The Formation of a "Primary Film" on Materials Submerged in the Sea at Port Hueneme, California[1]

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THE INITIAL STAGES OF FOULING, the accumulation of microscopic organisms, and formation of a primary film or slime layer, have received but sparse attention in the literature. To cite an example, the U. S. Naval Institute publication *Marine Fouling and Its Prevention* contains hundreds of references to fouling but only eight pertain to the primary film.

This area of concern, however, has not lacked for advocates of research. ZoBell, among his many notable contributions to marine microbiology, attributed considerable significance to the primary film and, in 1939, stated that "microorganisms merit considerable attention in investigating the exact cause and ultimate prevention of fouling." With E. C. Allen, ZoBell (1935) submerged glass slides for periods of a few hours to several days and noted that within an hour bacteria tenaciously attached to the slides. In a 24-hour period the number of bacteria adhering to a square centimeter of slide varied from 218,000 to 702,000. The number increased manyfold if the glass slides were treated with nutrient. Gram negative, rod-shaped organisms appearing singularly rather than in groups were predominant. The appearance of bacteria was followed by diatoms, which soon became the principal microorganism inhabitant on the slides. Wood (1950), in similar research off the coast of Australia, observed that diatoms and algae, not bacteria, were the predominant forms found on glass slides during the initial stages of fouling. In the Adriatic Sea, Cviic (1953) noted that, although bacteria were the principal components of the primary film, their number on test slides did not approach that cited by ZoBell. Certainly the numbers and kinds of organisms vary from site to site and, within a given area, the population and its components are subject to variations of the environment.

Hilen (1923) and Angst (1923) recorded the presence of unidentified yeasts in the early stages of fouling of ships' hulls in Puget Sound. ZoBell and Allen (1935) make the sole reference to Actinomycetes as components of the primary film. A few references pertaining to protozoan components of the primary film are available, notably those of Hentschel (1916), Waksman, Phelps, and Hotchkiss (1940), and Skerman (1956).

Because of the paucity of research and publications on the primary film, its relation to subsequent attachment of macroscopic organisms is not at all clear. Angst (1923) and Whedon (1937–1941) stated that primary film formation is a prerequisite to subsequent attachment of macroorganisms. Hilen (1923), Miller and Cupp (1942), Harris (1946), Miller (1946), and Skerman (1956), on the other hand, stated that, though a primary film may accelerate attachment of macroorganisms, its presence is not necessary for attachment. ZoBell (1939) suggested that the primary film may facilitate attachment by one or more of the following mechanisms: (1) enmeshing free-swimming larval forms, (2) discoloring bright or glazed surfaces, (3) protecting fouling organisms from toxic components of paints, (4) serving as a source of food, (5) increasing the pH of film surface interface, thus favoring deposition of calcareous cements by sessile organisms, and (6) influencing the potential of the surface, thereby expediting attraction and attachment.

This paper is concerned with primary film formation on structural materials in a marine environment. The changes of populations as a function of time are noted. The qualitative and quantitative differences of films formed simultaneously at different sites in the same harbor have been investigated, as well as films found at the same site in different years.

AREA OF STUDY

The study was conducted in the harbor at Port Hueneme, California, during the period

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of July to September 1965 and July to September 1966. Two sites were utilized during the investigation (Fig. 1):

Site *A:* Mock Ship Pier near entrance to harbor. The samples were exposed at the end of the 300-foot pier. The water depth varied from 4 feet at mean low tide to 12 feet at mean high tide. The samples were placed approximately 2 feet above the bottom.

Site *B:* Corrosion Dock located in the harbor channel. The samples were exposed at a distance of approximately 25 feet from the shore. The water depth varied from 13 feet at mean low tide to 20 feet at mean high tide. The samples were suspended about 5 feet from the bottom.

During the experimental periods the water temperature ranged from 13° C to 18° C, the oxygen concentration from 5.4 to 8.3 ppm, and the salinity from 32‰ to 33‰. Between the two sites the three physical parameters cited above did not significantly vary.

MATERIALS AND METHODS

Racks for test samples were constructed on Monel bars measuring 28″ × 1″ × 1/8″. Seven 3/8″ holes drilled 3″ apart served as attachment sites for the samples to be immersed and each sample was held in place by a numbered stainless steel tag. The seven different samples attached to each rack were as follows:

1. Glass slide, 4″ × 3¼″ × 1/8″
2. Polymethylmethacrylate (PMM) slides, 6″ × 1½″ × ¼″
3. Uncoated steel, 6″ × 2¾″ × 1/32″
4. Coated steel, as above, but pretreated with

