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Fiber glass-reinforced plastic immersed in an experimental estuarine mesocosm fouled at estimated rates of 0.5, 5.5, and 18.8 ng (wet weight) mm^{-2} day^{-1} over days 0 to 2, 2 to 6, and 6 to 14, respectively. Protists, dominated by diatoms, which developed between days 3 and 6 and covered 90% of the undisturbed surface in 2 weeks, were effectively removed by twice-weekly brushing of the surface to maintain an immature 3-day bacterial film which covered 12% or less of the surface and had a biomass 3 orders of magnitude smaller than surfaces with 2 weeks' unrestricted fouling. Direct brushing of the fiber glass-reinforced plastic tank walls of experimental estuarine mesocosms minimized the "wall effect" by keeping a surface that maintained a low biomass of a slowly accumulating bacterial film rather than a surface which supported the more rapid accumulation of protists which in turn may induce the settlement of invertebrates and macrophytes.

Microbial fouling of submerged surfaces in the sea (2, 3, 14, 20) is a precursor to macrofouling in the sea (5, 6, 21). This biological fouling is a major problem, causing a loss of heat transfer in heat exchangers such as those proposed for ocean thermal energy conversion, the loss of precision from submerged sensors, an increase in the hull drag of ships, and an undesirable effect on contained experimental planktonic ecosystems. It is primarily with the latter problem that this study was concerned, but the results are applicable to the limiting of fouling in general.

The use of large-volume enclosures for the study of biological and chemical processes taking place in the marine environment has received impetus in recent years (1, 16, 17). The study of any aquatic system in this manner has intrinsic in it the problem of containment. The introduction of walls around the study system introduces influences mediated by the presence of those walls, and this "wall effect" is of utmost concern if results obtained in these enclosures are intended to be applicable to the natural environment (18, 22).

This study evaluated the feasibility of limiting the accumulation of microorganisms on the walls of an experimental estuarine ecosystem by regular brushing. Experiments were carried out at the facilities of the Marine Ecosystem Research Laboratory (MERL) of the University of Rhode Island (19; M. E. Q. Pilson, C. A. Oviatt, and S. W. Nixon, Proc. Symp. on Microcosms in Eco-

logical Research, Augusta, Ga., in press). The experimental mesocosms at MERL are cylindrical tanks (inner diameter, 1.83 m; water column height, 5.0 m) composed of fiber glass-reinforced plastic. These tanks have been designed to simulate the planktonic ecosystem and its underlying benthos in nearby Narragansett Bay as a means of determining the effect and fate of various pollutants which might enter nearshore waters.

A cleaning procedure was adopted which consists of lowering and raising a circular brush assembly into the cylindrical tank to within 0.5 m of the bottom by means of a pulley system (see Fig. 420 in reference 9). The resulting friction between the 5-cm monofilament nylon bristles and the tank wall dislodges organisms fouling the surface of the wall. This cleaning procedure has been conducted at 3- to 4-day intervals in an attempt to limit the microorganisms colonizing the walls and prevent the more obvious fouling by invertebrates and macrophytes. The purpose of this paper is to document the effectiveness of this brushing procedure in preventing the development of the fouling community on the walls of the ecosystem tanks and to point out its potential for the disruption of fouling on any smooth surface submerged in the sea.

MATERIALS AND METHODS

This investigation was conducted in the late summer and early autumn months of 1976 when fouling was maximal. The fouling arrays were made of the same fiber glass material composing the tank wall, the innermost surface of which is a cross-linked polystyrene resin (Beetle Plastics Co., Fall River, Mass.). Disks 10 mm in diameter were cut from fiber glass

