Control of an Estuarine Microfouling Sequence on Optical Surfaces Using Low-Intensity Ultraviolet Irradiation

L. H. DiSALVO and A. B. COBET

Naval Biomedical Research Laboratory, University of California, Oakland, California 94625

Received for publication 11 September 1973

Ultraviolet light has been investigated as an active energy input for the control of slime film formation on optical surfaces submerged in San Francisco Bay for periods up to 6 weeks. Irradiation of quartz underwater windows was carried out from three positions: (i) exterior to the window, (ii) from directly behind the window, and (iii) from the edge of the window with the ultraviolet (UV) energy refracted through the front of the window. Internally administered irradiation reaching levels of 10 to 30 μW per cm² measurable at the glass surface was effective in preventing bacterial slime film formation and settlement of metazoan larvae. When administered from the external position, over one order of magnitude more (500 to 600 μW/cm²) UV energy was required to accomplish the same result. Irradiation from the edge position was most promising logistically and was effective in fouling control for 6 weeks. The results provide a preliminary quantification of the energy requirement for control of the marine microfouling sequence which precedes development of macrofouling communities.

Slime film formation is an early occurrence in the natural succession which precedes macroscopic biofouling on surfaces immersed in marine waters. Primary films are composed of microorganisms and larval forms of macroorganisms which commonly secrete adhesive substances aiding their attachment to surfaces. It has been debated whether or not larvae of the macroscopic fouling organisms require presence of a slime film for initial settlement (3).

Studies at our laboratory have been concerned with population dynamics of early microfouling on glass surfaces submerged in seawater. We have tested some antifouling effects of (clear) coatings which lower the critical surface tension of the glass. Hydrophobic fluoro-silane coatings (Lockheed rain repellant, Lockheed Georgia Co.; SC87, Pierce Chemical Co.) provide excellent draining properties for glass upon its removal from water; however, antiwetting properties of these coatings are lost after several days of submergence in seawater due to overgrowth and decomposition of the coatings by slime-forming microorganisms. An active input of disordering energy was required to prevent microbial growth.

It is well known that ultraviolet (UV) radiation is lethal to microscopic organisms, particularly when applied on a continuous basis (6). Macrofouling of glass surfaces has been prevented in marine waters for periods of up to 12 weeks by using high-intensity UV obtained from a 250-W mercury vapor lamp enclosed in quartz (11). Since even the more resistant microorganisms (e.g., UV-resistant fungi) are 99% killed after 10 min of continuous irradiation with 10 μW/cm² (6), it was reasonable to expect control of microfouling organisms by using low-level irradiation on a continuous basis. In this report we describe the UV irradiation requirements necessary to maintain unfouled optical surfaces and evaluate some methods of applying the energy to the surfaces.

In addition to practical goals of fouling prevention, the methods provided a unique opportunity to measure the disordering energy required to attenuate a primary ecological succession. Similar results have been obtained on a macroscopic scale by using gamma irradiation in forests (9). Field work was carried out on a pier at the Naval Supply Center (NSC), Oakland and at Great Lameshur Bay, St. John, V.I., by one of us (A.C.) during participation in the Tektite II project in 1970. Eutrophic conditions in San Francisco Bay provided an extremely favorable environment for slime film formation; control measures successful at this location were expected to provide efficient fouling control in cleaner waters of the open ocean.

The primary fouling sequence as discussed in this paper has been well described (2, 4, 10). The rate of slime formation and dominant
species in the fouling sequence vary with different environmental parameters; for example, fouling by diatoms is heaviest during the spring when nutrient levels are highest in Bay waters (2). Regardless of season, within a few hours of immersion, a glass surface becomes seeded with a diverse assemblage of bacterial cells. Within 16 to 48 h bacteria form filaments and microcolonies, and the number of individual cells continues to increase. Diatoms may adhere to and multiply on the glass at any stage of the fouling sequence, particularly Melosira spp. and naviculoid types. With the development of bacterial and diatom populations, stalked protozoans such as Vorticella spp. and Zoothamnium spp. arise and apparently feed on the cells of the microbial film. Among the metazoa, the hydroid Obelia dichotoma is an early colonizer of glass, although occurrence of larvae is particularly seasonal. Metazoan larvae and juveniles often occurring in the primary filming sequence include representatives of the sponges (various species), bryozoans (Bugula sp., Membranipora sp.), barnacles (Balanus glandula), tunicates (Ciona intestinalis, Styela sp., Botrylloides sp.), and bivalves (Mytilus edulis).