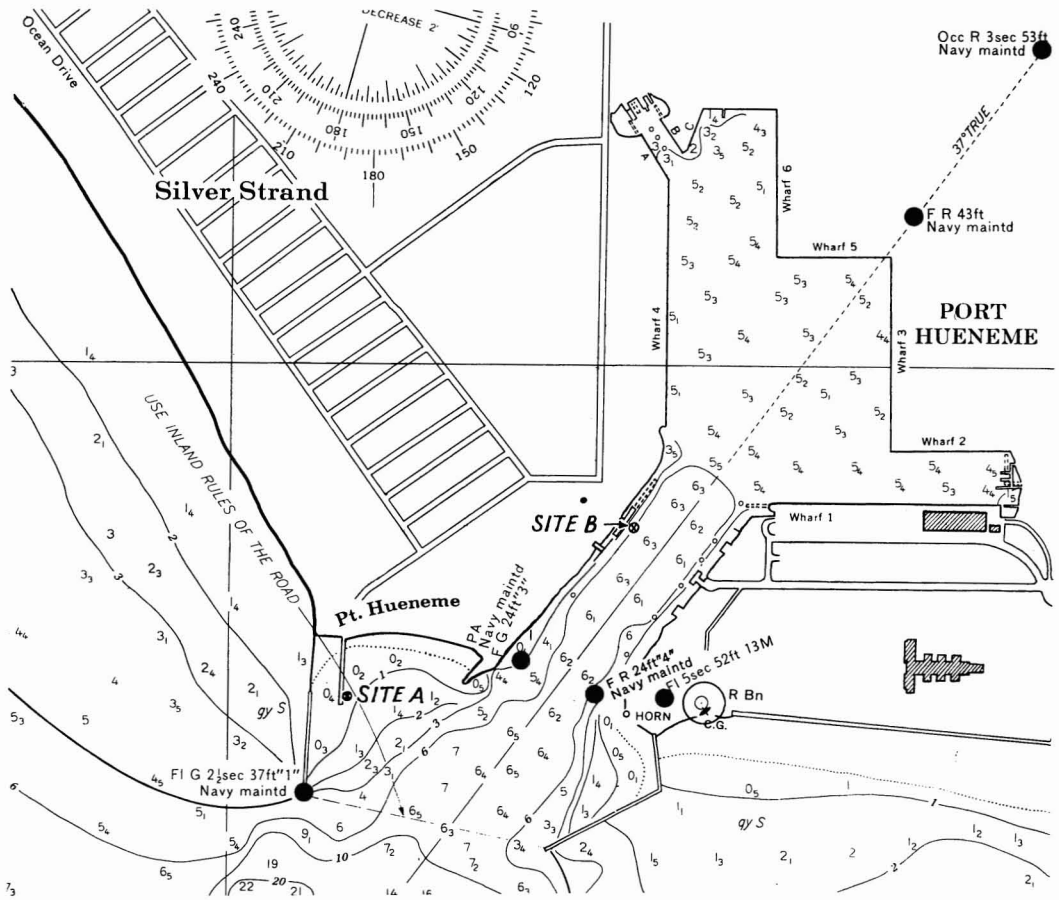

FIG. 1. Port Hueneme Harbor showing exposure sites *A* and *B*.

Formula 117, painted with vinyl red lead followed by a topcoat of white vinyl mastic
5. Douglas fir, 6″ × 1½″ × ¼″
6. Douglas fir, as above, but impregnated with 12 percent creosote
7. Douglas fir, as above, but impregnated with 100 percent creosote

Individual slides of glass and PMM suspended with nylon line and weighted with lead sinkers were extensively used and proved to be more readily manipulated than were the larger racks.

Prior to immersion in water, each sample was swabbed in 70-percent ethyl alcohol to remove the major portion of microbial contamination. The technique was not designed to be aseptic, though no growth resulted when newly swabbed glass slides were placed in dishes of nutrient agar and incubated.

Upon removal from the water, one side of each sample was stroked with sterile swabs which were then used to inoculate various media as follows: trypticase soy agar for bacteria, Starkey's medium for sulfate-reducing bacteria, and mycophil agar for fungi and yeasts. All media were prepared using aged seawater. Following this procedure, the swabbed side of the glass and polymethylmethacrylate (PMM) slides were cleaned in preparation for microscopic examination of the opposite side. A section of each slide was stained with Hucker's crystal violet for determination of microbial populations. The direct count method was used for the enumeration of bacteria and diatoms, and the number of fields counted complied with the statistical requirements of Hanks and James (1940). Because of their opacity, samples other than those of glass and polymethylmethacrylate could be examined only with low magnification and reflected light. The authors were concerned that manipulation of the slides might affect a removal of attached microorganisms and a consequent unreliable cell count. The results of two simple experiments allayed this apprehension. First, cell counts were made on two glass slides that had been immersed for 4 hours and 12 hours, respectively. After counting, the slides were vigorously washed and a second count was not appreciably different from the first. In the second experiment one-half of a glass slide that had been immersed for 4 hours was swabbed, washed, and stained. The cell count in the treated area was 1,325/cm² and in the untreated half was 1,500/cm². These results lend credence to the conclusion that microorganisms tenaciously adhere to surfaces.

