DISINFECTION OF SEA WATER BY
ULTRAVIOLET RADIATION

by

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by the
RESEARCH DEPARTMENT
SALT WATER FISHERIES DIVISION
FLORIDA BOARD OF CONSERVATION
TALLAHASSEE, FLORIDA

February 1965

Data from experiments in artificially and naturally
polluted sea water indicate that ultraviolet radiation
effectively destroys coliform organisms. The unit
developed for this purpose is described and the results
obtained are analyzed.

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The use of ultraviolet radiation for the disinfection of water has
been investigated primarily for its applicablity to fresh waters, although
in recent years investigations with sea water have been conducted in Japan
and in England, particularly in connection with water to be used for the
purification of shellfish. Studies on fresh water have been directed
primarily toward the sterilization of drinking water and swimming pools,
but the method has also been evaluated for the disinfection of small
containers used for dispensaries and surgeries and for biochemical products
when heat treatment would have an adverse effect.

Our interest in the ultraviolet treatment of sea water arose from
the need for a continuously flowing supply, having a uniformly low bacte-
rial content, for study of the accumulation and elimination of enteric
bacteria in oysters.

Luckiesh and Holladay[1], present fundamental data on the radiation
requirements for disinfection of fresh waters of specified physical and
chemical characteristics. The factors which influence the quantity of
radiation required were found to be those which affect the penetration of
ultraviolet energy through the water. Turbidity, color, and dissolved
iron salts were found to be among the naturally occurring qualities which
had the greatest effect on the absorption coefficient of the water.
Gillcreas and De Lalla[2] determined the efficiency of a commercial device in
treating Albany tap water from a surface supply and studied the inter-
ference of added color, turbidity, and colloidal iron compounds. They
found that with turbidity of 85 ppm, iron 3 ppm, color (inorganic) 90 ppm,
or with turbidity of 15 ppm, iron, 5 ppm, and color 150 ppm, effective
destruction of coliform organisms was accomplished at the rated capacity
of the equipment.

As to the relative efficiency of ultraviolet light in destruction
of micro-organisms other than those of the coliform group, Chaumeau[3] lists
the following comparative exposure times required for the destruction of
some common micro-organisms: staphylococci, 5-10 seconds; cholera, 10-15;
coli, 15-20; typhoid, 10-20; dysentery, 10-20; pneumobacilli, 15-30;
subtilis, 30-40; tetanus, 20-60. Buttolph, Haynes, and Matelsky[4] give the
following radiation requirements, in terms of microwatts per square centi-
meter to kill 63.2 per cent in one minute (1 leth): Alpha streptococci
and Staphylococcus aureus, 1.3; Beta hemolytic streptococci and Escherichia
coli, 5.3; molds, 10; and mold spores typical, 28. Kawabata and Harada[5]
reported on the relative resistance of four groups of typical micro-
organisms in aqueous suspension in a unit utilizing a 15-watt germicidal
lamp delivering an ultraviolet intensity of 90 microwatts per square
centimeter.

Gram-negative bacteria were found to be the least resistant. The time required to kill 99.9 per cent for gram-negative strains was found to be as follows: Proteus sp., 42 sec; Shigella sp., 47; Eberthella typhosa, 49; E. coli, 60. Gram-positive organisms had the following requirements: Group A Streptococcus, 83 seconds; Staph. aureus, 103; S. fecalis, 165; Bac. subtilis sawamura 240; Bac. subtilis spores 369. The resistance of yeasts was slightly greater, Saccharomyes sake requiring approximately 217 seconds and Willia anomala 420 seconds.

Pilot plant studies were conducted on sea water by Public Health and Fisheries Authorities of Hiroshima[6] in an apparatus designed to investigate methods for the purification of the Pacific oyster, Crassostrea gigas, using three germicidal units similar in design to that of Kawabata and Harada,[5] with rather high rates of flow however. Water from a tank containing oysters was recirculated to provide a replacement of about one-half of the total volume of the tank per hour. A reduction of approximately 90 per cent in coliform density was accomplished in one passage through the ultraviolet unit. Pilot scale studies on the efficiency of ultraviolet radiation in the sterilization of sea water were also conducted by Kamimura and Suzuki[7] at Matoya Bay, Japan, utilizing a unit provided with a total of six 15-watt germicidal lamps. Flow through the unit was at the rate of 12 gallons per minute. Approximately 90 per cent of the bacteria were killed by a 15-second exposure.

