine dioxide achieves up to 70% removal. An even more dramatic example of the relative efficiencies of the two compounds is seen with chlorine at 5.0 ppm, which obtains 90% reduction after 5 min, compared with chlorine dioxide at 2.0 ppm, which obtains approximately 100% kill in 30 sec.

Generally, reports of chlorine efficiency are stated in terms of residual chlorine values. We found that residuals are not in themselves indicative of the concentration required for kill. Similarly, the initial concentration is not the killing dose. We feel that the actual value lies between the two. The amount of material used is probably closer to the true killing value.

Figure 5 presents residual values obtained for chlorine and chlorine dioxide in the effluent studies. For complete removal of organisms in 30 sec, about 0.9 ppm of ClO_2 was used when dosed with an initial concentration of 2.0 ppm. In the case of Cl_2, 2.25 ppm were actually used of an initial dose of 5.0 ppm to obtain 90% removal in 5 min.

Our data indicate that, even though residuals are present after 5 min, major reductions occur within the first minute of contact and do not occur appreciably thereafter.

This would suggest that residuals actually have little disinfectant value. Thus, reports of killing concentrations based on residual values seem unrealistic.

It should be borne in mind that the plateau effects in the case of chlorine are probably due to the formation of monochloramines (*p*H 8.5) and other chloro compounds which are of little value as disinfectants. In the case of chlorine dioxide, however, reaction with ammonia to form chloramines has not been reported.The fact that 100% kill has not been obtained with residuals up to 0.5 mg per liter, as measured by *o*-tolidine, which is nonspecific, would suggest that ClO_2 undergoes reaction to form organo-chloro compounds that have little disinfectant ability.

Additionally, Fig. 5 shows that less chlorine dioxide was used to obtain greater bacterial reductions. This may be due to fewer side-reactions at *p*H 8.5 that tie up the essential disinfecting material. As fewer molecules of chlorine dioxide were present initially, 1.4×10^{-5} as opposed to its usual 1.5×10^{-5} moles per liter, the results would tend to support even more strongly the conclusion that ClO_2 exhibits greater bactericidal activity than chlorine.

ACKNOWLEDGMENT

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