Algalicidal Activity of a Surface-Bonded Organosilicon Quaternary Ammonium Chloride

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The hydrolysis product of a quaternary amine-containing organosilicon salt, 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride, was found to exhibit algalicidal activity while chemically bonded to a variety of substrates. Six representative species of Chlorophyta, Cyanophyta, and Chrysophyta were used to evaluate the algalicidal activity. Substrate-bonded 14C-labeled organosilicon quaternary ammonium salt when attached to nonwoven fibers was durable to repeated washings, and algalicidal activity could not be attributed to slow release of the chemical.

A wide variety of chemicals are used for the control of algal growth in water intended for domestic and industrial uses. The most widely used agents contain chlorine, copper salts, or quaternary ammonium compounds (2, 3, 5, 8).

Stroganov et al. (15) reported that three alkoxysilanes, (EtO)3Si, CH3:CHSi(OMe)2, and CF3CH:CH2SiMe3(OEt)2, when added to reservoirs and inland lakes, controlled algal blooms of Schenedesmus quadricauda and Chlorella vulgaris at minimal inhibitory concentrations of 0.1 to 1.0 mg/liter. The algalicidal activity reported for the above alkoxysilanes was confirmed in our laboratory, against six different representative species of Chlorophyta, Cyanophyta, and Chrysophyta. The algalicidal activity of 3-(trimethoxysilyl)-propyldimethyl-alkyl ammonium chlorides, with alkyl chain lengths varying from 6 to 22 carbons, when added to concentrations of algae, gave a greater degree of control against the six representative species of algae. Preliminary investigation in our laboratory demonstrated that these compounds (Fig. 1) retained biological activity when durably attached to a surface. This study (presented in part at the Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., April 1972) concerns the surface-bonded algalicidal activity of 3 - (trimethoxysilyl) - propyldimethyloctadecyl ammonium chloride (Si-QAC).

MATERIALS AND METHODS

Source of algal cultures. The unialgal cultures used in this experiment were Oscillatoria borneti strain LB 143 (Indiana University, Bloomington), Anabaena cylindrica strain B 1446-lc (Culture Collection of Algae and Protozoa, Cambridge, England), Selenastrum gracile strain B 325 (Indiana University), Pleurococcus sp. strain LB 11 (Carolina Biological Supply Company, Burlington, N.C.), Gonium sp. strain LB 9c (Carolina Biological Supply Co.), and Volvox sp. strain LB 9 (Carolina Biological Supply Co.).

Source of chemicals. Si-QAC and 14C-labeled Si-QAC were prepared (J. R. Malek, Radiation Chemistry Laboratory, Dow Corning Corp.) as methanolic solutions containing 50% solids (w/v). The 14C label was present in the octadecyl portion of the molecule. Benzalkonium chloride (Winthrop Laboratories Division of Sterling Drug Inc., New York, N.Y.) was obtained commercially.

Culture medium. Stock cultures were maintained on Allen's (1) medium as 1.5% agar slant cultures. Liquid cultures were placed in 5-liter glass tanks containing a combination of distilled water and Alga-Gro Concentrate (Carolina Biological Supply Co.) as a source of sterile enrichment for algal growth. Each aqueous culture was aerated to ensure agitation at room temperature (23 ± 2 C), and the cultures were placed under Gro-lux lamps (Sylvania Electric Products Inc., New York, N.Y.) at 150 ft-c on a 12-hr photoperiod. Daily cell counts were made with a hemocytometer to maintain the cultures at a concentration of about 1,000,000 cells/ml for use as a source of inocula.

Glass slide test. Si-QAC was applied to the lower half of cleaned glass slides from a 1% aqueous solution, and the slides were allowed to air-dry. In this method, the upper portion of each slide thus served as an untreated control. The slides were then maintained in a vertical position in paraffin floats (Fig. 2) and suspended in covered 1-pint (473-ml) wide-mouth jars (height, 11.4 cm; inner diameter, 7.6 cm). Each jar contained 5,000,000 cells of a mixed algal culture/ml (S. gracile, Pleurococcus, Gonium, etc.)
and A. cylindrica). Algal cultures were aerated to ensure a constant movement of algae past the surface of the slide. Illumination consisted of 150 to 200 ft-c of Gro-lux light at a 12-hr photoperiod at 24 ± 2 C. Benzalkonium chloride was evaluated on a glass slide under identical conditions. Slides were removed from the algal cultures at 7 and 14 days, and were examined for growth and attachment of algae on both the treated and nontreated portions of each slide.

**Algicidal surface bonding with ¹⁴C-labeled Si-QAC to nonwoven fibers.** Cotton (Johnson and Johnson, North Brunswick, N.J.), polyester (Wellman Industries, Johnville, N.C.), and secondary cellulose acetate (Celanese Corp. Summit, N. J.) of approximately equal denier were treated from a 0.4% aqueous solution containing ¹⁴C-labeled Si-QAC (specific activity, 0.71 mCi/g). The fibers were dried and washed repeatedly to remove any unbound chemical. The washing consisted of placing the radioactive treated fiber in a closed beaker with water at a water to fiber ratio of 1,000:1. Subsampling the fiber and wash water, with subsequent ¹⁴C analysis, determined the amount of ¹⁴C-labeled Si-QAC retained on the fiber.

