Effects and Fate of a Surfactant in Cultures of the Red Time Organisms, *Gymnodinium breve*

William S. Hitchcock* and Dean F. Martin

Department of Chemistry
University of South Florida
Tampa, Fla. 33620

Sporadic over-bloom of a planktonic organism, red tide, off the west coast of Florida have had an adverse effect on tourism in the area. This, coupled with clean up costs, associated with the fish-killing phenomenon, has spurned much interest in the causative organism, Gymnodinium breve, an unarmoured dinoflagellate (DAVIS, 1948). It was estimated that total losses associated with the June-August 1971 outbreak along a seven county area were in excess of $20 million (HABAS and GILBERT, 1975).

The possibility of management has been considered. While studying the effects of treated sewage on the growth of G. breve, DOIG and MARTIN (1974) noted that some phosphate-containing detergents were lethal to the organism, and later studies (KUTT and MARTIN, 1974) showed that a surfactant, a linear alkylbenzene sulfonate (C_{13}LAS), was highly toxic to G. breve. It was also observed that the surfactant was rapidly degraded in sea water, which is in agreement with observations of GOODNOW and HARRISON (1972), who reported that C_{13}LAS was degraded by a large number of bacteria. In addition, a surface-active material was isolated from red tide water containing a blue green alga (Gomphosphaeria aponina) and was found to be cytolytic toward G. breve (KUTT and MARTIN, 1975).

It is not surprising to find surfactants affecting the growth of G. breve since surface processes have long been known to be involved in red tides. WOODCOCK (1948) suggested that toxins from red tide blooms were transferred to the atmosphere from bursting bubbles. Production of aerosols from bursting bubbles is now well known fact (BLANCHARD, 1975). Also it has been noted that G. breve tends to concentrate in the top few millimeters of a culture illuminated from above and the side, and this concentration would enhance the effects of a surfactant.

This paper deals with the study of the fate of a model surfactant in a water column, with the effect on G. breve cultures in that column, and with the inadvertant utility of an environmental contaminant.

---

* Present address: Department of Chemistry, University of Missouri-Rolla

Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 3 © 1977 by Springer-Verlag New York Inc.

MATERIALS AND METHODS

Cultures. Axenic cultures of Gymnodinium breve were obtained through the courtesy of S. M. Ray and W. B. Wilson (Texas A & M Marine Station, Galveston) and were maintained in B-5 enriched aged sea water (cf BRYDON et al., 1971, for details of culturing). Cells were enumerated electronically using a Model B Coulter counter equipped with a C-1000 Channelyzer.

Surfactant. Material used, C-13 linear alkylbenzene sulfonate, was provided by Proctor & Gamble as 49.67% active material (the rest being water and sodium sulfate). The material was used without further purification.

Study systems. These consisted of Pyrex glass columns, 150 cm (51 mm, o.d.) stoppered at the top and closed at the bottom with a Teflon stopcock; two other stopcocks were affixed at right angles 60 and 120 cm from the bottom.

Diffusion studies. The study columns were filled with sea water ($S = 34^{o}/oo$) and surfactant stock solution was introduced specifically at the top or bottom. Samples (10-25 ml) were withdrawn daily from each of three stopcocks and were analyzed for surfactant using a methylene-blue photometric procedure (LONGWELL and MANIECE, 1955; KUTT and MARTIN, 1975). Absorbance measurements were made using 5-cm cells and a Beckman DB-GT spectrophotometer. Standard solutions were prepared daily and were analyzed with the samples. Total initial concentrations were 2-10 ppm surfactant.

Surfactant culture studies. Columns were carefully cleaned (with 12 M HCl, followed by repeated rinsing with distilled water, then doubly distilled water, then sea water) prior to innoculation with G. breve. Cultures were allowed to enter log-phase growth before introduction of the surfactant. Initial concentrations of surfactant were 6, 12, 26 ppm. At appropriate times, samples of culture were withdrawn from each of the three stopcocks and cells were enumerated.