MATERIALS AND METHODS

Irradiation fixtures. Methods of application of UV light to underwater windows included placement of the UV source (i) external to and facing the glass surface; (ii) behind a UV transmitting window; and (iii) at the (polished) edge of a UV transmitting window, so that radiation was refracted onto the surface. Figures 1 to 3 illustrate these three configurations. In all cases, UV sources were low-pressure, cold cathode mercury vapor lamps (model series SC-1, Ultra Violet Products Co.). UV measurements were made either with a model J-222 meter (Ultra Violet Products) or on an IL 600 research photometer (International Light Co.). For irradiation from the external position (Fig. 1), UV lamps were enclosed in a case made of Naval bronze and fitted with a removable quartz window sealed with "O" rings. Gas valves were installed to allow flushing of the case with nitrogen prior to use. This device allowed for irradiation of surfaces with up to 2,000 µW/cm², depending on the size and number of lamps installed and the distance from the target surface. Target surfaces in the external irradiation series were either 25- by 165-mm glass plates or underwater windows as used with the internal device (below).

An underwater window mounted on a Plexiglas case was used for application of UV from the internal position (Fig. 7A). Underwater windows employed in these experiments were made of fused silica, 3.4-cm thick, set in a stainless-steel frame (see Fig. 3). The window transmitted approximately 10% of the incident UV energy when measured in air. The internal device fitted with a 16-cm UV lamp showed maximum UV intensity of about 200 µW/cm² measurable at the surface of the window. Variations in UV intensity were obtained by using lamps of different sizes; in one instance, to obtain an extremely low emission, a lamp was used which had acquired a partial coating of (unidentified) UV-occluding material. UV intensities were measured in the laboratory at the beginning and end of all experiments. Field checks were made by us-

FIG. 1. Front side (F) and cutaway side view (S) of a fixture for UV irradiation of underwater windows from the external position. (a) Pen Ray brand UV lamps, 16 cm; (b) brass case; (c) quartz window; (d) gas valves; (e) target glass surface.

FIG. 2. Front view (F) and cutaway side view (S) of an underwater fixture for irradiation of a (quartz) window from the internal position. (a) Pen Ray brand UV lamp; (b) Plexiglas case; (c) stainless-steel frame; (d) target surface.
ing the J-222 UV meter enclosed in a waterproof case. One experiment with the internal device was made by using a 16-cm UV lamp and a window which was half covered on the inside with aluminum foil (Fig. 4) so as to produce a gradient of UV energy beginning at the border of the foil and proceeding toward the darkened (upper) end of the window. Most experiments with internal irradiation were carried out by using an 8-cm UV lamp which provided 10 to 20 μW/cm² at the external surface. Two 4-week trials were run at the NSC pier, Oakland, and one 2-week trial was run at Great Lameshur Bay, St. John, V.I. Additional measurements were made with both internal and external irradiation devices at Oakland for periods ranging from 7 to 30 days.

Irradiation from the edge of an underwater window was accomplished by grinding and polishing shallow grooves in the edges of a window (Fig. 3) and affixing the window in a Plexiglas case with the UV lamps positioned over the grooves. After several preliminary trials, two 4-cm lamps equipped with model G-275 filters (U.V. Products Co.) were installed in the fixture, and a 6-week test was carried out at the NSC pier, Oakland. The filters screened out a majority of wavelengths other than shortwave UV at 253.7 nm. Less than 5 μW/cm² of UV was measurable (in air) escaping any point on the surface of this window.

