APPLIED MICROBIOLOGY, Jan., 1966

Vol. 14, No. 1
Printed in U.S.A.

Algicidal Effect of Bromine and Chlorine on *Chlorella pyrenoidosa*

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Received for publication 17 May 1965

ABSTRACT

KOTT, YEHUDA (Israel Institute of Technology, Haifa, Israel), GALILA HERSHKOVITZ, A. SHEMTOB, AND J. B. SLESS. Algicidal effect of bromine and chlorine on *Chlorella pyrenoidosa*. Appl. Microbiol. **14**:8–11. 1966.—*Chlorella pyrenoidosa* was found to grow rapidly in tap water. Peak growth was reached after 2 to 3 days. Chlorine and bromine, added to such water, were shown to be effective inhibitors of algal growth. Bromine and bromamine were primarily algicidal, whereas chlorine and chloramines were mainly algistatic. It is assumed that the mechanisms of action of these halogens on *Chlorella* are not the same.

Of the halogen group, only bromine and chlorine are used for disinfection of water. Chlorine is used extensively in water treatment plants; however, the use of bromine is limited to disinfection of swimming pools, and even then is practiced only on a small scale (11).

The bactericidal effects of both halogens have been investigated in a large number of studies (5, 6, 15, 16). From these, it is evident that lower concentrations of bromine than of chlorine are necessary to obtain maximal kill of bacteria. This is attributed to the fact that bromine introduced to water produces bromamines, which are much more active than chloramines (4, 9–11, 13).

The algicidal effects of chlorine have been studied by a number of investigators (7, 12); however, the algicidal effects of bromine have received little attention. In the literature, there is only one reference on the effect of bromine on algae (1). Complete kill of a filamentous alga growing in a water-cooling tower was achieved, whereas treatment with chlorine was ineffective.

Since, in public water supplies, pollution is caused by both algae and bacteria, considerable interest should be attached to the use of a disinfectant which has maximal bactericidal and algicidal effects.

The purpose of this study was to compare the algicidal effects of chlorine and bromine on *Chlorella pyrenoidosa* under laboratory conditions.

MATERIALS AND METHODS

Source of culture material. The algal species *C. pyrenoidosa* (a strain isolated by the Sanitary Engineering Laboratories) was selected for the study. Algal cultures were grown in 50-ml flasks in Allen's (2) medium at a temperature of 25 to 28 C and with a fluorescent illumination of 1,500 lux at the surface of the flasks.

Algicidal effects of halogens. Fresh cultures of the algae, not more than 10 days old, were used for inoculation of 100-ml Erlenmeyer flasks and 1- and 7-liter containers.

Chlorine and bromine were added separately in various concentrations and under different conditions as described below. The effects of the halogens were determined at hourly or daily intervals by counting in a hemacytometer. Algal samples were collected after thorough stirring, and counts were made either immediately or the following day on samples preserved in 4% formaldehyde.

The bioassay was carried out according to Fitzgerald's (8) procedure. Local tap water (25 ml), to which 120 mg/liter of $NaNO_3$ and 25 mg/liter of K_2HPO_4 had been added to promote algal growth, were introduced to 100-ml Erlenmeyer flasks. (The chemical analysis of the tap water used is given in Table 1). To these, 1 ml of algal culture was added so as to give an initial concentration of 300 cells per cubic millimeter. Sodium hypochlorite or sodium hypobromite was added to these flasks so as to give final concentrations ranging from 0.18 to 0.42 mg/liter of the halogen. Flasks similarly inoculated with algae, but without halogens, served as blanks. Algal concentrations were determined 24 hr later, and every day subsequently for a period of 5 days.

Seven-liter containers were filled with tap water, and algae were inoculated in each vessel to provide an initial concentration of approximately 100 cells per cubic millimeter. Hypobromite or hypochlorite was then added once a day to give a 0.5 mg/liter residual concentration. Chlorine and bromine concentrations were determined daily by use of a comparator (W. A.