RESULTS AND DISCUSSION

At present there is no available method to measure concentrations of C_{13}LAS in the ppb range which are lethal to G. breve. For this reason, diffusion studies were run at higher concentrations.

When the surfactant was carefully introduced into the column from the bottom, it typically diffused upward and evenly through the column. No attempt was made to measure surface concentration. A typical run for 10 ppm (mean concentration throughout column) is shown in Table 1. Within 2 days, the surfactant was evenly distributed and remained that way for up to 10 days when the experiment was terminated.

TABLE I

Distribution of C_{13}LAS surfactant in water column as a function of time

Day	Relative[a] surfactant concentration[b]		
	Bottom	Middepth	Top
0.04	1.0	0.85	0.845
1.0	0.86	0.74	0.56
2.2	0.69	0.66	0.71
5	0.72	0.68	0.69
7	0.68	0.68	0.69
10	0.68[c]	0.68	0.68

[a] relative to initial bottom sample = 14.6 ppm

[b] average of determinations in triplicate

[c] final value 10 ppm, calculated; 9.9 observed

If the surfactant is introduced at the top of the column, similar results are not obtained. Immediately upon addition of the surfactant, a cloudy layer is formed at the top. Even at lower concentrations (2 ppm), the layer formed, and the surfactant remained at the top of the column. Over a 10-day period, the concentration at the top increased (by 15% from initial value) and the concentrations at mid-depth and bottom remained essentially constant (30 $\pm$2 and 27 $\pm$2% of initial surface value, respectively).

This is an interesting and not completely unexpected result. Owing to the high ionic strength of seawater large micelles are readily formed. It is reasonable to assume that the micelles have a density less than that of the water and would rise to the surface in a column of water. Therefore, if the surfactant were introduced into the bottom of a column, micelles may form (indeed they were observed in some experiments), but they would rise to a region of lower surfactant concentration and would dissolve there owing to the lower concentration. If, however, the surfactant were introduced into the top of a water column any micelles formed would have a tendency to rise. This rise to the surface would not bring the micelles into a region of lower concentration, but to a region of higher concentration, and would be prevented from evenly diffusing through the column.

The effect of surfactant on viability of G. breve in the water column was studied using sub-lethal (6, 12 ppb) concentrations and two modes of introduction (surface and bottom).

When enough surfactant, introduced into the bottom of the surfactant column, was added to give a mean concentration of 6.25 ppb, fatality of the culture did not occur, but the number of cells at the bottom of the column increased. These cells, when observed with a microscope, were found to be intact cells. They were not, however, motile nor were they encysted. They seemed to be "anesthetized." After four days, the culture was once again motile. With an average concentration of 12.5 ppb, the effects were similar, but more pronounced (Figure 1). Again after four days, the culture was back to normal.

When the surfactant (average concentration 12.5 ppb) was added to the top of the column there was a large drop in the number of cells at the top of the column and a corresponding rise in the number of cells at the bottom of the column. Once again, recovery was complete after four days.

Concentrations of 25 ppb were lethal to the G. breve culture (Figure 2). Death occurred rapidly throughout the column and implies that the surfactant diffused rapidly upward. When surfactant (26 ppb) was introduced into the top of the column, cell mortality was also rapid (24 hr). Large apparent cell counts in samples from the top of the column were caused by cell fragments. This destruction of the cell membranes occurred rapidly. Regardless of the mode of introduction at 26 ppb, there was no recovery by the culture. These results agree with the results of KUTT and MARTIN (1974) in which 26 ppb was effective in controlling the growth of G. breve.

Results of the present study indicate the useful mode of introducing a proper surfactant in the water column to manage a red tide bloom, and they indicate the rate of movement of surfactant in non-turbulent water. Further, the results indicate the basis of studying naturally occurring surfactant that could be environmentally acceptable and that could manage a red tide. Finally, the results with sub-lethal concentrations indicate the possibility of inactivating G. breve cells, presumably without releasing toxin into the water column. Reactivation of the cells is due to degradation of surfactant, or conversion of surfactant micelles, or, possibly, adaption of the cells.