Each opaque sample was uniformly stroked with a sterile cotton swab which was then used to prepare appropriate dilutions. This technique is not designed to yield accurate quantitative results but provides an estimate of relative population sizes and a means of isolating microbial constituents of the primary film to obtain pure cultures for identification. On all slides the presence of fungi and algae was determined by noting hyphae or filaments, respectively, rather than spores, unless the latter were present in large numbers.

RESULTS

For the sake of clarity, the results are given in three different sections as follows: (*a*) comparison of samples from a single site—Site *B;* (*b*) comparison of glass slides placed at the same site in two different years; and (*c*) comparison of glass slides simultaneously placed at two different sites.

Comparison of Samples from a Single Site—Site B

1-HOUR IMMERSION: After this minimal exposure, no apparent macroscopic changes were recorded except that the uncoated steel surfaces were dotted with early signs of corrosion. Microscopic examination of the PMM and glass slides revealed a flora consisting solely of gram-negative bacteria—medium-size rods, occurring singly, and identified as pseudomonads. The number of bacteria per square centimeter of glass and PMM (Plexiglas) was 620 and 860, respectively. The slightly lesser number recorded from the glass slide might well be due to the smoothness of the surface, an influence cited by Pomerat and Weiss (1946). A sample of water adjacent to the racks had a bacterial count of 247 per ml. The comparative paucity of bacteria in water adjacent to a submerged surface having a larger number of organisms was cited by ZoBell and Allen (1935) as attesting to the importance of surfaces for microbial attachment.

2-HOUR IMMERSION: Macroscopically the samples did not differ from those previously described, though the corrosion of uncoated steel was more pronounced. Again, bacteria, specifically pseudomonads (Fig. 2), were the sole microorganisms on the glass and PMM samples, where their population was 710 per cm^2 and 1,260 per cm^2, respectively.

4-HOUR IMMERSION: Macroscopic study of the samples indicated no conspicuous change of the surfaces, though again the notable exception was uncoated steel, which was severely corroded. The majority of bacteria observed were again pseudomonads. The number of bacteria removed by swabbing untreated wood was much greater than from any other samples. The number of bacteria removed from swapped glass slides was greater than the number removed from swabbed PMM slides, though direct counts yielded the opposite results. Perhaps the bacteria of the primary film are less tenacious on the smoother glass slide than on the PMM slide. The maximum number of genera isolated from a single sample (wood) was six, including *Pseudomonas, Achromobacter,* and *Flavobacterium. Pseudomonas* was the one genus common to all samples having bacteria. The genera of fungi identified after isolation and growth on mycophil agar were *Alternaria* and *Helicoma.* The former was isolated from wood samples, the latter from the glass. Yeasts were identified as *Rhodotorula* sp. and *Torula* sp. Both genera were isolated from the untreated wood, whereas *Rhodotorula* was common to all samples from which yeasts were isolated. Table 1 summarizes the observations after immersion for 4 hours.

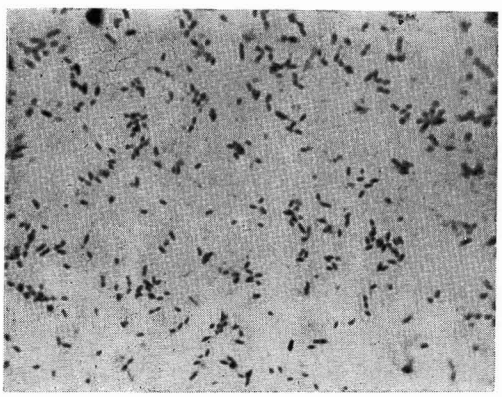

FIG. 2. Bacteria (pseudomonads) on glass slide after 2 hours' immersion in seawater. ×1200

6- AND 8-HOUR IMMERSION: These two time periods are simultaneously discussed since differences between the two groups of samples were primarily quantitative. Similarly, differences between those two groups of samples and those in the 4-hour immersion were principally quantitative, though some significant qualitative differences existed. Foremost of these was the appearance of diatoms after 6 hours of immersion for the PMM sample and after 8 hours of immersion for the glass, untreated wood, and coated steel samples. *Navicula* and *Nitzschia* were the genera observed, the former in greater abundance on all samples. The diatoms were not in clusters on the glass and PMM slides but were more or less evenly distributed. The diatoms did not appear to be associated with aggregates of bacterial cells, nor was there any accumula-

TABLE 1

SUMMARY OF OBSERVATIONS OF SAMPLES IMMERSED FOR 4 HOURS IN SEAWATER

TYPE OF ORGANISM	TYPE OF SAMPLE						
	WOOD UNTREATED	WOOD 12% CREOSOTE	WOOD 100% CREOSOTE	GLASS	POLY-METHYL-METHA-CRYLATE	STEEL UNCOATED	STEEL COATED
Fungi (other than yeasts)	+ (1)	+ (1)	—	+ (1)	—	—	—
Yeasts	+ (2)	+ (1)	—	+ (1)	+ (1)	—	+ (1)
Bacteria	+ (6)	+ (2)	+ (1)	+ (4)	+ (3)	+ (2)	+ (1)
Diatoms	—	—	—	—	—	—	—

NOTE: +, presence of organisms; —, absence of organisms. Numbers in parentheses refer to numbers of genera isolated.

tion of detritus evident in the vicinity of diatoms. The surfaces of samples were neither slippery nor gritty to the touch.