In all of the semicommercial size units designed for the purification of shellfish, including one recently developed in England,[8] there appears a similar principle. The ultraviolet units are of such capacity that by recirculation of the water there will be accomplished a destruction of bacteria concurrent with the use of the water for purification of shellfish. The water is not completely sterilized in the initial passage through the treatment system. The rate of destruction, although slow, accomplishes purification of the sea water to a low acceptable coliform level by continuous recirculation during the 24-hour period of treatment, and the water presented to the oyster some four to six hours prior to the completion of the treatment period is practically zero in coliform content.

The principle is not acceptable in American practice. The Public Health Service Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry[9] stipulates that water used for purification of shellfish, if treated,[10] should meet the Public Health Service Drinking Water Standards. Thus, the coliform density of the water must be such that not more than 10 per cent of the 10 milliliter portions tested shall show the presence of organisms of the coliform group. Occasionally three or more of the 10 milliliter portions may be positive for coliform organisms, provided that this density shall not occur in more than 5 per cent of the samples when 20 or more of the samples are examined per month. To meet this bacteriologic requirement, the ultraviolet treatment unit would need to be of much greater killing power than those used in commercial or semicommercial establishments in foreign countries.

During the course of laboratory experiments on shellfish conducted at Pensacola, Fla., and Purdy, Wash., we have had occasion to design and to evaluate ultraviolet treatment units of a high degree of efficiency in the sterilization of sea water. The units were designed primarily for use in laboratory experiments to determine the rate of bacterial accumulation by shellfish. In these experiments, oysters in laboratory tanks were furnished flowing sea water artificially polluted by the continuous addition of suspensions of coliform organisms. Samples of water and oysters were examined periodically for coliform density and from these tests the rate and extent of accumulation of bacteria by the oysters were determined. Obviously, a requirement for the proper operation of these studies was a constant load of coliform organisms in the water. Since the sea water proved to be extremely variable in coliform content it became necessary to treat the water to reduce the native coliform content to a constant level. Reduction to zero density was considered to be the easiest and most practical.

Preliminary experiments indicated that chlorination resulted in the production of a water not acceptable to the oysters. Studies at Pensacola[11] demonstrated that the feeding activity of oysters was adversely affected by chlorination and dechlorination of sea water. Therefore, other methods of removal or destruction of coliform organisms were considered. In an effort to avoid the harmful effects of chemicals on the activity of the oysters, it was decided to evaluate ultraviolet radiation. After having first demonstrated that ultraviolet treatment of sea water did not produce the same objectionable effect as chlorination and dechlorination, the bactericidal efficiency of the treatment units were determined.

MATERIALS AND METHODS

Description of Ultraviolet Treatment Units

The ultraviolet treatment units used in these studies were of two different designs. The unit used in the studies at Pensacola is a modification of an apparatus suggested by Buttolph[4]. It consists of a bottom section, essentially an open trough through which the water passes, and a top section, a reflector unit housing the ultraviolet lamps. The trough is 36 inches long, 8 inches wide and 6 inches high, with a longitudinal baffle 2 1/2 inches high placed along the center line of the trough. Intake and discharge pipes, 2 inches in diameter, are placed on the long sides of the tank at diagonally opposite corners.

The lamp housing, fitting on the trough, is a semicylindrical archshaped reflector, lined with specular finish aluminum material. The unit is provided with two 30-watt ultraviolet germicidal lamps, placed so that the lamps are approximately one-half the distance from the lower edges to the center line of the reflector. When the housing is in place on the trough, the distance from the center of the lamps to the water level is 6 1/8 inches.