**Evaluation of UV effects.** Development of slime film on irradiated and control glass surfaces was generally recognized by retention of water on the surfaces when they were lifted from the water. Quantitative estimation of the fouling was made by direct counting of fouling organisms. Upon recovery from each test run, glass surfaces were rinsed with membrane-filtered (0.45 μm; Millipore Corp.) seawater, fixed in 10% buffered Formalin for 5 min, stained for 2 to 3 min with 0.25% Safranin "O," rinsed in distilled water, and air-dried. Surfaces were observed with light microscopy at ×100 and ×400, and the organisms in at least 10 representative microscope fields were counted. Several incidental observations were made concerning fouling by metazoan larvae.

**RESULTS**

Preliminary results using the internal device and a prototype external irradiation device were positive. Two 4-week trials in San Francisco Bay and one 2-week trial at St. John, V.I., with the internal device produced apparently clean, nonwettable windows compared with non-irradiated controls which were heavily obscured.
Table 1. Effect of UV irradiation on the development of microbial fouling on glass quartz surfaces

| Expt         | Duration (days) | UV intensity (µW/cm²) | Microorganism counts per cm² | Wettability |
|--------------|-----------------|-----------------------|-----------------------------|-------------|
|              |                 |                       | Bacterial cells | Bacterial colonies | Sessile protozoa |           |
| Internal configuration |                 |                       |                             |             |             |
| I             | 10              | 1-5°                  | 10⁴-10⁵                  | 10³         | U           | +          |
| II            | 10              | 10-20°                | 10⁴                       | ND          | U           | -          |
| III           | 24              | 800°                  | 10⁴                       | ND          | 10⁴         | +          |
| External configuration |                 |                       |                             |             |             |
| I             | 24              | 80-60°                | 10⁴                       | 10³         | 10³         | ±          |
| II            | 30              | 100-200°              | 10⁷                       | 10³         | 10³         | ±          |
| III           | 30              | 500-600°              | 10⁵-10⁶                  | 10³         | O           | -          |

*Abbreviations: U, not detected; ND, no data; O, overgrown with macrofouling.
*UV measured in air at surface.
*Wettability key: +, wetted; ±, incomplete wetting; -, nonwettable.
*Three replicate tests.
*Experimental.
'--', Control.
*External surface of quartz window of irradiation device.

by slime and macroorganisms. Similar results were obtained in San Francisco Bay with a prototype external irradiation device which delivered approximately 300 µW/cm² to the target surface during a 1-week test.

**Internal irradiation.** Measurements using the internal device established the threshold of control in the vicinity of 10 µW/cm² measurable in air at the outside surface of the glass. Below this irradiation level, the fouling sequence proceeded slowly, resulting in the development of wettable films; above the threshold, slime formation was prevented and only minor amounts of microscopic particulate matter adhered to the glass. Table 1 summarizes these results. The lowest value achieved for cell settlement during internal UV irradiation (100 cells per cm²) was observed on the outside surface of the external UV irradiation device which was subject to about 800 µW/cm². This surface held approximately 500 nanoflagellates per cm², a phenomenon also observed at the highest intensity levels in the UV gradient experiment.

The UV gradient experiment graphically illustrated the threshold concept, with only a slight continuum between slimed and clean areas of the glass. The bacterial count on the glass surface decreased with increasing UV irradiation (Fig. 5). Toward the non-irradiated end of the window, the population of stalked protozoans increased markedly, and there was a decrease in numbers of bacterial cells (Fig. 6).

**External irradiation.** Irradiation of glass surfaces from the exterior was never as efficient as the internal methods. Almost two orders of magnitude more UV energy were needed to produce the same effects (Table 1).

In spite of the large amount of external irradiation, a detrital (nonwetting) film formed on the glass surface. Microscope observation of this film showed large numbers of microscopic particles not identifiable as bacterial cells, bacterial microcolonies were absent, and the film remained nonwettable to seawater although it drained more slowly.

