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Survival of Poliovirus in Flowing Turbid Seawater Treated with Ultraviolet Light

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The effectiveness of a model ultraviolet (UV) radiation unit for treating flowing turbid seawater contaminated with poliovirus was determined. At a turbidity of 70 ppm, the observed survival ratios ranged from 1.9×10^{-3} (99.81% reduction) to 1.5×10^{-4} (99.98% reduction) at flow rates ranging from 25 to 15 liters/min; no virus was recovered at flow rates of 10 and 5 liters/min. At a turbidity of 240 ppm, the observed survival ratios ranged from 3.2×10^{-2} (96.80% reduction) to 2.1×10^{-4} (99.98% reduction) at flow rates ranging from 25 to 5 liters/min. As expected, turbidity had an adverse influence on the effectiveness of UV radiation; however, by adjusting the flow rate of the seawater through the treatment unit, adequate disinfection was shown to be predictable.

In studies undertaken to develop an artificial purification system for shellfish (referred to as depuration) in which natural seawater is used, each component of the system must be tested for its effectiveness. One such component is the seawater treatment unit, since seawater to be used in a depuration system must be adequately disinfected. The treatment of choice presently under consideration is ultraviolet light (UV).

In 1961, Kelly (8) described a UV radiation treatment unit designed for the purpose of disinfecting flowing seawater polluted with raw sewage. The use of this unit was also in connection with the depuration of shellfish. Evaluation of the unit by Kelly showed that the unit was not only highly efficient but also that UV radiation was an effective means for destruction of coliform organisms. This UV treatment unit has not been evaluated as to its ability to inactivate viruses in flowing seawater. Consequently, a study was undertaken to ascertain the effectiveness and predictability of a model UV seawater treatment unit for inactivating poliovirus. This unit was similar in design to the unit described by Kelly. Experimentally, the tests were set up to study the efficacy of the UV unit when the seawater exhibited a high turbidity. Natural turbidity in seawater is caused by the presence of suspended material coming from the bottom sediments as a result of wave action or from material transported by fresh water runoff. This heterogeneous material, composed of both organic and inorganic particulate matter, occurs more often than not in estuarine waters adjacent to the Gulf of Mexico. This paper reports the results of these experiments.

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MATERIALS AND METHODS

Virus. The LSc2ab strain of poliovirus type 1 was used throughout the study. The virus was propagated in HEp2 cells. The virus stock was stored at −70 C until used.

Cell culture. HEp2 cells were used for plaque assays. The cells were grown in 32-oz (about 900 ml) screw-cap prescription bottles as a monolayer in a growth medium consisting of Eagle's basal medium (BME) and 10% calf serum with penicillin (100 units/ml), streptomycin (100 μg/ml), neomycin (200 mg/ml), and amphotericin B (1.0 μg/ml). Cell overlay medium was a maintenance medium consisting of BME and 5% chicken serum with 0.0017% neutral red, 1.1% purified agar (Difco), and the above-named antibiotics.

Plaque assay. The plaque assay procedure of Dulbecco and Vogt (3), as modified by Hsiung and Melnick (7), was used throughout the study. Plaques were permanently marked and counted daily as described by Berg et al. (2). Cell monolayer bottles exhibiting the greatest number of plaques short of overcrowding were recorded and used for computations. For purposes of assay, serial 10-fold dilutions of virus were made in Hanks' balanced salt solution, and 1 ml of virus at each dilution level in duplicate was inoculated onto three cell monolayer bottles [3-oz (about 85 ml) prescription size].