Preliminary experiments with the ultraviolet treatment unit indi-
cated that at least 150 liters (39.6 gallons) per minute could be treated
with a high degree of destruction of coliform organisms. At this rate of
flow the theoretical detention time in the treatment trough is 20 seconds,
and the actual detention time as determined by dye tests is 15 seconds.
The amount of ultraviolet energy then reaching the water at this rate of
flow is 960 microwatts per square centimeter per minute. We realize that
this is considerably in excess of the design data furnished by some of the
manufacturers as well as the intensities prevailing in other installations.
However, as previously mentioned we were interested in accomplishing as
near 100 per cent kill as possible, with an extremely high degree of
confidence.

RESULTS

Influence of Chlorination of Sea Water on Feeding Activity of Oysters.

Experiments at Pensacola[11] demonstrated that chlorinated and
dechlorinated sea water had an adverse effect on feeding activity of the
eastern oyster, Crassostrea virginica. Similar studies conducted at Purdy
with the Olympia oyster, Ostrea lurida, demonstrated the same effect. Com-
parative activity of Olympia oysters furnished untreated and chlorinated,
dechlorinated sea water is presented in Table 1. Chlorine dose was
sufficient to produce a residual of 0.2 to 0.5 ppm after five minutes
contact and the water was dechlorinated with an excess of sodium thiosul-
fate. Activity was determined by the presence of fecal deposits. The
adverse effect of chlorination can be seen by the distinct difference
between per cent activity observed in the "treated" and "untreated" flats.

Similar experiments, using ultraviolet treated water, demon-
strated that this method of treatment did not produce the same effect.
The results of a series of four experiments are presented in Table 2.
It can be seen that there was little if any difference in the per cent
activity between oysters receiving treated and untreated water.

Germicidal Efficiency of UV Treatment Units.

The efficiency of the units at Pensacola was first determined
in preliminary experiments with artificially polluted sea water. Sea
water was seeded with sufficient cotton-filtered raw sewage to produce
coliform loads up to MPN 7,900 per 100 ml. The sewage was introduced
continuously into the sea water by means of a proportionating pump. The
polluted sea water was then allowed to flow through the ultraviolet treat-
ment units at the rate of 40 liters per minute. Twenty-five sets of
samples were examined during the course of experiments conducted on three
days. The units consistently reduced the coliform density of the water
to less than 1.8 organisms per 100 ml (negative in five 10 ml volumes
tested). The raw water coliform MPN's ranged from 79 to 7,900, with a
median of 2,200 per 100 ml.

Table 1--Influence of Chlorinated and Dechlorinated Sea Water on Feeding
Activity of Olympia Oysters

Treatment of Water for Test Tank	Test Period Hours	Water Temp. ° C	Per cent activity	
			Treated	Untreated
Chlorination and dechlorination; water flowing through	12	9	21	57
	11	8	29	50
	31	8.5	30	57
	23	8	40	71
None (both tanks supplied untreated water)	21	8	59	60
	23	8.5	66	65

Table 2--Influence of UV Treated Sea Water on Feeding Activity of Olympia
Oysters

Test Period Hours	Water Temp. °C	Per cent activity	
		Treated	Untreated
23	10	91	88
22	10	90	87
22	9	90	88
34	9	94	96

The apparatus used at Purdy was also evaluated prior to operating
the unit in the routine experiments. The results of these tests are pre-
sented in Table 3. Five series of tests were conducted. As in the experi-
ments at Pensacola, the sea water was seeded with cotton filtered raw
sewage to produce the coliform densities shown. The Purdy experiments,
however, were augmented to allow the collection of more information
concerning the efficiency of this method of sterilization. In the coliform
determinations, combinations of 100, 10, and 1 ml portions, five replicates
per dilution, were planted in lauryl sulfate broth. In the Pensacola
experiments the largest portion planted was 10 ml. In addition to the
coliform tests, plate counts at 35°C and at 20°C were determined on the raw
water and the treated water.