**Edge irradiation.** A 6-week test using UV energy administered at the edge of the window resulted in a generally nonwetting surface (Fig. 7A). Wettability appeared to increase with distance from the lamps, as observed in the UV gradient test. Microscope observation showed about 5 x 10⁴ bacteria-size (approximately 1- by 2-µm) particles per cm² with less than 2 x 10⁸ microcolonies per cm². Microcolonies were poorly developed, as evidenced by small size and compactness. No protozoa or diatoms were found, and metazoan juveniles were only noted after 5 weeks of immersion. At 6 weeks, the only metazoans present were tunicates; these were about 1 mm in diameter and were distributed away from the UV lamps. The control window in this experiment (Fig. 7B) showed an exten-
Fig. 5. Relationships between bacterial count, UV intensity, and wettabiliy of underwater window surface in relation to locus on glass as shown in Fig. 4. High power (HP) fields were at $\times 400$; approximately 400 $\mu$m in diameter.

Fig. 6. Comparative counts of protozoa, bacterial colonies, and bacterial cells occurring on gradient UV window (see Fig. 4).

Fig. 7. A. Plexiglas fixture with underwater window which was side irradiated for 6 weeks in San Francisco Bay in April and May, 1973 (see Fig. 3). Note water droplets remaining on surface immediately after removal from water. (a) Position of 4-cm Pen Ray UV lamps. B. Control underwater window immersed for 6 weeks near UV-irradiated window (A). (a) Barnacles; (b) hydroids.

Fig. 8. Aspects of microfouling on glass surfaces resulting from a 10-day immersion in San Francisco Bay in 1972. A. Quartz surface, internally irradiated with approximately 10 $\mu$W/cm² to show: (a) juvenile ascidian, (b) surface devoid of microfouling (from $\times 400$). B. Non-irradiated (control) surface to show: (b) edge of juvenile barnacle, (p) sessile protozoan, (d) diatom, and (m) bacterial cells (from $\times 400$).

**Effects of metazoa.** Several informal observations were made on the UV plates regarding the metazoa. Although the antiwetting coatings produced a surface slightly wettable to water (approximate surface tension about 25 dynes/cm², water contact angle about 90°), barnacles, sponges, tunicates, and hydroids attached to the surfaces, although they could be removed with less difficulty than those attached to clean glass surfaces. In some cases, with the internal UV applied to surfaces, juvenile tunicates (probably *C. intestinalis* and *Botrylloides* spp.) were found attached to the glass, although bacterial settlement and growth were inhibited (Fig. 8A, B). This phenomenon occurred in some instances where irradiation was 10 to 30
μW/cm². Barnacles were more sensitive to UV irradiation and were rarely found in 10 to 30 μW/cm² irradiation fields. When UV irradiation was not enough to prevent their settlement and growth, barnacles and mussel larvae were attracted to the visible light emitted by UV lamps. Table 2 summarizes the results of a typical 10-day irradiation experiment at 10 to 30 μW/cm² to show the relation between numbers of metazoans and unicellular organisms on irradiated and control surfaces.

DISCUSSION

The results suggest that low-power UV lamps (5 to 10 W) could effectively prevent fouling of glass surfaces in an enriched estuarine environment. The greater effectiveness of UV light coming from inside the window (in contrast to externally applied UV) may have been because particle shading effects were avoided. Bacteria, barnacles, and other organisms require intimate physical contact as they attach to surfaces by using their adhesive secretions. Shading out of externally applied UV by detrital particles or resistant organism integuments could allow adhesive interaction between fouling organism and glass surface. Future research should test the hypothesis that the UV energy interferes with functions of secretory organelles or causes molecular denaturation of adhesive substances produced by microscopic fouling organisms. The numerous particles observed on irradiated surfaces using internal and edge irradiation concurred with previous findings that nonliving cells were able to rapidly adhere to glass immersed in seawater (4). This settlement is probably the base-line value for electrostatic attraction of particles to glass (our unpublished data; see also Marshall et al., reference 7).

