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Naval Ship Research and Development Center Washington, D.C. 20007

> REPORT COVER NDW-NSRDL(A) 3960/164 (Rev 2, 6/7

# ANTISLIME COATINGS Part I - Primary Marine Fouling

By E. J. Dyckman and V. John Castelli

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June 1971

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## ABSTRACT

Initial investigations on primary fouling (slime generation) on submerged surfaces and the development of a transparent antifouling coating system for submerged optics are summarized. The chronological succession of bacterial sliming on submerged-glass coupons has been recorded. Pure bacterial cultures isolated from these slimes have been subjected to preliminary identification and their response to several toxic organometallic compounds has been tested. The facile synthesis of various organometallic acrylic monomers suggests their polymers as foremost candidate materials in the search for antifouling underwater optics and coatings.

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## ADMINISTRATIVE INFORMATION

This work is authorized under Task Areas SF 11 552 101 and ZF XX\_412\_001, Task 12874, Work Units 1\_937\_101 and 1\_937\_105 as descr'bed in the 1 November 1970 Program Summary. The latter task area is concerned with the development of optical coatings.

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#### INTRODUCTION

Microorganisms, their viscous bio-organic products and absorbed organic matter, 1-4 constitute a tenacious, opaque slime which forms on submerged surfaces. The initial organisms in this fouling sequence are bacteria<sup>5</sup> followed by a biotic progression of diatoms, hydroids, algae, bryozoans, protozoans, and finally macrofoulants.<sup>6-10</sup> Crisp<sup>11</sup> has shown that macrofoulants tend to be rugophilic, settling on roughened surfaces in preference to smooth glass and acrylic surfaces.<sup>12,13</sup> It is thought that primary marine slimes precondition the surface in some manner thus stimulating the settling of macrofoulants.14-18 Skerman<sup>17</sup> demonstrated barnacle settlement is less frequent on clean glass surfaces compared to those covered with emollient films high in particulate matter. This film may provide a physical substrate and/or a nutritive source which encourages the attachment of macroscopic plants and animals.<sup>15, 18</sup> In order to determine the necessity of bio-organic slimes as preconditioning agents, a controlled laboratory procedure is essential. A procedure for barnacle rearing developed originally by Freiberger<sup>19</sup> has been continued at this activity and will be used in later experiments on marine slimes.<sup>20, 21</sup> From a practical standpoint, slime films affect the performance of antifouling coatings to some extent by physically overlaying the toxic surface.12,16,21,22 However, specific slimicidal coatings are virtually nonexistent.

Various authors have pointed out the inherent value of organometallic compounds as biocides.<sup>22-29</sup> Their studies indicate that toxicities of several selected organometallic compounds are at least an order of magnitude greater than classical copperbased agents. Initial studies to determine the feasibility of optical antifouling agents were limited to bis-(tributyltin) oxide (TBTO). Metzler<sup>10</sup> coated glass slides with this agent,

<sup>&</sup>lt;sup>1</sup>Superscripts refer to similarly numbered entries in the Technical References at the end of this report.

and Muraoka<sup>30</sup> similarly treated acrylic polymers, in addition with hydrostatic pressure to increase diffusion of the toxin into the polymer matrix. Samples treated both ways fouled within 3 weeks through rapid depletion of the TBTO. A novel approach undertaken at this activity to increase the long-term serviceability of such coatings is to incorporate the toxin chemically on a synthetic polymer backbone. This provides minimum dissolution of toxin through surface hydrolysis, while hopefully maintaining an effective contact-toxic for primary fouling organisms. Therefore, in addition to increased service life of antifouling coatings, this provides a new generation of coatings which do not represent an input to marine pollution, particularly in harbors where antifouling agents would be concentrated.

### MATERIALS AND METHODS

Standard glass microscope slides were used as test surfaces for slime development. These coupons were exposed on submersible experimental units (SEU), as shown in figure 1. Each SEU can support sixty 1- x 3- x 3/32-inch coupons at any depth and angle. Several SEU's were moored from a buoy array (figure 2) at a depth of 1 to 4 feet in Carr Creek (figure 3), a local estuary with average salinity of 11%. Prior to exposure, all test coupons were cleaned in chromic acid, rinsed profusely in distilled deionized water, and maintained at 110° C until used.\* The coupons were mounted on the SEU with adequate precaution taken to prevent contamination of the surface. SEU's were then placed in a sterilized plastic bag for transport to the exposure site. Random samples proved sterile when checked prior to immersion. After varying exposure periods up to 96 hours, the coupons were retrieved and microfouling organisms quantified and isolated. This was accomplished by first rinsing the slides with 30 cc of sterile Carr Creek water, followed by gently pressing the face of the slide on marine agar plates (Difco) for 5 minutes. Then the slide was removed and the inoculated agar incubated for 48 hours at 25° C. Developing colonies were counted when possible. Pure cultures were subjected to cursory identification by standard methods using Eugon Agar prepared with artificial seawater (Rila Marine Mix). These microorganisms were subsequently used for determining the relative toxicity of various organometallics by the "ring of inhibition" method. Candidate compounds (TBTO, bis-(tripropyltin) oxide (TPTO), tetramethyl lead (TML)) dissolved in ligroine (boiling point 35°-60° C), a very inocuous solvent to all the microorganisms

<sup>\*</sup>Abbreviations used in this text are from the GPO Style Manual, 1967, unless otherwise noted.

tested, were used to saturate standard size disks (5-mm diameter) of absorbent paper. These were placed on the surface of marine agar plates uniformly inoculated with one of the slime-associated microorganisms. The extent of inhibition was determined after 48-hours incubation at 25° C.