Table 3--Bactericidal Efficiency of Purdy Ultraviolet Treatment Unit

Exp. No.	Flow L/Min	Lamps	Raw Water			Treated Water			Per cent Reduction		
			Coliform MPN per 100 Ml	Plate Count Per Ml 35°C	Plate Count Per Ml 20°C	Coliform MPN Per 100 Ml	Plate Count Per Ml 35°C	Plate Count Per Ml 20°C	Coliform MPN per 100 Ml	Plate Count Per Ml 35°C	Plate Count Per Ml 20°C
1	60	8	390	49	160	<0.18	2	3	>99.95	95.9	98.1
2	60	8	600	210	220	<0.18	0.6	1.2	>99.97	99.71	99.4
3	100	8	510	27	200	<0.18	4	14	>99.96	85.2	93.0
4	100	8	580	150	290	<0.20	1.4	7	>99.96	99.1	97.5
5	100	13	280	78	740	<0.18	0.2	0.5	>99.94	99.74	99.93

The 35°C plate counts were conducted on tryptone glucose extract agar, incubated for 24 hours. The 20°C plate counts were conducted on MacLeod's[12] basal medium incubated for five days. Water flow through the unit was maintained at 60 liters per minute in two of the experiments and at 100 liters per minute in three. The experiments conducted with eight lamps at a flow of 100 liters per minute showed a lower per cent removal of plate count, although the destruction of coliform organisms appeared to be not affected by the higher rate of flow. In experiment 5, the UV radiation was increased by the addition of five lamps and the results indicate that this added radiation produced exceedingly high reductions in 35° and 20° plate counts as well as in coliform organisms.

In an attempt to determine the bacterial types that were resistant to the ultraviolet radiation, identification was made of the types of organisms present in colonies on the tryptone glucose agar and the MacLeod's agar plates inoculated with ultraviolet treated water. Thirty such cultures were examined. Nineteen were found to be Pseudomonas, two were Achromobacteria, one Flavobacterium, one Micrococcus, and one Cytophaga. Although cultures from the untreated water were found to contain Bacilli and Vibrio in addition to the above, the latter two bacterial types were not isolated from the ultraviolet treated water.

Results of Pilot Plant Studies.

Information on the efficiency of UV treatment under pilot plant conditions was obtained during the course of experiments on the accumulation of bacteria by oysters. In the Pensacola experiments, the performance of the unit was tested periodically by the examination of samples of water, as discharged from the treatment unit and water and oysters in a tank used as a control, which was fed UV treated

water. A summary of the results obtained in 18 experiments, conducted during a period of nine months, is presented in Table 4.

Of the 176 samples of UV treated effluent, 98.3 per cent showed the absence of coliform organisms from three 100 ml portions, equivalent to MPN less than 0.41. Two, or 1.2 per cent, of the samples showed one of the three 100 ml portions to be positive for coliform organisms and one showed an MPN of more than 0.41 per 100 ml. Of the 257 samples of water examined from control flat C, 95 per cent showed the absence of coliform organisms from five 10 ml volumes, 3.1 per cent showed coliform MPN's between 2.0 and 4.5 (one or two 10 ml portions positive) and 1.9 per cent showed coliform MPN's in excess of 7.8. Oysters in the control tank were also examined. More than threequarters of 257 samples tested showed the absence of coliform organisms from 1 gm quantities, equivalent to MPN less than 18, and 95 per cent showed coliform densities of less than 45.

At Purdy, the comparative raw water and UV treated water MPN's were determined during the course of eight experiments on the rate of accumulation and elimination of bacteria by oysters conducted from November 1959, to June, 1960. The results are shown in Table 5. A total of 95 samples of raw water were tested for coliform density. The coliform MPN of the raw water ranged from less than 1.8 to 2,400 and the geometric mean was 32. All but four of the 95 samples of the effluent from the ultraviolet treatment system collected simultaneously showed the absence of coliform organisms from five 100 ml portions, equivalent to an MPN of less than 0.18. Each of the four samples showing positive coliform results were found to have one of the five 100 ml portions positive, equivalent to an MPN of 0.2 per 100 ml.