The number and kinds of bacteria present were significantly different from samples examined previously. *Micrococcus* sp. and *Sarcina* sp., both coccoid organisms, were detected, as well as those previously identified. Of particular interest to the authors was that *Pseudomonas creosotensis* was found on all three wood samples after 8 hours of immersion. Because of time limitations, no attempt was made to determine the identity of all the bacteria isolated. At least 30 different species of bacteria were isolated from glass, which yielded the largest number. The wood impregnated with 100-percent creosote had the fewest number of different species, three; and wood impregnated with 12-percent creosote the next fewest, five. The technique of swabbing samples recently removed from the water does not precisely differentiate between the organisms that may be attached to the samples and those that are transients.

The increase in number and kind of yeast species appears to parallel closely the increase of bacteria. Three additional species of yeasts were isolated from wood and two from glass. Of interest was the isolation of *Pullularia* sp. from both samples of wood impregnated with creosote after immersion for an 8-hour period. *Hansenula* sp. was also isolated from untreated wood samples.

Several additional fungi were isolated from the samples. Of these *Penicillium* sp. and *Aspergillus* sp. were identified. The summary of observations of samples immersed for 8 hours is given in Table 2.

1-DAY IMMERSION: Samples viewed with the unaided eye revealed that the differences between these and the 8-hour samples were considerable. The untreated wood, glass, PMM, and coated steel samples bore a moist or slimy scum quite homogenous in appearance and smooth to the touch. The uncoated steel showed extensive corrosion, and large fragments of rust exfoliated from the samples as they were removed from the water. The now-exposed metal surface was dark in color but had no perceptible slime layer. Fungi, yeasts, bacteria, and diatoms were present on all samples, whereas sulfate-reducing bacteria, protozoa, and algal filaments were absent on all. Microscopic examination of the slime revealed that it primarily consisted of bacteria and, to a somewhat lesser extent, organic detritus and diatoms. The bacteria and diatoms were not as evenly distributed as they had been on previously examined plates, but rather were in clusters or aggregates in masses of detritus. The number of bacteria and diatoms was greater than in samples previously examined, but the number of different kinds did not appear to vary extensively. Freehand sections of wood were stained with cotton blue in lactophenol and examined microscopically, but no intracellular lignicolous fungi were detected.

Among the bacteria isolated on the test specimens for the first time were the genera *Vibrio* and *Bacillus*. Different species of genera previously cited in this report were noted, though species identifications were not made.

The diatom genera initially observed on these samples were *Grammatophora* and *Striatella*, both of which occurred in short chains.

The fungus *Cephalosphorium* was isolated from untreated wood samples.

TABLE 2

SUMMARY OF OBSERVATIONS OF SAMPLES IMMERSED FOR 8 HOURS IN SEAWATER

TYPE OF ORGANISM	TYPE OF SAMPLE						
	WOOD UNTREATED	WOOD 12% CREOSOTE	WOOD 100% CREOSOTE	GLASS	POLYMETHYL-METHACRYLATE	STEEL UNCOATED	STEEL COATED
Fungi (other than yeasts)	+ (3)	+ (1)	—	+ (5)	+ (4)	—	—
Yeasts	+ (5)	+ (1)	+ (1)	+ (3)	+ (5)	—	+ (2)
Bacteria	+ (20)	+ (5)	+ (3)	+ (30)	+ (26)	+ (7)	+ (18)
Diatoms	+ (2)	—	—	+ (2)	+ (2)	—	+ (1)

NOTE: +, presence of organisms; —, absence of organisms. Numbers in parentheses refer to numbers of genera isolated.

The yeasts isolated from the 24-hour samples were identified as genera previously noted.

A summary of observations is given in Table 3.

2-DAY IMMERSION: The slime, so characteristic of the 1-day samples, became more pronounced after 2 days. The untreated wood, glass, PMM, and coated steel bore a rather heavy slime which seemed to be homogenous to the touch, though visually the slime consisted of darker areas scattered throughout a lighter colored mass. The uncoated steel, more heavily corroded than in previous samples, bore no slime and the woods impregnated with creosote possessed but a faint hint of slime. Microscopic observations of slime revealed amounts of detritus and microbial constituents which were similar in quantity and quality to those of the 1-day samples. Protozoa, sulfate-reducing bacteria, algal filaments, and fungal hyphae were not observed, though representatives of the latter two groups were isolated by the swabbing technique.