**Surface-bonded algicidal control in a closed water system.** Five-gallon (18.9-liter) aquariums were filled with aged tap water and aerated for 24 hr previous to the introduction of any chemical treatment. Each tank was landscaped with gravel and four aquarium plants: Cabomba sp., Elodea anachairs, Sagettaria microfolia, and Myriophyllum (Carolina Biological Supply Co.). The tanks were covered with clear plastic tops and were illuminated under Gro-lux lamps at 150 ft-c on a 12-hr photoperiod. The temperature was maintained at 24 ± 2 C during the course of the experiment. The following species of tropical fish were introduced into each aquarium in triplicate: Hyphessobrycon innesi, Pterophyllum scalare, leibistes reticulatus, Xiphophorus helleri, and Xiphophorus maculatus. Normal tank maintenance conducted during the experiment included daily fish feeding, maintaining and adjusting water levels, and periodic cleaning or removal of fish wastes and plant debris. Each tank was uniformly inoculated with mixed cultures of A. cylindrica, Pleurococcus sp., S. gracile, and Gonium sp. to give a final cell count of 1,000,000 cells per 5-gal aquarium. A recirculating water filter (Metaframe Corp., Maywood, N.J.) was side-mounted on each aquarium and contained 100 g of charcoal and 10 g of nonwoven cellulose acetate fiber filter.

Three systems for control of algae were tested in triplicate and evaluated over a 14-day period. The first was an untreated control which received no chemical treatment. The second was treated with water-soluble tablet algicide containing: 0.75% Monuron (3-[p-chlorophenyl]-1,1-dimethylurea), 0.55% Simazine (2-chloro-4,6-bis-ethylamino-s-triazine), 0.2% Atrazine (2-chloro-4-ethylamino-6-isopropyramino-s-triazine), 0.15% Dichlorone (2-3-dichloro-1,4-naphthoquinone), and 98.4% inert ingredients. In the third system, cellulose acetate fiber, treated with Si-QAC as previously described, was substituted for the untreated cellulose acetate fiber in the side-mounted water filter. Daily visual and photographic comparisons of algae control were made, as well as algae cell counts with a hemocytometer.

**RESULTS AND DISCUSSION**

The nontreated control portion of the glass slide shown in Fig. 2 was covered with attached viable algae, whereas the treated portion demonstrated the presence of attached nonviable cells as determined by subsequent subculture.
Visual examination of the three jars containing the mixed algal cultures, with the suspended treated glass slides, demonstrated the following effects. The free-floating algae in jars containing the untreated control and the surface-treated Si-QAC glass slides were not affected. In contrast, treatment of the slide in the third jar with benzalkonium chloride resulted in algicidal solution activity, indicating that the chemical treatment did not remain on the glass surface.

This bonded algicidal activity is not limited to glass surfaces. Successful attachment of Si-QAC to natural, synthetic, and reconstituted fibers has been accomplished with nonwoven cotton, polyester, and secondary cellulose acetate. As the surface chemistry of each fiber varies, so does the amount of chemical that can be bonded to that surface. The retention of 14C-labeled Si-QAC was greater for cellulose acetate and cotton than for polyester (Fig. 3). After two wash cycles, the cellulose acetate fiber retained 99% of the initial 14C activity, and no more chemical was released into the wash water during subsequent wash cycles. Cotton exhibited a constant value of 95% retained activity after three washes. Polyester fiber released 14C-labeled Si-QAC until the eighth wash, after which the amount on the surface remained constant through subsequent washing. The radioactivity data provided information that allowed us to calculate the approximate number of monolayers of Si-QAC on fibers as well as other substrates, assuming uniform distribution of the treatment. Subsequent electron microscopy verified this assumption. Procedures and techniques evolved during the radioactivity retention experiment were utilized for testing nonlabeled Si-QAC-treated fibers under simulated usage conditions.

Evaluation of chemicals for algicidal activity by direct addition to recirculating water systems has been described by several authors (5, 7, 11-13). These authors examined the effects of chemicals in solution upon algae suspended in water, and their methodology is not applicable to Si-QAC-treated substrates or surfaces. Conditions and concentrations under which the Si-QAC was evaluated had to be selected to test the unique proprieties of the chemical pertinent to its actual usage applications.

Figure 4 illustrates the degree of algicidal control obtained with Si-QAC bonded to cellulose acetate filter fiber (C). A comparison is made with an untreated control (A) and a chemical treatment with a solution algicide (B). Tanks containing the Si-QAC-treated cellulose acetate had an algal cell count of 60,000 cells/ml after 14 days, whereas the control tank and the tank treated with solution algicide had reached this level of algal growth by the eighth day. All algal species contained in the original inoculum were equally reduced. The algicidal activity of the treated fiber diminished with time. This decrease in activity of the treated fiber may be due to masking of the active surface by the accumulation of dead algae. Further work is necessary to substantiate or refute this hypothesis.