UV treatment unit. The UV treatment unit (Fig. 1) consisted of two 30 amp UV bulbs (91 cm long) running side by side, 11.4 cm apart, over a chamber 91 cm long and 30.5 cm wide. Six wooden baffles (2.5 cm high) were inserted at 15.2-cm intervals at right

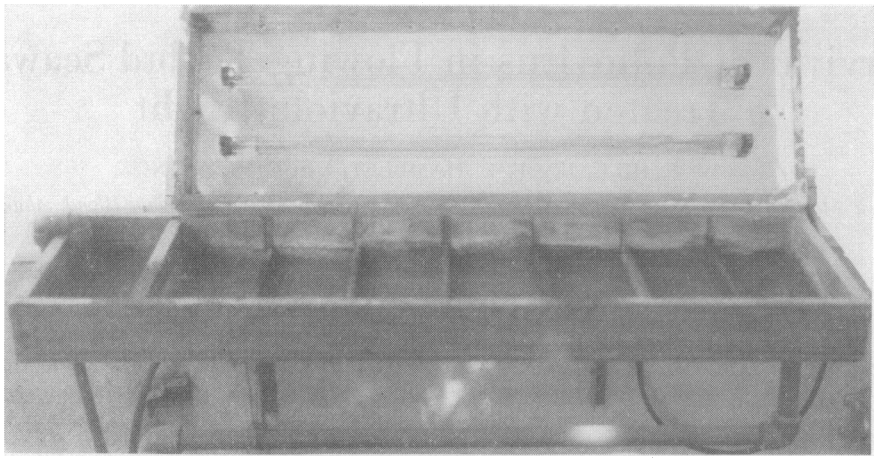

FIG. 1. *UV treatment unit.*

angles to the direction of the flow, to provide a stirring effect. The distance from the bulbs to the surface of the water was approximately 5 cm. The depth of the seawater was 0.6 cm at the top of the baffles and 3.2 cm at the baffle interspace. A reflective aluminum sheet covered the inside top of the bulb unit. A three-view drawing of the UV seawater treatment unit is shown in Fig. 2. The UV lamps were checked for intensity with a Westinghouse SM-600 UV meter prior to each experiment. The average UV intensity of the two lamps used in experiment 1 was 71.6 $\mu w/cm^2$. The average UV intensity of the two lamps used in experiment 2 was 67 $\mu w/cm^2$.

Seawater supply. The seawater used in the experiments was pumped into the wet laboratory from Dauphin Island Bay, Alabama. The intake lines were fiber glass and extended 21 m into the Bay, reaching a maximal depth of 0.9 m below sea level. The flow of seawater was regulated by a polyvinyl-chloride ball valve. All seawater contact surfaces in the system were nonmetallic. The salinity of the seawater was 20.9 parts per thousand in experiment 1 and 26.4 parts per thousand in experiment 2, as measured hydrometrically (9).

Experimental. The experiments were conducted in a seawater flow-through system consisting of an epoxy resin-coated mixing tank (61 by 61 by 61 cm), containing 200 liters of seawater; a positive-flow pumping system; and the UV treatment unit. In this system, seawater could either be pumped through the UV treatment unit or diverted back to the original mixing tank. This allowed time for flow-rate adjustment between runs. Turbidity was adjusted with "marine silt," which consisted of natural bottom mud. The marine silt was "washed" and autoclaved prior to use. The marine silt was added to the mixing tank so that turbidity measured 70 and 240 parts per million (ppm) in experiments 1 and 2, respectively. Turbidity was