4-DAY IMMERSION: The conspicuous feature of these samples was the texture of the slime on the glass, PMM, untreated wood, and coated steel samples. The panels, formerly smooth to the touch, were somewhat granular and coarse. Visually they appeared as before; that is, light amber colored with circular darker areas. Microscopically a large number of colonial diatoms were discernible, the most prominent of which was *Licmorphora flabellata* (Fig. 3), a fan-shaped aggregate occurring in very dense tufts

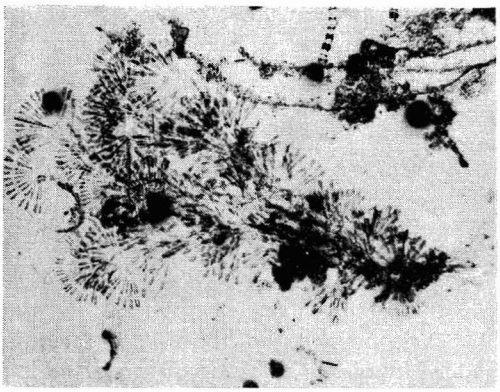

FIG. 3. Stalked colonial diatom *Licmorphora flabellata* on a glass slide after 4 days' immersion in seawater. ×100

and attached to the surfaces by means of a gelatinous stalk. The stalks appeared to be a collecting site for bacteria, solitary diatoms, and considerable detritus. In some areas of the glass slide other diatoms seemed to predominate. In the same glass slide containing many colonies of *Licmorphora,* pure cultures of the unicellular solitary *Cocconeis scutellum* (Fig. 4) were present. The average size of *C. scutellum* is 50 μ by 30 μ and in regions where this form predominated it occupied over 50 percent of the sample area. Fungal hyphae were observed growing on glass and PMM slides for the first time. Species identification of the fungi could not be made because of the absence of reproductive structures.

TABLE 3

SUMMARY OF OBSERVATIONS OF SAMPLES IMMERSED FOR 24 HOURS IN SEAWATER

TYPE OF ORGANISM	TYPE OF SAMPLES						
	WOOD UNTREATED	WOOD 12% CREOSOTE	WOOD 100% CREOSOTE	GLASS	POLYMETHYL-METHACRYLATE	STEEL UNCOATED	STEEL COATED
Fungi (other than yeasts)	+ (4)	+ (1)	—	+ (6)	+ (4)	—	+ (2)
Yeasts	+ (5)	+ (1)	+ (2)	+ (5)	+ (6)	—	+ (3)
Bacteria	+ (22)	+ (5)	+ (4)	+ (37)	+ (31)	+ (8)	+ (22)
Sulfate-Reducing Bacteria	—	—	—	—	—	—	—
Protozoa	—	—	—	—	—	—	—
Algae (other than diatoms)	—	—	—	—	—	—	—
Diatoms	+ (4)	+ (2)	+ (2)	+ (4)	+ (5)	+ (2)	+ (5)

NOTE: +, presence of organisms; —, absence of organisms. Numbers in parentheses refer to numbers of genera isolated.

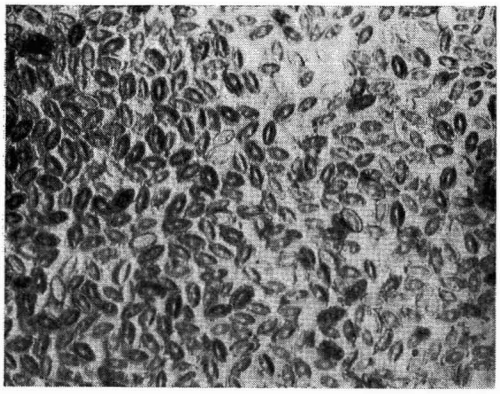

FIG. 4. Pure culture of solitary diatom *Cocconeis scutellum* on glass slide after 4 days' immersion in seawater. ×100

Sulfate-reducing bacteria were isolated for the first time and only on uncoated steel.

Protozoa, algal filaments, and macroscopic organisms were not discernible on the samples.

8-DAY IMMERSION: The large assemblage of different diatoms that so characterized the 4-day samples was again apparent in the 8-day samples. The number of diatoms and different species appeared to increase. Again colonial forms predominated and new species recorded were *Chaetoceros* sp. and *Melosira* sp. The coarse granular texture of previously examined samples was intensified. The uncoated steel had but a faintly detectable slime layer, a characteristic shared with the creosote-impregnated woods. Sulfate-reducing bacteria were isolated from the uncoated steel and the untreated wood sample, but from no other samples.

Algal filaments were isolated from glass, PMM, untreated wood, and coated steel samples. Most of the filaments were found on the edge of the samples where saw blades made rougher surfaces during fabrication. *Enteromorpha* sp., a filamentous green alga, was also found on the untreated wood samples.

Stalked colonial protozoa of the genus *Zoothamnium* were found on wood, glass, and PMM slides, thus marking the first appearance of protozoa.