During the same experiments, 78 samples of water were taken from the control tank containing oysters and receiving ultraviolet treated water. The distribution of coliform MPN's is shown in Table 5. Seventy-three, or approximately 94 per cent, showed the absence of coliform organisms from 100 ml volumes. Of the remaining five samples, two were at MPN 0.2 and one each at MPN 0.68, 2.3 and greater than 13.

The turbidity of 91 raw water samples was determined by comparison with standard turbidity suspensions prepared as described in Standard Methods.[13] To test the effect of turbidity on the efficiency of ultraviolet radiation, the samples were sorted into four turbidity ranges; i.e., zero to trace, 1 to 5, 6 to 10, and 11 to 20. From these plots the average and 90 per cent probability range of occurrence were derived.

The ranges of coliforms MPN of the raw water and the geometric averages at three turbidities are presented in Table 6. A progressive increase in coliform MPN, parallel with the increase in turbidity, can be seen. The results obtained on the samples of treated water are also presented in Table 5 in terms of percentages of samples in each turbidity group showing coliform MPN's less than 0.18, 0.2, and greater than 0.2.

Table 4--Coliform MPN's of ultraviolet Treated Sea Water and of Water and Oysters from Control Flat C.

	Water, UV Effluent MPN			Water, Flat C MPN			Oyster, Flat C MPN			
	<0.41	0.41	>0.41	<1.8	2.0-4.5	>7.8	<18	20-45	78-490	>490
No.*	173	2	1	244	8	5	200	45	11	2
% +	98.3	1.2	0.5	95	3.1	1.9	77.5	17.5	4.3	0.8

*Number of samples showing stated coliform MPN's or less.
+Per cent of total samples of each type examined.

Table 5--Coliform MPN of UV Treatment Tank Effluent and Water in Control Oyster Tank

MPN per 100 Ml	Number of Samples		Per Cent		Cumulative Per cent	
	UV Treated	Tank C	UV Treated	Tank C	UV Treated	Tank C
<0.18	91	73	95.8	93.6	95.8	93.6
0.20	4	2	4.2	2.56	100.0	96.1
0.68	0	1	- -	1.28		97.4
2.3	0	1	- -	1.28		98.7
>13	0	1	- -	1.28		100.0
Total samples	95	78				

The effect of turbidity on the germicidal efficiency of the treatment unit can be seen only from the increase in percentage occurrence of MPN 0.2 with increases in turbidity. It should be noted that, although coliform organisms were recovered from treated water samples, the highest density determined was 0.2 per 100 ml, even in the presence of turbidities up to 20 ppm.

DISCUSSION

The use of chlorine for the sterilization of sea water in shellfish purification plants is contraindicated, since it has been established that chlorine has an adverse effect on the feeding activity of both the Eastern oyster and the Olympia oyster. Further, the experiments have demonstrated

Table 6---Effect of Turbidity on Efficiency of UV Treatment Units

Turbidity Range	Number of Samples	Raw Water Coliform MPN/100 Ml		UV Treated Water Per Cent of Samples Showing Coliform MPN		
		Average	Range	<0.18	0.2	0.2+
0-trace	40	17	<1.8- 330	100.0	0.0	0
1-5	24	17	<1.8- 240	92.0	8.0	0
6-10	18	61	7.8- 920	94.4	5.6	0
11-20	9	160	23.0-2400	87.5	12.5	0
0-20 (All samples)	91	32	<1.8-2400	95.8	4.2	0

that dechlorination does not remove the factor which influences the feeding activity. On the other hand, it has been demonstrated that ultraviolet treated water does not have a similar effect and there is experimental evidence to demonstrate that oysters will purify themselves in such water. Turbidities of up to 20 ppm did not seriously interfere with efficiency of the Purdy ultraviolet treatment unit. Obviously, a water containing gross particles of particulate matter should be clarified by settling to be acceptable for ultraviolet treatment.

There are at the present time means for measuring the efficiency of operation of the ultraviolet lamps. A germicidal watt meter is available to measure the direct ultraviolet output of the lamps. Instruments for measuring ultraviolet intensity at short distances from bare tubes such as might be desired in ultraviolet treatment units are available and more sensitive meters can be obtained to measure the intensity at greater distances. All can be combined with suitable electronic recording devices to produce a continuous record of the UV energy applied to the water.