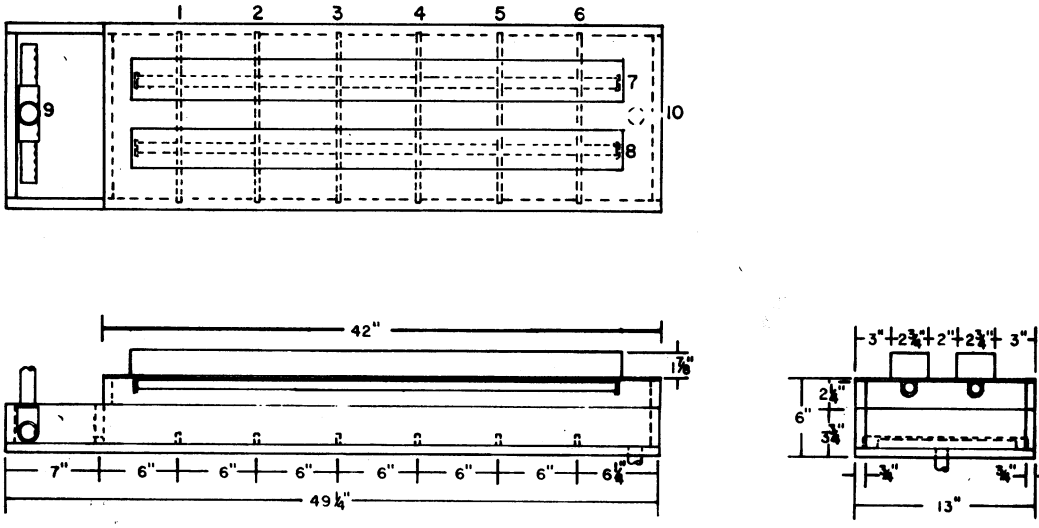

FIG. 2. *Three-view drawing of UV treatment unit.*

determined spectrophotometrically by measuring transmittance of light in a Spectronic-20 colorimeter (5). Poliovirus was then added to the mixing tank at a multiplicity of approximately 5×10^3 plaque-forming units (PFU)/ml (final volume). The contaminated seawater was then stirred for 10 min by a mechanical mixer and by flowing the seawater through the system, diverting the flow so as to bypass the UV unit. During the experiments, virus-contaminated seawater was pumped from the mixing tank through the UV treatment unit. The flow rate of the seawater was adjusted so that the flow through the UV unit would be at rates of 5, 10, 15, 20, and 25 liters/min. Samples of untreated and UV-treated seawater were collected from the influent and effluent ends of the UV unit simultaneously.

RESULTS AND DISCUSSION

The logarithm of the virus plaque survival ratios plotted against the UV dosage purportedly manifests linearity. For purposes of presentation, such data may conveniently be treated as a first-order reaction, recognizing, of course, that approximation of first-order kinetics or obvious departure from linearity (or both) may be the more frequent observation. This subject has been adequately reviewed by Hiatt (6). We decided to present our findings graphically as if first-order kinetics applied. We well appreciate that the physical state of the virus (e.g., single virus particles, aggregated virus particles, single-aggregate particle ratio, and uniformity of aggregates), as well as multiplicity reactivation, influences both the shape and slope of virus survival curves (4). The physical state of poliovirus suspended in seawater was not known and multiplicity reactivation was ignored when plotting the survival curves, to facilitate comparison of the derived slopes. The equation for a reaction that follows first-order kinetics may be expressed as:

$$Nt/No = e^{-k\bar{I}t}$$

where No is the virus population size at time zero; Nt is the virus population size at time t (time of exposure); $\bar{I}$ is the mean UV intensity; and k is the inactivation rate (slope). In this study, the expression $\bar{I}t$ relates to flow rate of seawater through the UV treatment unit and represents the UV dosage function.

The survival ratios, Nt/No, were plotted on semilogarithmic graph paper. The straight line of best fit (prediction curve) was fitted to the observed points by the graphic method of least-squares regression as described by Askovitz (1); each point representing the mean virus plaque count of two replicate counts.

At a turbidity of 70 ppm, the observed survival ratios ranged from 1.9×10^{-3} (99.81% reduction) to 1.5×10^{-4} (99.98% reduction) at flow rates ranging from 25 to 15 liters/min; no virus was recovered at flow rates of 10 and 5 liters/min (Table 1). The survival ratios, as determined by a least-squares regression prediction curve, ranged from 1.9×10^{-3} (99.81% reduction) to 1.2×10^{-5} (> 99.99% reduction) at flow rates ranging from 25 to 5 liters/min, respectively (Fig. 3). At a turbidity of 240 ppm, the observed survival ratios ranged from 3.2×10^{-2} (96.80% reduction) to 2.1×10^{-4} (99.98% reduction) at flow rates ranging from 25 to 5 liters/min (Table 1). The survival ratios as determined by a least-squares regression prediction curve ranged from 5.6×10^{-2} (94.40% reduction) to 2.5×10^{-4} (99.98% reduction) at flow rates ranging from 25 to 5 liters/min, respectively (Fig. 3).