![Fig. 3. Effect of repeated washings upon the surface retention of 14C-labeled 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride on secondary cellulose acetate (A), cotton (B), and polyester (C).](image)

![Fig. 4. Algicidal control determined by cell counts taken from their aquarium treatments at daily time intervals. Treatments consisted of an untreated control (A), a solution algicide in tablet form (B), and 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride surface-bonded on cellulose acetate (C).](image)
Surfaces of materials such as siliceous stone, sand, and gravel were successfully treated with Si-QAC and rendered algicidal. Data suggest that the amount of Si-QAC bonded to a surface is in part dependent upon the availability of bonding sites (10). This bonding selectivity may explain why cellulose acetate and cotton are similar in surface attachment compared to polyester. The “bonding” to polyester may occur as an encapsulation of the fiber by the mechanism of alkoxysilane condensation.

The mechanism of killing by the bonded Si-QAC is not understood at this time. It is postulated that the algicidal activity may be due to disruption of membrane function with possible cell lysis caused by the high concentration of charged chemical on the substrate. This area remains for future investigation.

Aquariums used in the simulated usage test are in effect small closed water systems. The data generated with these small-scale closed recirculating systems should be useful in extrapolation to large industrial and domestic water problems. The Si-QAC compounds possess algicidal activity similar to that of benzalkonium chloride (3) when added directly to algae solutions:

Experiments with either Si-QAC or benzalkonium chloride (14) added directly to the water in the aquarium have shown that a concentration of 2 μg/ml is an LD₅₀ in species of tropical fish used in the experiment. Fish used in the aquarium with Si-QAC-treated cellulose acetate were not effected in any way by the presence of the bonded chemical.

Currently available chemical algicides are employed by direct addition to algae solutions (6, 9). In contrast, the algicidal surface-bonded chemical described in this communication is unique. The ability of the Si-QAC to form durable algicidal surfaces, without release of chemical to the surrounding environment, offers a new approach to water treatment.

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LITERATURE CITED

1. Allen, M. B. 1962. The cultivation of Myxophyceae. Arch. Mikrobiol. 17:34-53.
2. Eipper, A. W., and H. B. Brumsted. 1959. Control weeds and algae in farm ponds. p. 1-32. Cornell Agriculture Bulletin, Ithaca, New York.
3. Fitzgerald, G. P. 1959. Bactericidal and algicidal properties of some algicides for swimming pools. Appl. Microbiol. 7:206-211.
4. Fitzgerald, G. P. 1960. Loss of algicidal chemicals in swimming pools. Appl. Microbiol. 8:269-274.
5. Fitzgerald, G. P. 1962. Bioassay for algicidal chemicals in swimming pools. Water Sewage Works, p. 361-363.
6. Fitzgerald, G. P., and M. E. Devartianian. 1967. Factors influencing the effectiveness of swimming pool bactericides. Appl. Microbiol. 15:504-509.
7. Fitzgerald, G. P., and S. L. Faust. 1963. Bioassay for algicidal vs. algicidal chemicals. Water Sewage Works, p. 296-298.
8. Hueck, H. J., D. M. Aolema, and J. P. Weigmann. 1966. Bacteriostatic, fungistatic, and algicidal activity of fatty nitrogen compounds. Appl. Microbiol. 14:308-319.
9. James, G. V. 1971. Water treatment—a survey of current methods of purifying domestic supplies and of treating industrial effluents and domestic sewage. Technical Press Ltd., Edinburgh, Scotland.
10. Johansson, O. K., F. O. Stark, E. G. Vogel, and R. M. Fleischmann. 1967. Evidence for chemical bond formation at silane coupling agent interfaces. Composite Materials 1:278-292.
11. Kaye, S. 1971. Evaluating algicidal activity of films. Modern Plastics, p. 78-79.
12. Koski, T. A., L. P. Ortenzio, and L. S. Stuart. 1967. Effect of algicidal quaternaries on the germicidal activity of chlorine on swimming pool water. Appl. Microbiol. 15:1291-1295.
13. Maloney, T. E., and C. M. Palmer. 1966. Toxicity of six chemical compounds to thirty cultures of algae. Water Sewage Works, p. 509-513.
14. Rucker, R. R., H. E. Johnson, and E. J. Ordal. 1949. An investigation of the bactericidal action and fish toxicity of two homologous series of quaternary ammonium compounds. J. Bacteriol. 57:223-234.
15. Stroganov, N. S., V. G. Khobot’ev, L. V. Kolosova, and M. A. Kadina. 1968. Application of alkoxysilanes for reducing the abundance of phytoplankton during water reservoir “bloom.” Dokl. Akad. Nauk SSR 181:1257-1259.