Accurate measurement of internally delivered UV at the surface of the glass is as yet problematical because of inability to quantitate internally refracted UV light available at particle attachment sites. The measurement of UV escaping the glass surface is probably an underestimation of the effective dose of the UV energy available at the surface. UV energy coming through the window of the external device was apparently responsible for the formation of an extremely thin coating of (unidentified) brown material on the surface of the target window, as well as on the window of the device. This was probably a coating of oxides resulting from ozonization of oxygen and the subsequent reaction with metals in the water (e.g., iron, manganese). This film formation seriously reduced the effectiveness of the device when run at intensities over 1,000 μW/cm². Similar problems have been encountered on quartz sleeves protecting UV lamps in large-volume seawater-purifying systems (5). Preliminary experimentation suggests this problem will be alleviated by the use of filters to screen out ozonizing UV energy (185 nm).

Effects on organisms. The inhibition of fouling by UV energy was a broad-spectrum effect crossing phylogenetic lines. The occurrence of about 10³ bacteria per cm² as found on "clean" irradiated surfaces is typical of the settlement obtained by using killed bacterial cells to evaluate fouling uptake by glass surfaces (4). We cannot explain the presence of nanoflagellates in our most intense radiation fields where very few other microorganisms were found. The phenomenon was consistently noticed in both preliminary and definitive experiments and suggested high resistance of this type of organism to UV irradiation. In one experiment, an O. dichotoma colony survived in a radiation field of about 10 to 30 μW/cm², reaffirming the high radiation resistance reported for hydroids (12).

Of greatest importance to prevention of the slime filming process, the low-level UV irradiation from both internal and external positions prevented multiplication of slime-forming bacteria. Bott and Brock (1) have used UV irradiation as a research tool in determining multiplication rates of bacterial microcolonies in freshwater environments. Our study reaffirms the value of UV irradiation as a research tool in this type of basic research. Figure 6 provides indirect evidence of a trophic link between the sedentary ciliate protozoa and fouling bacteria on glass surfaces. When the irradiation level was high enough to kill the protozoa, the bacterial single-cell population reached a high point. In the presence of the protozoa, the

---

**Table 2. Primary fouling on internally UV-irradiated and non-irradiated quartz surfaces**

| Organism type       | No. per cm² |         |         |
|---------------------|-------------|---------|---------|
|                     | Control     | UV irradiated |
| Ascidians           | 3.43*       | 0.50*   |
| Barnacles           | 0.033       | None    |
| Sessile protozoa    | 7 × 10⁸     | None    |
| Bacteria            | 3.3 × 10²*  | 1.8 × 10¹* |
| Gross appearance    | Fouled, occluded | Clean, clear |

* UV intensity, 10 μW/cm², measurable in air escaping glass surface; immersion for 10 days in August 1972 in San Francisco Bay (see Fig. 8).
* Diameter, 0.2 to 3.5 mm.
* Diameter, 0.2 to 0.5 mm.
* Microcolonies and recognizable bacterial cells.
* Scattered bacterial-sized particles; no microcolonies.
standing crop of bacterial single cells was markedly smaller.

Some evidence obtained in the present research suggested that barnacles, mussels, tunicates, sponges, and hydroids were able to settle on surfaces where bacterial settlement and growth had been inhibited by UV irradiation. This evidence supports the contention that slime filmning of surfaces is not a requisite for the settlement of many important fouling organisms. UV irradiation techniques promise to be a valuable investigative tool in further evaluation of this aspect of the microfouling sequence.

Practical application of these findings rests on the ability to employ UV-transmitting glass (e.g., Corning Co. no. 7940) in underwater optical devices to provide requisite amounts of antifouling energy to the critical surface.