TABLE 1. *Analysis of Technion tap water**

Component	Amt	Form
	mg/liter	
Fluorides	0.05	F
Chlorides	141.8	Cl
Sulfates	20.2	SO_4^{-2}
Nitrates	14.1	NO_3^-
Nitrites	0.007	NO_2^-
Bicarbonates	390.2	$CaCO_3$
Phosphates	0.032	PO_4^{-3}
Copper	0.003	Cu
Iron	0.065	Fe
Calcium	93.7	Ca
Magnesium	37.2	Mg
Potassium	2.7	K
Sodium	74.5	Na

* This analysis was carried out by the chemical laboratory of "Mekoroth" Water Co. The $KMnO_4$ demand of this tap water was 590 ml of 0.01 N $KMnO_4$ per liter. The conductivity was 1,111 μmho/cm.

TABLE 2. *Effect of various concentrations of halogens in bioassay flasks on Chlorella pyrenoidosa**

Time	Control flasks	Bromine (ppm)			Chlorine (ppm)		
		0.18	0.24	0.42	0.18	0.24	0.42
days							
0	300	300	300	300	300	300	300
1	397	473	480	26	190	145	14
2	1,620	1,670	1,920	74	730	1,850	100
3	2,110	1,680	1,820	146	1,110	2,500	163
4	2,383	2,100	1,850	270	1,850	2,750	500
5	2,510	—	1,840	270	1,900	—	530

* Results are expressed as the number of *Chlorella* cells per cubic millimeter.

Taylor and Co., Baltimore, Md.) under standard conditions (3). The halogens were added after algae sampling so that the initial concentration of halogens was maintained. Algal concentrations were determined every 24 hr for 6 days.

Tests in 1-liter vessels were carried out in order to follow the algicidal effects of the halogens both in free and combined form at frequent time intervals over 24-hr periods; for the same purpose, 5-liter vessels were filled with buffered tap water at *p*H 7.0, and NH_4Cl was added. Algae were inoculated so as to give initial concentrations of 140 to 150 cells per cubic millimeter in each of these vessels. Bromine and chlorine, as hypobromite and hypochlorite, were added to provide 0.4 mg/liter of combined or 1.0 mg/liter of free halogen. Also, a control vessel was inoculated to follow algal growth. Algal concentrations were determined at time intervals of 1 to 3 hr.

Determination of volatility of halogens. Tap water (6.5 liters) was placed in six two-gallon (7.5-liter) cylindrical Pyrex jars in an outdoor location exposed to sunlight and wind; the temperature of the water was 34 C. Halogens were added to each of the vessels so that three of the vessels contained 0.5 mg/liter of chlorine, and three contained 0.5 mg/liter of bromine. The degree of volatility was determined by measuring the concentration of the halogens every 10 minutes for 2 hr.

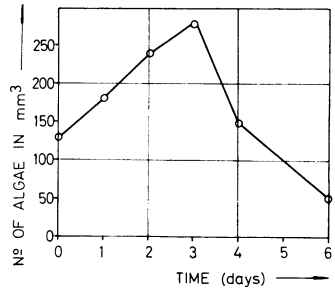

FIG. 1. *Growth of Chlorella pyrenoidosa in tap water.*

RESULTS

Results of bioassay experiments (Table 2) showed that, in the lower concentrations, chlorine had an inhibitory effect in the first 48 hr whereas bromine had none. Thereafter, algal growth was of the same order as that recorded in the control vessels. With concentrations of 0.42 mg/liter bromine had a markedly more inhibitory effect than chlorine; at higher concentrations, complete algal kill was obtained with both halogens.

In control vessels, algal concentrations increased eightfold in 5 days. Since such development does not normally occur in reservoirs, it was considered desirable to examine this phenomenon. Tap water was used as a culture medium to provide the same nutritive elements as are found in nature.

