

Inactivation of *Giardia lamblia* Cysts with Ozone

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***Giardia lamblia* cysts were inactivated in water with ozone at pH 7.0 and 5 and 25°C. The concentration-time products for 99% inactivation were 0.53 and 0.17 mg-min/liter at 5 and 25°C, respectively. These products were significantly lower than those reported for chlorine.**

Several waterborne outbreaks of giardiasis have occurred as a consequence of the inability of conventional chlorination practices to inactivate *Giardia lamblia* cysts present in public water supplies (1). This may be attributed to the reported high resistance of *G. lamblia* cysts to chlorine, especially at low temperatures (2). The present study was conducted to investigate the effectiveness of ozone as an alternative disinfectant for the inactivation of *G. lamblia* cysts at 5 and 25°C.

Fecal specimens obtained from a donor with symptomatic giardiasis were washed with distilled water, and the cysts were isolated by flotation on a 1 M sucrose solution. The cysts were suspended in distilled water and stored at 5°C for 7 days before being used in inactivation studies.

Two borosilicate reactors, one as the inactivation reactor and the other as the control reactor, were used in the experiments. Each reactor contained 1 liter of ozone-demand-free 0.01 M phosphate buffer (pH 7) and was kept in a constant-temperature water bath. Ozone was continuously bubbled through the contents in the inactivation reactor to

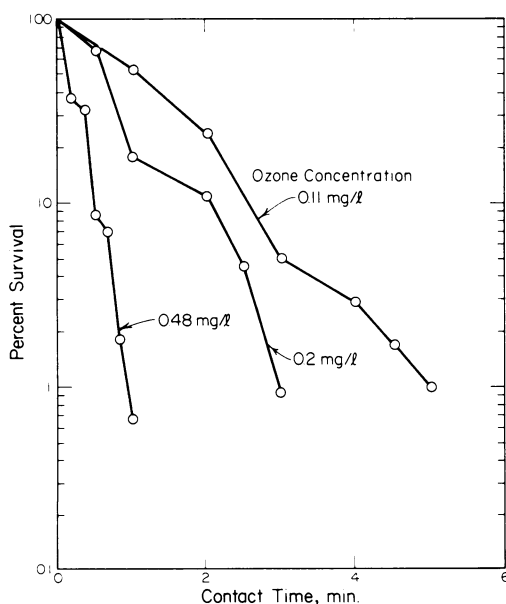


FIG. 1. Inactivation of *G. lamblia* cysts by ozone at pH 7 and 5°C.

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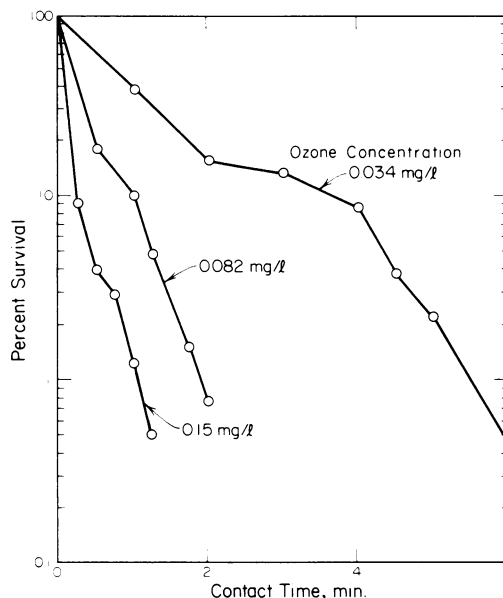


FIG. 2. Inactivation of *G. lamblia* cysts by ozone at pH 7 and 25°C.

maintain an approximately constant ozone concentration. Oxygen was used instead of ozone in the control reactor. The contents in both reactors were kept completely mixed by mechanical stirring. The ozone concentration was measured spectrophotometrically (7).

When the ozone concentration in the inactivation reactor reached a steady state, sufficient *G. lamblia* cysts to obtain a final density of about 10,000 cysts per ml were injected into the reactor. Samples (15 ml) were transferred every 10 to 30 s from the reactor to test tubes containing 1.5 ml of sodium thiosulfate solution (0.0206 g/liter) to neutralize the residual ozone. The cysts in each sample were concentrated by centrifugation and induced to excyst by the procedure suggested by Rice and Schaefer (4). The induction step of this method was modified by treating the cysts with aqueous hydrochloric acid for 15 min before adding the remaining solutions. No sodium bicarbonate was added to induce the cysts. The excystation was determined by a microscopic counting method. The survival fraction of each sample was computed relative to the viability in the controls, arbitrarily designated to be 100%.

G. lamblia cysts were inactivated at three different ozone concentrations at 5 and 25°C. The results are presented as

TABLE 1. Comparative concentration-time data for 99% inactivation

Organism	Disinfectant	pH	Temp (°C)	Concn (C) (mg/liter)	Time (t') (min)	C · t' (mg-min/liter)	Reference (no. of samples in average)
<i>G. lamblia</i> cysts	Ozone	7	25	0.03–0.15	5.5–1.06	0.17	Present study (3)
		7	5	0.11–0.48	5.0–0.94	0.53	Present study (3)
	Chlorine	7	25	1.5	<10	<15	2
		7	5	2.0–4.0	45–40	~125	2 (2)
Poliovirus 1	Ozone	7.2	20	0.15	0.5	0.08	5
		7.2	5	0.15	1.45	0.22	5
	Chlorine	6	5	0.5	4.0	2.0	6
<i>E. coli</i>	Ozone	7.2	1	0.065	0.33	0.02	3
	Chlorine	6	5	0.1	0.4	0.04	6

semilog plots of the percentage of cysts surviving against contact time (Fig. 1 and 2). The average concentration-time products ($C \cdot t'$) for 99% inactivation were 0.17 and 0.53 mg-min/liter at 25 and 5°C, respectively.

Representative $C \cdot t'$ products for ozone and chlorine inactivation of several organisms are presented in Table 1. When more than one concentration was used, a mean $C \cdot t'$ value was estimated from the number of samples tested. The data are not exactly comparable, as there were minor differences in pH and temperature, but the differences do not seriously detract from the comparison. *G. lamblia* cysts were only about twice as resistant to ozone inactivation as poliovirus 1 at a neutral pH and 5 and 25°C. The reported data for the inactivation of these two organisms by chlorine show that the cysts are about 60 times more resistant than the virus at 5°C (2, 6). *G. lamblia* cysts were more resistant to inactivation by ozone and chlorine than *Escherichia coli* by about 1 and 3 orders of magnitude, respectively, at low temperatures. Thus, the small range of $C \cdot t'$ products for ozone inactivation of cysts, viruses, and bacteria given in Table 1 show that there are fewer differences in resistance among these three organisms to ozone inactivation than to chlorine inactivation. These data also indicate that, at both 5 and 25°C, ozone is far more effective than chlorine for the inactivation of *G. lamblia* cysts, as the former has relatively low $C \cdot t'$ products.

The results of the present study indicate that the resistance of *G. lamblia* cysts to ozone inactivation at 5°C is nearly three times greater than that at 25°C. Although the available data are insufficient to make an exact estimate for

chlorine inactivation of *G. lamblia*, the resistance of the cysts appears to increase by ca. 1 order of magnitude as the temperature drops from 25 to 5°C at pH 7 (2). Thus, when compared with chlorine for the inactivation of *G. lamblia* cysts, ozone is less affected by temperature changes.

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