Two of the above mentioned organometallic compounds (TBTO and TPTO) were utilized in the formulation of experimental antislime coatings. The toxic agents were dissolved in an acrylic monomer solution and polymerization induced with a catalyst. The resultant polymers were coated on glass slides previously treated with a coupling agent to permit adequate adhesion of the polymer to the glass.

### RESULTS AND DISCUSSION

During the first 24 hours of sliming on glass surfaces, there was a numerical increase in microorganisms with time, coupled with a decrease in diversity of species (figures 4-6). Of 13 different organisms isolated from the slime and adjacent water, only one (X3) showed repeated dominance after 96 hours. Preliminary characterizations of all isolates are listed in table 1. Biochemical differentiation proceeded with difficulty owing to incompatability between various constituents of the test media and salt water. Since this problem has recently been overcome, however, biochemical tests are presently underway.

	Pigmen_	Eleva_	Agar		
Organism	tation	tion	Stroke	Form	
X2	Orange	Flat	Effuse	Punctiform	
X3	Cream	Convex	Filiform	No individual colonies	
x9	Yellow	Raised	Beaded	Punctiform	
X16	Green	Convex	Beaded	Punctiform	
X17	Red	Convex	Beaded	Circular	
x19	Yellow	Raised	Beaded	Punctiform	
x20	Brown	Convex	Effuse	Circular	
X22	Green	Convex	Beaded	Punctiform	
x25	Brown	Convex	Beaded	Circular	
X27	Green	Raised	Beaded	Punctiform	
x29	Brown	Convex	Effuse	Filamentous	
x30	Black	Convex	Beaded	Punctiform	
x31	Tangerine	Convex	Beaded	Filamentous	

Table 1 Characteristics of Estuarian Sliming Bacteria

Note: Anaerobicity for all organisms is +.

Twelve of the thirteen slime-associated microorganisms, tested by the "ring of inhibition" method (figure 7), are susceptible to the toxic properties of TBTO and/or TPTO, whereas all are unaffected by TML (figure 8). These results agree with previous studies23 which showed that the most toxic organometallic compounds are of the type R<sub>z</sub>MX, where R is an organic radical, M is the metal atom, and X can be any electronegative inorganic or organic radical (e.g., oxide, methacrylate). The radical X does not affect the toxicity of the compound to any significant degree, whereas the organic radical R has great influence on toxicity.<sup>23</sup> Organometallic acrylic monomers, when polymerized, exhibit satisfactory physical properties for use as underwater optics or optical coatings. These polymers are readily formed into complex shapes, machineable, colorless, transparent, strong, and lightweight. The organometallic polymer can be visualized as a backbone acrylic polymer with side\_chain organometals attached, as shown below.



In contrast to the current antifouling coatings, these organometallic polymers are expected to maintain a constant surface toxicity. Toxin is released here by means of metabolic degradation of the polymer upon contact by the sliming microorganisms.

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#### CONCLUSIONS

• The methods developed for the preparation, exposure, and retrieval of test coupons are reliable procedures for studying the progression of sliming by microorganisms on submerged surfaces.

• There is a numerical increase in microorganisms with time, coupled with a decrease in species diversity in the primary fouling sequence.

• Organometallic compounds (TBTO and TPTO) may be used effectively as combatants against microorganisms involved in the sliming of underwater surfaces.

• The physical properties of acrylic organometallic polymers suggests their use as slimicidal underwater optics and optical coatings.

#### FUTURE WORK

The biochemical differentiation of microbial slime isolates will be completed. The biotic progression of primary fouling organisms will be closely followed, and representative species of each stage in this progression will be isolated and studied. Synthesis of organometallic polymeric materials will be continued and their performance evaluated. Statistical methods of bioassay using barnacle cyprids and microorganisms will be used to evaluate the antifouling performance of the organometallic polymeric biocides. Additional emphasis will be placed on the utility of the "contact toxic" principle for future antifouling coatings in general.

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Figure 1 - Submersible Experimental Unit



Figure 2 SEU Mooring Buoy Array



Figure 3 - Carr Creek, Annapolis, Maryland



Figure 4 Sliming Culture from Glass Surface 5-Minute Submergence; 10 Diverse Organisms



Figure 5 Sliming Culture from Glass Surface l-Hour Submergence; 6 Diverse Organisms



Figure 6 Sliming Culture from Glass Surface 24-Hour Submergence; Predominantly 2 Diverse Organisms



# Figure 7

Inhibition of a Marine Bacterial Isolate Culture; Determined by the "Ring of Inhibition" Method Using Organometallic Compounds (Scale 1:2)



Figure 8 Relative Annular Toxicity of Organometallic Compounds to Sliming Microorganisms

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KET WORDS	ROLE	wт	ROLE	wт	ROLE	wт
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Slime						
Bacteria						
Diations						
Hudroida						
Algae						
Bryozoans						
Protozoans			•			
Macrofoulants						
Barnacles						
Emolient films						
Coatings						
Biocides						
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