NOTES

New Technique for Microscopic Examination of the Fouling Community of Submerged Opaque Surfaces

GARY E. SECHLER AND K. GUNDERSEN

Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822

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The fouling film formed on surfaces of opaque materials submerged in seawater could be almost quantitatively removed for microscopy by application of a Parlodion film.

It has been well established (1) that in the initial stage of fouling of submerged surfaces bacteria and the primary film of slime formed by them promote the attractiveness of the surfaces to other microorganisms and to sessile aquatic invertebrates. Such films also act as a trap for organic and inorganic particulate matter suspended in the surrounding water and, in turn, enmesh not only the associated bacteria but the entire microecosystem of the submerged surfaces.

In situ microscopic studies on the populations of natural surface films have classically been limited to glass (2, 3). The present study shows, however, that the attached microcommunity may be removed intact from a variety of test surfaces in a Parlodion film and subsequently be stained and mounted for microscopy. Small qualitative and quantitative differences can easily be followed on any surface which is not highly porous or absorbent, i.e., soft wood. Bacteria, diatoms, various filamentous algae, and attached extraneous matter have been observed in their natural spatial states as clearly as if attached directly to glass.

Panels (25 by 75 by 1 mm) of four metal alloys, including aluminum 5052 (Reynolds), stainless-steel 304 (ALSC Corp.), nickel-copper alloy 400 (“Monel,” Inco Corp.), phosphor-bronze (type A, Porter), and two nonmetals, including fiberglass (Filon type 140, with polyester resin) and glass (as standard microscope slides), were used.

After immersion in the sea for different periods of time, the panels were rinsed gently with sterile seawater and left under cover at room temperature for 2 to 3 days to dry thoroughly. Neither desalination nor chemical fixation was done on any of the test surfaces after immersion.

A 10% solution of Parlodion (Mallinckrodt) was prepared with amyl acetate as solvent; gentle overnight agitation on a shaking machine was used to dissolve the plastic.

Approximately 40 ml of Parlodion solution was added to a 50-ml beaker and placed into a liter jar with a plastic screw cap, the latter with a covered opening (2 by 4 cm) for inserting and removing test panels. It was found important to insure complete saturation of the atmosphere inside the jar at all times.

The dried test surfaces were inserted through the hatch and immersed into the Parlodion solution for at least 2 min. Without removing them from the jar, the panels were then raised from the solution and braced beneath the lid above the beaker for about 1 min to drain off excess Parlodion, withdrawn from the jar, blotted several times at the draining end on absorbent paper, and placed upright in a chemical hood for at least 2 hr to dry thoroughly.

Portions of the filmed surfaces convenient for mounting were excised with a razor blade, cutting deeply enough to contact sufficiently the test surface. One corner of the excised strip was then freed by gently inserting the blade beneath the film, and, by using blunt forceps, the latter was carefully peeled away with its embedded microcommunity. The strip was then immersed intact into a staining solution, rinsed, held with forceps in an air jet until dry and permanently mounted in Permount (Fisher). The side adjacent to the test surface must be mounted upwards, and a no. 1 thickness cover slip should be used to allow for oil immersion.

Crystal violet was the only stain used that did not fade after mounting. Those fading within 24
hr were toluidine blue, methylene blue, Loeffler's methylene blue, and safranin.

Difficulties involving the inability to remove the entire excised portion of the film intact were the result of a too short drying period, extensive absorption into a surface layer largely composed of a metallic oxide (mainly seen in aluminum surfaces after extended periods of immersion), or a too thin Parlodion film. Shortening the draining period or refilming a second time with thorough drying has been found to alleviate such problems, should they occur. Conversely,

FIG. 1. Glass surfaces, 9 days of immersion. (A and B) Direct observation of glass surface. (C and D) Parlodion mount of removed surface material. (E and F) Glass surface after Parlodion film removal. Note similarity of (A, C) and (B, D) and sparcity of remaining organisms in (E, F). (A, C, E) × 300; (B, D, E) × 2,600.
such materials as stainless steel, which retain their chemical passivity in seawater for extended periods of time, are among the easiest test surfaces from which to withdraw the film, which usually separates spontaneously when completely dry. Of several concentrations tested, 10% Parlodion was found to have the most suitable viscosity to permit intercellular infiltration, while allowing for convenient film removal. Refilming the various varieties of surfaces

![Image with figures A through F]

**Fig. 2.** Representative fields from stained Parlodion preparations after 14 days of immersion. Note obvious differences in varieties, numbers, and distribution of attached organisms on (A) glass, (B) fiberglass, (C) stainless steel, (D) Monel, (E) aluminum, and (F) phosphor-bronze. Heavy dark residue in Monel and phosphor-bronze preparations is exfoliation from surface. × 300.
from which Parlodion films have been previously removed demonstrated that essentially all adsorbed material is removed in the initial film- ing (Fig. 1). Examination of the test surfaces after film removal by using oblique lighting with a low-power magnifier showed all the opaque materials apparently to reacquire their original cleanliness and gloss. In a few cases, Monel was found to have pitted quite extensively; the exfoliation is likewise seen as clumps fairly evenly distributed within the mount (Fig. 2D). Phosphor-bronze, on the other hand, exfoliated extensively but evenly (Fig. 2F).

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