**Stress energy of antifouling.** A common property of different types of energy which cause biological stress is that their inflow to the biological system results in internal systems disorder (8) such as denaturation of deoxyribonuclease and enzyme molecules, which, in turn, result in interruption of cell division and interference with metabolic processes. Stress energy flowing into various systems can be compared by conversion of energy values into comparable units. Our research is the first case to our knowledge where the stress energy requirement for fouling prevention has been quantitatively determined in one type of environment (estuary). This determination was analogous to similar measurements made on macro-ecosystems where ecological succession was arrested by using biologically disruptive energy inputs (gamma radiation; reference 9, 13). Odum (9, p. 1-269) reported that, in a tropical rainforest which had been experimentally irradiated, an energy equivalent of about 6.07 joules per m² per day of gamma irradiation was required to overbalance the repair rate of the forest. A range of 1 to 50 μW/cm² (10 to 500 ergs per cm² per s) of UV energy was needed to arrest the microfouling sequence. This can be converted to 8.79 to 439.53 joules per m² per day, showing the similarity in magnitude at the lower range of limiting stress energy requirement between the forest macrocommunity and the fouling microcommunity. If this relationship holds true in future research, it may provide a unifying biological principle based on the laws of thermodynamics. It is reasonable, however, to expect a higher requirement of stress energy to arrest succession in the high entropy, rapid turnover, poorly structured fouling microsystem when compared with the more stable and more highly structured forest macrosystem. Future research is therefore being designed to measure stress effect per unit of energy flow, as measured by community metabolism.

**ACKNOWLEDGMENTS**

We gratefully acknowledge support by the Office of Naval Research through a contract with the Regents of the University of California and by ONR Contract N00014-69-A-0200-1001. We also acknowledge the technical assistance rendered by D. M. Scheller, HM2, USN, and M. Roles.

**LITERATURE CITED**

1. Bott, T. L., and T. D. Brock. 1970. Growth and metabolism of periphytic bacteria: methodology. Limnol. Oceanogr. 15:333-342.
2. Campbell, S. A., and A. B. Cobet. 1970. An investigation into primary fouling of glass surfaces in San Francisco Bay. p. 375-402. 47th Tech. Prog. Rep. Naval Biomedical Research Laboratory, University of Calif., Berkeley.
3. Crisp, D. J., and J. S. Ryland. 1960. Influence of filming and surface texture on the settlement of marine organisms. Nature (London) 185:119-121.
4. DiSalvo, L. H. 1972. Early steps in the microbial fouling of optical surfaces in the marine environment. p. 357-378. 47th Tech. Prog. Rep. Naval Biomedical Research Laboratory, University of Calif., Berkeley.
5. Herald, E. H., R. F. Dempster, and M. Hunt. Sept. 1970. Ultraviolet sterilization of aquarium water, p. 57-71. In W. Hagen (ed.). Aquarium design criteria (special edition of Drum and Croaker). U.S. Department of the Interior, Washington, D.C.
6. Koller, L. R. 1965. Ultraviolet radiation, 2nd ed. John Wiley and Sons, N.Y.
7. Marshall, K. C., R. Stout, and R. Mitchell. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. 65:337-348.
8. Odum, H. T. 1968. Work circuits and systems stress p. 81-138. In H. E. Young (ed.), Mineral cycling and productivity. University of Maine Press, Orono.
9. Odum, H. T. 1970. A tropical rainforest. no. TID 24270. USAEC. Div. Tech. Inf. Ext., Oak Ridge, Tenn.
10. O'Neill, T. B., and G. Wilcox. 1967. The formation of a primary film on materials submerged in the sea at Port Hueneme, California. Pac. Sci., 25:1-12.
11. Plotner, R. 1968. Sensor fouling on deep submergence vehicles, p. 267-295. In F. Alt (ed.). Marine sciences instrumentation, vol. 4. Plenum Press, Inc., New York.
12. Strehler, B. L. 1961. Aging in coelenterates, p. 373-398. In H. M. Lenhoff and W. F. Loomis (ed.). The biology of hydra and some other coelenterates. University of Miami Press, Coral Gables.
13. Woodwell, G. M. 1967. Radiation and the patterns of nature. Science 156:461-470.