The number of bacteria and diatoms was so great as to nullify accuracy of counting. In brief, the samples of untreated wood, uncoated steel, coated steel, glass, and PMM slides possessed vast aggregates of microbial populations in masses of organic debris though, as yet, no macroscopic forms were evident.

12- AND 16-DAY IMMERSION: Because of the great similarity of these samples, they are discussed simultaneously. The large populations of diatoms and bacteria previously reported were found in still greater numbers. The slime layer, extremely coarse and granular on the glass and PMM slides as well as the untreated wood and coated steel, was thicker than in previously examined samples. The creosoted woods possessed a greater slime than previously, though the nature of this slime was mucoid rather than coarse. Microscopic examination of the swabbing from such samples revealed a microbial constituency similar to that which occurred in early stages of fouling of untreated wood, coated steel, glass, and PMM slides.

Sulfate-reducing bacteria were isolated from all samples except the glass slide. Of considerable interest was the initial appearance of macroscopic animals on samples of both age groups. The untreated wood, coated steel, glass, and PMM slides all had growths of the hydroid *Obelia dichotoma* and the bryozoan *Lichenopora radiata.* The coated steel, after 16 days of immersion, had a single specimen of the tubeworm *Eupomatus gracilis,* and the glass slide had a single specimen of the barnacle *Balanus tintinnabulum.*

Because subsequent observations will emphasize microbial populations on glass slides, a summary of results for 16 days of immersion is given in Table 4. For comparative purposes, the table includes a summary of results on PMM slides.

LONGER PERIODS OF IMMERSION: After approximately 3 weeks of immersion, hydroids appeared on the surfaces and soon became the conspicuous and dominant organism. By the end of the 6th week, algae, notably the brown alga *Ectocarpus* sp. and the green alga *Ulva* sp., became the conspicuous component of the primary films. At the end of the 9 weeks when the surfaces were covered with algae, the barnacle *Balanus tintinnabulum* was evident on the samples and thus initiated macroscopic fouling.

DISCUSSION: A film of microorganisms began to form on the surface of structural materials within an hour after immersion in seawater. Initially, the film consisted of bacteria and

TABLE 4

SUMMARY OF OBSERVATIONS OF SLIDES OF GLASS AND POLYMETHYLMETHACRYLATE AFTER IMMERSION IN SEAWATER

PERIOD OF IMMERSION	GLASS				POLYMETHYLMETHACRYLATE			
	BACTERIA NO. PER CM^2	DIATOMS NO. PER CM^2	ALGAL FILAMENTS	FUNGAL HYPHAE	BACTERIA NO. PER CM^2	DIATOMS NO. PER CM^2	ALGAL FILAMENTS	FUNGAL HYPHAE
1 hour	620	—	—	—	860	—	—	—
2 hours	710	—	—	—	1,260	—	—	—
4 hours	1,500	—	—	—	7,500	—	—	—
6 hours	8,700	—	—	—	53,600	7	—	—
8 hours	189,000	10	—	—	110,400	30	—	—
1 day	300,000	78	—	—	655,000	37	—	—
2 days	605,000	320	—	—	1,274,300	410	—	—
4 days	1,580,000	1,780	—	present	2,897,600*	2,150	present	present
8 days	3,570,600*	9,630*	present	present	4,685,400*	15,700*	present	present
12 days	5,671,700*	11,500*	present	present	top numerous	17,500*	present	present
16 days	2,650,000*	18,300*	present	present	top numerous	21,900*	present	present

NOTE: —, absence of organisms.

* Counts inaccurate because of accumulation of organisms and detritus.

detritus. Within a day after immersion, solitary diatoms became common on the surfaces and after several days were the principal constituent. Concomitant with an increase in the amount of detritus and in the number of bacteria and solitary diatoms was the appearance of a slightly brown mucoid film. As colonial diatoms accumulated the film became gritty to the touch and less uniform in appearance.

The succession of microorganisms appeared to be very regular, to an extent that one can speak of phases of succession somewhat analogous to succession of land plants on disturbed soil. The different phases are respectively termed bacterial, diatom, hydroid, algal, and barnacle. The phases cited refer to the predominant organisms and do not imply the absence of other microorganisms on the surfaces. Fungi and protozoans were present after 4 days of immersion, but never in conspicuous or even significant numbers.

The quantity and quality of the setting organisms differed from sample to sample. The initiation and subsequent development of a primary film on untreated wood occurred at a much more rapid rate than on wood impregnated with creosote. The rate of settling on a rough surface was more rapid than on a smooth surface.

The simultaneous apppearance of detritus with microorganisms is of interest and at this time a determination cannot be made as to whether the accumulation of detritus was the cause or effect of microbial settling or if, indeed, there was a relationship.

These observations also demonstrate the effect of the presence of surfaces for bacterial reproduction. The number of bacteria in water adjacent to the samples is dramatically lower than on the surfaces of the samples.