As was expected, turbidity had an adverse influence on the effectiveness of UV radiation; however, by adjusting the flow rate, adequate disinfection of poliovirus-contaminated seawater was shown to be predictable. The inactivation rate of poliovirus, as shown by the parallel slope of the survival curves plotted by least-squares

TABLE 1. *Poliovirus multiplicities of untreated and UV-treated seawater*

Expt[a]	Flow rate	Untreated	UV-treated	Survival ratio	Reduction
	liters/min	*PFU/ml*	*PFU/ml*		%
1	5	3.3×10^3	0	—	—
	10	4.2×10^3	0	—	—
	15	6.9×10^3	1.0×10^0	1.5×10^{-4}	99.98
	20	5.4×10^3	3.0×10^0	5.5×10^{-4}	99.94
	25	7.1×10^3	1.4×10^1	1.9×10^{-3}	99.81
2	5	4.8×10^3	1.0×10^0	2.1×10^{-4}	99.98
	10	5.1×10^3	4.0×10^0	7.8×10^{-4}	99.92
	15	4.4×10^3	3.0×10^1	6.8×10^{-3}	99.32
	20	3.8×10^3	8.3×10^1	2.2×10^{-2}	97.80
	25	3.5×10^3	1.1×10^2	3.2×10^{-2}	96.80

[a] In experiment 1, the turbidity was 70 ppm; in experiment 2, it was 240 ppm.

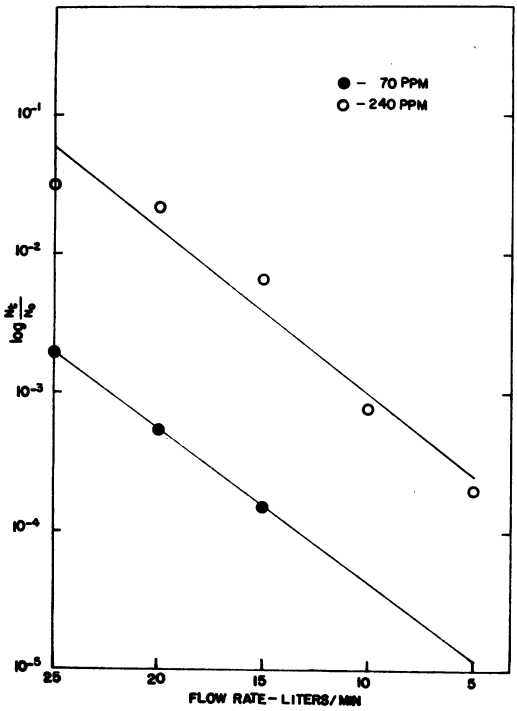

FIG. 3. *Survival of poliovirus type 1 in turbid seawater (70 and 240 ppm) treated with ultraviolet light.*

regression, was the same under both turbidity conditions. However, effective disinfection of the seawater occurred at a flow rate of 15 liters/min when the turbidity was 70 ppm, as compared to a flow rate of 5 liters/min when the turbidity was 240 ppm. This observation has practical application in that seawater turbidity does not remain stable in the Gulf of Mexico region. Consequently, for adequate treatment of seawater supplies to be used in shellfish depuration plants, turbidity will have to be monitored. Our data indicate that desirable levels of treatment may be achieved by adjusting the flow rate of the seawater through the UV treatment unit. Obviously, additional experimentation must be accomplished before definitive prediction curves can be prepared. Nevertheless, the data reported herein provide supportive evidence that UV light is a practical and effective means for treating seawater.

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