Results of *Chlorella* growth in tap water (Fig. 1) show that logarithmic reproduction continued for 3 days, at which time the algae reached maximal growth; thereafter, concentrations declined, apparently because of depletion of nutrients.

In the control vessel, maximal algal growth also occurred after 3 days of incubation, and subsequent decline in concentrations was recorded (Table 3). Jars to which 0.5 mg/liter of chlorine were added showed a slight decline in algae concentrations, in contrast to beakers in which the same concentration of bromine was added daily. A gradual progressive decline in algal concentrations was recorded.

The disinfecting efficiency of these halogens was measured also by their volatility in water. Although there was not very much difference in the volatility of these halogens under experimental

TABLE 3. *Effect of bromine and chlorine on chlorella in 7-liter vessels of tap water**

Time	Control	Chlorine	Bromine
days			
0	130	120	120
1	180	100	90
2	240	120	100
3	280	180	90
4	150	80	50
5	140	100	40
6	50	80	50

* Chlorine and bromine were added daily at a level of 0.5 ppm. The control received no halogen. The results are expressed as the number of *Chlorella* cells per cubic millimeter.

TABLE 4. *Volatility of chlorine and bromine in 6.5-liter vessels*

Contact time	Residual bromine (ppm)			Residual chlorine (ppm)		
	1*	2	3	1	2	3
min						
0	1.0	1.0	1.0	1.0	1.0	1.0
10	0.6	0.7	0.7	0.7	0.6	0.6
20	0.4	0.5	0.6	0.6	0.4	0.3
30	0.3	0.4	0.4	0.3	0.3	0.2
40	0.2	0.2	0.2	0.2	0.2	0.1
50	0.2	0.2	0.2	0.2	0.1	0.1
60†	0.2	0.1	0.2	0.1	0.1	0.1
70	0.2	0.1	0.2	0.1	0.1	0
80†	0.1	0.1	0.2	0	0	0
90†	0.1	0.1	0.1	0	0	0
120†	0.1	0.1	0.1	0	0	0

* Vessel number.
† The water was stirred.

conditions, residual bromine was detected for a longer period (Table 4).

The various results obtained showed that there is no major difference in activity between these halogens. However, as these halogens are known to be highly reactive, it was suspected that a 24-hr sampling program would not enable detection of an immediate effect of the halogens on *Chlorella*. It therefore seemed desirable to investigate the effect of a short contact time, and to compare the effects of free available halogens and combined residual halogens.

The results obtained with 0.4 ppm of combined residual chlorine and bromine show that bromamines had a much more pronounced effect on the algae than did chloramines (Fig. 2). The number of *Chlorella* cells dropped almost immediately, and progressively decreased up to 10 hr after the halogen was introduced. Thereafter, the algae

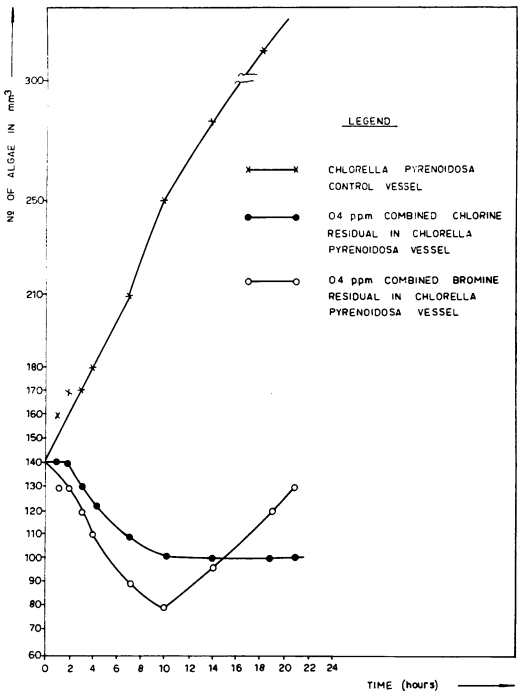

FIG. 2. *Effect of 0.4 ppm of combined residual chlorine and bromine on Chlorella pyrenoidosa.*