Comparison of Glass Slides Placed at the Same Site in 2 Different Years

In both the summers of 1965 and 1966 the environmental parameters at the corrosion dock (Site *B*) were similar as was the bacterial population of the seawater adjacent to the immersed samples. The quality, quantity, and sequence of succession of the principal microbial components of the primary film formed in 1966 appeared to differ from that occurring at the same site the previous year. Table 5 summarizes the significant differences. In 1965, the succession was very regular with bacterial, diatom, hydroid, and algal phases clearly delineated, a characteristic that was not conspicuous in 1966. In the earlier year the glass slides, for example, were uniformly covered with what appeared to be an almost pure culture of bacteria after a relatively short period of immersion. Diatoms, which appeared later, likewise were in almost pure culture. Yeast cells were very rare.

In 1965, the plates were covered with a homogenous and mucoid film after 1 day. The following summer, after immersion for 1 day, a macroscopically visible slime layer was not present, nor was the slide slimy or gritty to the touch. Both the bacteria and diatoms tended to be in clumps, a characteristic shared with yeast cells. After an initial 3-week period of immersion during both summers, a similar succession was observed. The hydroids *Obelia dichotoma* and *Eudenderium rameum* appeared on the surfaces and soon became the dominant organisms. At this time the bacteria population was too large to be enumerated (greater than 5×10^6 /cm^2). The brown alga *Ectocarpus* sp. was present in small numbers (eight per slide) after 3 weeks' immersion, but after 5 weeks was the dominant organism. Another algal species, the red alga *Polysiphonia,* was also present but not in large numbers.

By the 8th and 9th weeks the barnacle *Balanus tintinnabulum* became the conspicuous form, thereby indicating macroscopic fouling. At this time tubeworms and bryozoans were also found on the slides.

DISCUSSION: The differences in events of the two summers appear not to be attributable to those environmental characteristics at the time of observation. Microenvironmental differences on the surfaces of the slides may account for differences. Macroenvironmental differences, preceding the period of observation, that might alter the flora and fauna of the water adjacent to the slides might also produce the differences noted.

The rapid and complete colonization of the slide by bacteria immediately after immersion in water appears to delay attachment of diatoms and thus provides a more clearly delineated succession. If space is not occupied by bacteria, diatoms rapidly settle. It thus appears that the

TABLE 5

SUMMARY OF MICROBIAL SUCCESSION ON GLASS SLIDES

TIME	TYPE OF ORGANISM	JULY TO SEPTEMBER 1965	JULY TO SEPTEMBER 1966
1 Hour	bacteria	620/cm^2 *Pseudomonas*	6,400/cm^2 *Pseudomonas*
	yeasts	none	6,000/cm^2 *Torula*
	diatoms	none	5/cm^2 *Surirella*
2 Hours	bacteria	710/cm^2 *Pseudomonas*	8,700/cm^2 *Pseudomonas, Achromobacter, Micrococcus, Actinomyces*
	diatoms	none	*Surirella, Navicula*
	yeasts	none	8,000/cm^2 *Torula*
4 Hours	bacteria	1,500/cm^2 *Pseudomonas, Micrococcus, Achromobacter, Flavobacterium*	17,200/cm^2. Same as 2-hour sample
	diatoms	none	*Acnanthes, Chaetoceros, Cocconeis, Grammatophora, Navicula, Nitzschia, Surirella*
	yeasts	*Rhodotorula*	*Torula* (clumping made enumeration impossible)
	fungi (other than yeasts)	*Helicoma*	none
8 Hours	bacteria	189,000/cm^2 30 species	29,700/cm^2
	diatoms	10/cm^2 *Navicula, Nitzschia*	Same as 4 hours, but also *Licmorphora*
	yeasts	3 species	*Torula, Rhodotorula*
	fungi (other than yeasts)	5 species	none
24 Hours	bacteria	300,000/cm^2 37 species	430,000/cm^2
	diatoms	78/cm^2 4 genera	12/cm^2 10 genera
	yeasts	5 species	—
	fungi (other than yeasts)	6 species	—

NOTE: —, absence of organisms.

presence of bacteria is not necessary for the subsequent attachment of other microorganisms. The presence of yeast cells in large numbers during 1966 cannot be explained, though the introduction of organic substrates into the harbor may provide a basis for speculation.

Comparison of Glass Slides Placed Simultaneously at Two Different Sites, Site A and Site B

The temperature, salinity, and oxygen concentration of the seawater at sites *A* and *B* did not significantly differ. However, a meaningful difference in the biological characteristic of the two sites was the large amount of macroscopic fouling at Site *A*. The piling was covered with goose barnacles, *Pollicipes polymerus;* California mussels, *Mytilus californianus;* acorn barnacles, *Balanus glandula;* the tubeworm *Eupomatus gracilis;* the bryozoan *Bugula neritina;* and the striped barnacle *Balanus tintinnabulum.*