SUMMARY

Data from results of experiments on both artificially polluted sea water, and on sea water containing coliform organisms up to a density of 2,400 occurring naturally in the water, indicate that ultraviolet radiation is an effective means of destruction of coliform organisms. The unit developed for the treatment of sea water in the laboratory experiments on oysters at Purdy, Wash., is of a design and capacity sufficient to evaluate the applicability of ultraviolet radiation in at least small scale commercial installations. The unit accomplished more than 99.96 per cent reduction of coliform organisms from a sea water seeded with sewage and having an average coliform MPN of as high as 580 per 100 ml. Percentage reduction in 35° and 20° plate counts were of the same order.

Pilot scale studies conducted over extended periods have demonstrated that, with proper maintenance, the unit will operate at a high degree of dependability. With raw water ranging in coliform MPN's up to 2,400 per 100 ml, and turbidity up to 20 ppm, only 5 per cent of 91 samples of UV treated water examined during a period of eight months showed the presence of coliform organisms in 100 ml volumes tested. The maximum MPN of the treated water was 0.2 per 100 ml. The maintenance requirements of these units are of the simple housekeeping nature. For proper operation the units should be flushed periodically and the lamps and reflectors cleaned to remove accumulated deposits.

REFERENCES

1. Luckiesch, M., and Holladay, L. L. Disinfecting Water by Means of Germicidal Lamps. General Electric Rev. 47:45-50, 1944.

2. Gilcreas, F., and DeLalla, L. Application of Ultraviolet Ray Sterilization to Water Treatment. New England Water Works A. (June), 1953, pp. 130-139.

3. Chaumeau, R. Sterilisation De L'Eau par les Radiations Ultra-violettes. L'Eau 46:263-264 (Nov.), 1959.

4. Buttolph, L.; Haynes, H.; and Matelsky, I. Ultraviolet Product Sanitation. Bull. LD-14, Cleveland, Ohio: General Electric Co., 1953.

5. Kawabata, T., and Harada, T. The Disinfection of Water by Germicidal Lamp. J. Illumination Soc. (Japan). 36:89-96 (Mar.), 1959.

6. Okinami, M.; Sakai, M.; Kurata, Y.; Fujita, T.; Massaubara, T.; and Furno, H. Public Hygienic Study of Cultured Oyster on Hiroshima Bay. The Artificial Purificatory Experiment of Oyster. Hiroshima Prefectural Public Health Section. Rep. No. 4, 1952.

7. Kamimura, M., and Suzuki, N. The Investigated Report on the Purification Facility of Cultured Oyster Institute. Sato Oyster Nursery at Matoya Bay Japan Export Frozen Marine Products Inspection Corp., 1954.

8. Wood, P. C. The Utilization of Ultra-violet light in the Purification of Oysters. International Council for the Exploration of the Sea. C. M. 1959, Shellfish Committee, No. 41, 1959.

9. Sanitary Control of the Shellfish Industry. 1959 Manual of Recommended Practice. Part I: Growing Areas, p. 21. Washington, D. C.: Department of Health, Education, and Welfare, PHS, 1959.

10. Public Health Service Drinking Water Standards. Pub. Health Rep. 61:11 (Mar. 15), 1946.

11. Kelly, C. B.; Arcisz, W.; Presnell, M. W.; and Harris, E. K. Bacterial Accumulation by the Oyster Crassostrea Virginica on the Gulf Coast. Cincinnati, Ohio: Robert A. Taft Sanitary Engineering Center, Tech. Rep. F60-4, 1960.

12. MacLeod, R. A.; Onofrey, E.; and Norris, M. E. Nutrition and Metabolism of Marine Bacteria: I. Survey of Nutritional Requirements. J. Bact. 68:680-686, 1954.

13. American Public Health Association. Standard Methods for the Examination of Water, Sewage, and Industrial Wastes(Tenth Ed.). New York, N. Y.: The Association, 1955.