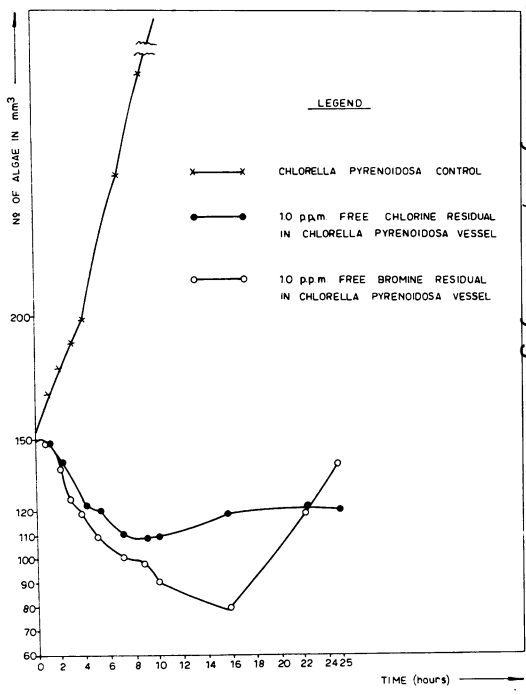

FIG. 3. *Effect of 1.0 ppm of free residual chlorine and bromine on Chlorella pyrenoidosa.*

began to multiply as if they had entered the logarithmic phase.

The number of algae in the experimental vessel 22 hr after bromine was added was less than at the start. Observations of chloramine action on the algae show that, although the number of algae decreased less than with bromine, the effect was more prolonged; 22 hr after the halogen was added, the number of algae was less than in the bromine vessel. Similar results were obtained when *C. pyrenoidosa* was exposed to free halogens under the same experimental conditions (Fig. 3). The effect of 1.0 ppm of bromine on algae kill was much more apparent in the first 16 hr, during which the number of algae dropped steadily. After this period, new algal growth followed, and, 24 hr after the start of the test, the number of algae in the experimental container was only slightly less than at the beginning of the experiment. The effect of chlorine on algae kill was similar to that of bromine during the first 5 hr of the test. But, later, chlorine acted as an algistat; 24 hr after the start of the test, the number of algae was much less than at the beginning, in comparison with 1.0 ppm of bromine.

DISCUSSION

The experiments described show that *C. pyrenoidosa* is able to grow readily in tap water. The fact that the algae grew logarithmically in tap water for 3 days enabled the experimental conditions to closely simulate nature. The 3-day growth span also limited the length of each experiment to less than 3 days.

The results obtained from daily sampling of the chlorine and bromine vessels showed no drastic difference in algicidal effect. However, the results of hourly sampling showed that there is a clear difference in mode of action of the two halogens. Bromamines and free bromine have an algicidal effect, with the number of algae dropping very rapidly as long as the bromine is active. At the moment that bromine is unavailable, the algae resume reproduction in logarithmic phase. Chloramines and free chlorine have a much lower killing effect, but act as a long-term algistat.

From the above discussion, it can be inferred that results obtained from daily sampling in small-scale tests might lead to a wrong interpretation, especially when applied to continuous unit operations.

By maintaining a constant level of 0.2 ppm of bromamines, it would be possible to kill algae in water. By using 0.2 ppm of free chlorine, one might expect to keep the population of algae constant, but not to reduce their numbers appreciably.

In aqueous solution the volatility of bromine is less than that of chlorine. The fact that bromine acted as an algicide and chlorine as an algistat suggests that there are two different mechanisms of inhibition. It therefore seems to be desirable to investigate the mechanisms of action of these halogens on *C. pyrenoidosa*.

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