After immersion for 1 hour at Site *A,* the dominant organisms were not bacteria, as at Site *B,* but yeasts of the genus *Torula* (Fig. 5). Their distribution was generally not uniform but, rather, they appeared in aggregates upon the slide. Within the aggregate many budding cells were evident. Organic matter was common on the slide and appeared as a gathering site of yeast cells. Bacteria were present, though in small numbers (780/cm^2) when compared to the bacterial populations (6,400/cm^2) recorded at the same time at Site *B*. No diatoms nor mi-

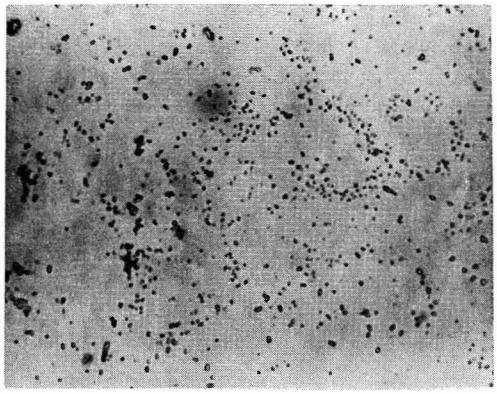

FIG. 5. Yeast *Torula* sp. on a glass slide after 1 hour's immersion at Site *A*. ×400

croorganisms other than yeasts and bacteria were found on this slide. This condition predominated during the first 24 hours of immersion, and at the end of this period the yeast population was 17,600/cm² and the bacterial population 10,300/cm². Diatoms were noted only after 4 days of immersion, at which time the diatom flora was sparse and the number of individuals was equaled by the number of different genera recorded: *Melosira* sp., *Licmorphora* sp., *Grammatophora* sp., *Navicula* sp., *Chaetoceros* sp., and *Striatella* sp.

After but 1 day of immersion, a strong buildup of barnacle population was noted. Two barnacle larvae were found after 1 day and after immersion of 2, 3, 4, and 5 days the barnacle count was 44, 51, 55, and 63, respectively. All of the barnacles were in the cyprid stage. Concomitant with the attachment of barnacles to the slide was the appearance of algal spores. After 1 day of immersion, 12 spores were found on the slide and subsequently 27, 68, 75 and 112 spores were found on slides after immersion of 2, 3, 4, and 5 days, respectively.

At Site *A* the hydroid *Obelia dichotoma* was noted on slides after 4 days of immersion, though it did not appear in large numbers until 5 weeks of immersion, at which time it was not the dominant organism. The hydroid phase so characteristic at Site *B* did not occur at Site *A*. Furthermore, algae which were the successors of hydroids at Site *B* were also not a dominant group at Site *A*.

A macroscopically conspicuous film was evident on slides after immersion for 8 days. The film so formed was different from those at Site *B* in that it was much coarser both in appearance and to the touch. Microscopic examination suggested that this difference was due to the presence of colonial diatoms to an extent not previously observed, lengthy colonies of *Chaetoceros* sp., *Navicula ramosissima, Melosira* sp., *Grammatophora* sp., *Acnanthes longipes,* and *Licmorphora flabellata.* All of these genera had been previously seen on other slides but not in the lengthy filaments found at Site *A*. Solitary cells of these genera or other genera were rare. The copepod *Calanus* sp. was common on the 8-day slide and appeared to be grazing upon diatoms. An unidentified polychaetous worm and cyprid larvae of barnacles also grazed upon the diatoms. After the 9th week of immersion, a few algae did appear, notably the brown alga *Ectocarpus* sp. and the green alga *Enteromorpha* sp. The presence of green algae might be explained by the location of samples at a depth shallow enough to permit the photosynthesis of these organisms.

Another organism not previously seen on other slides in the early stages of fouling but found at Site *A* after 2 weeks of immersion was the encrusting bryozoan *Lichenopora radiata.* Similarly, colonial protozoans such as *Zoothanmium* sp. and the foraminiferan *Foraminifera pulvinulina* were also found on this slide.

At Site *A,* macroscopic fouling organisms such as barnacles, *Balanus tintinnabulum;* mussels, *Mytilus californianus;* acorn barnacles, *Balanus glandula;* and goose barnacles, *Pollicipes polymerus* appeared in significant numbers at the end of the 5th week of immersion.

DISCUSSION: The quantitative and qualitative differences between sites *A* and *B* are believed to be due to the differences in biological parameters. At Site *A,* the slides were placed in water adjacent to pilings with considerable macroscopic fouling. This fouling was similar to that found at Site *B* but was much more extensive. The biological population at Site *A* was also reflected in the slower rate of formation of the primary film. There were more yeast cells at Site *A* and relatively fewer bacteria and diatoms. When diatoms occurred they were colonial forms. The early appearance of barnacle larvae at Site *A* indicates that a primary film is not

necessary for attachment; in fact, the physical presence of bacteria could make barnacle attachment more difficult. The advanced stages of fouling already present at Site *A* negate somewhat the importance of the primary film, for example, as a food source for macroscopic fouling organisms. The lack of a rich biotic community at Site *B* necessitates a primary film and could account for the orderly succession of organisms.

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