As with any environmental problems, great care must be taken so that the solution is not more damaging than the problem. Although C_{13}LAS is biodegradable, it has been shown that long-term exposure to sub-lethal concentrations can have a deleterious effect on marine organisms (BELLAN et al., 1972). Obviously, the effects of any surfactant on a range of marine organisms would need to be investigated before further consideration of actual utility. Nevertheless, the present results, coupled with a past observation that C_{13}LAS did not have a deleterious effect on the blue-green alga, Gomphosphaeria aponina, (KUTT and MARTIN, 1974) suggest those experiments should be undertaken.

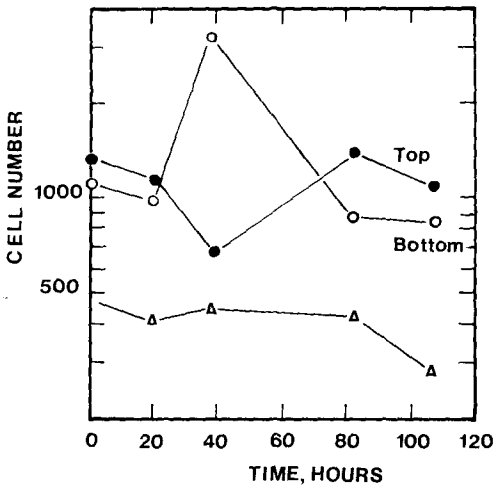

Fig. 1. Apparent distribution of G. breve as a function of time. An average concentration of 12.5 ppb C_{13}LAS was introduced at the bottom of the study column, and samples were obtained at the top (closed circles), bottom (open circles) and mid-point (triangles) of the column at indicated times.

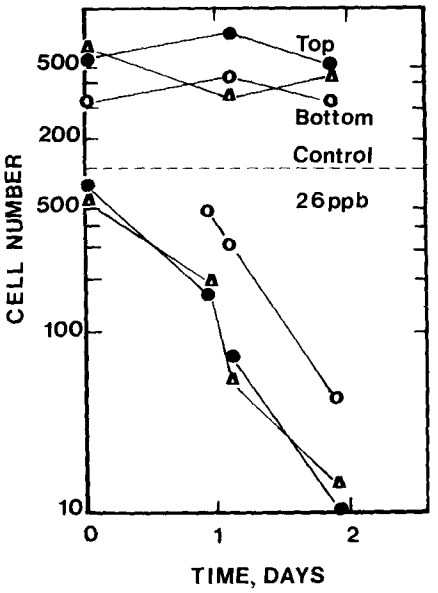

Fig. 2. Apparent cell number of G. breve culture in study column as a function of time after an average concentration of 26 ppb C_{13}LAS was introduced in the bottom of the study column. Cells were counted over a 10-sec interval using the C-1000 Channelyzer attachment. Results for the control column are shown for comparison.

LITERATURED CITED

BELLAN, G. D., D. J. REISH, and J. P. FORET, Internat. J. Life Oceans Coastal Waters, 14, 183 (1972).

BLANCHARD, D. C. Adv. Chem. Ser., 145, 360 (1975).

BRYDON, G. A., D. F. MARTIN, and W. K. OLANDER, Environ. Letters, 1, 235 (1971).

DOIG, M. T., III and D. F. MARTIN, Mar. Biol., 24, 235 (1974).

DAVIS, C., Bot. Gaz., 109, 358 (1948).

GOODNOW, R. A. and A. P. HARRISON, Appl. Microbiol., 24, 555 (1972).

HABAS, E. J. and C. K. GILBERT, Environ. Letters, 6, 138 (1974).

KUTT, E. C. and D. F. MARTIN, Mar. Biol., 28, 253 (1974).

KUTT, E.C. and D. F. MARTIN, Environ. Letters, 9, 195 (1975).

LONGWELL, J. and W. D. MANIECE, Analyst, Lond., 80, 167 (1955).

WOODCOCK, A. H., J. Mar. Res., 7, 56 (1948).