sheets and attached to rectangular sheets of fiber glass (20 by 20 cm). Two of these experimental fouling arrays were hung in each of the three control tanks (no. 3, 5, and 7) of the MERL facility. The fouling surface was oriented vertically facing the center of the tank at a depth of 1.5 m. The experimental tanks used for this study were not stressed with pollutants at any time during this investigation. Just before each sampling, one fouling array in each tank was brushed in a cleaning procedure which closely mimicked that used in the tanks, whereas the other array received no cleaning throughout the study period. Immediately upon sampling the disks were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer plus 0.25 M sucrose and processed in the usual manner by using critical point drying for scanning electron microscopy (20). Counts were made by scanning electron microscopy of both bacterial and protist cells (autotrophic and heterotrophic single-celled eucaryotes) per unit area by scanning at a 0° angle at a fixed magnification. Replicate counts were made at randomly selected sites on the fouling disk to insure representative results. In most cases two or more disks from each array were observed. The results from similarly treated fouling disks held in different experimental tanks showed no significant differences and were thus combined in the determination of all parameters (i.e., cell density, species diversity, biomass, and percent cover). Species identification of the protists was based on scanning electron micrographs of the fouling disks in conjunction with light microscopic examination of organisms colonizing glass microscope slides exposed in the experimental tanks during the study period. Diatoms, the only algal group observed, were identified by reference to Cupp (7) and Hendey (11). Heterotrophic eucaryotes were identified by reference to Grell (10), Kahl (12), and Kudo (13). The surface area of fouling disks covered by microorganisms and debris was measured for three or more micrographs per fouling disk. A grid of 100 evenly-spaced points was overlaid on each micrograph. The number of these locations covered by material was used as the estimate of percent cover. Volume estimates were calculated from scanning electron micrographs for each protist species observed based on its shape and dimensions. For bacterial estimates, the cells were grouped into one of four categories depending upon size and shape. A volume was determined for each of these bacterial "types," and the bacterial volume present per unit area was determined (8, 23). Bacteria occurring in chains or filaments or both were enumerated separately and placed into the appropriate category. Biomass for bacterial and protist populations per unit area, expressed in wet weight, was converted from volume estimates on the basis of 1 cm^3 = 1 g. In instances where protists strongly dominated the fouling film, as with the uncleaned fouling arrays after 10 days of exposure, bacterial cells were not enumerated because of their low contribution to total biomass and because they were obscured by the larger protists.

RESULTS

A simulation of the brushing procedure employed by MERL was effective in the disruption of the microbial fouling process on the resin surface of the fiber glass tank material and returned the microbial fouling process to a relatively early stage. Figure 1 presents scanning electron micrographs of the surface when allowed to foul naturally (Fig. 1, A, B, and C) and when allowed to foul for the same period but brushed as described just before sampling (Fig. 1, D, E, and F). Virtually all protist cells were removed by the brushing assembly. Figures 2 and 3 show the effect of the brushing process on protist species diversity and total protist cell density, respectively. Periodic brushing of the fouling arrays drastically reduced colonization. Unbrushed surface material was densely populated by protists after 16 days of exposure (1,950 cells mm^{-2}), whereas material subjected to the brushing process showed very few (if indeed any detectable) protists. Day 6 and day 10 values in Fig. 3 for brushed arrays fell below the level of detectability (1 protist per 2 mm^2) by the methods used here. This is further illustrated by the surface area covered by fouling shown in Fig. 4. Brushing kept the surface cover below 12%, whereas about 90% of the surface was covered on the unbrushed array. In contrast to the unbrushed fouling arrays, bacterial contribution to the living biomass on the brushed arrays was significant throughout the study period. This was not due to an increase in bacterial biomass (which in fact remained relatively constant), but rather to the more efficient removal of protists from the surface. Figure 5 shows the changes in biomass per unit area for brushed and unbrushed fouling arrays. The log scale is deceptive, and although the initial rate of biomass accumulation appears most rapid and to slowly fall off to a plateau after day 14, the converse is true. Total fouling accumulated at a rate of 0.5 ng (wet weight) mm^{-2} day^{-1} over the first 2 days, 5.5 ng mm^{-2} over the next 4 days, and at 18.8 ng mm^{-2} day^{-1} over the next 8 days.

DISCUSSION

Colonization of the unbrushed fiber glass-reinforced plastic surface during this period (Fig. 1) was strongly dominated by nitzschioid diatoms consisting of larger *Nitzschia*-like forms and a smaller fusiform species similar to *Cylindrotheca* which accounted for the low species diversity values of Fig. 2. Pennate diatoms were the major component of the primary fouling film throughout this study, due undoubtedly to the light intensity at the shallow water depth used to immerse the test panels and the white color of the fiber glass wall material. The brushing process used at the MERL facility was not only effective in the removal of these gliding pennate
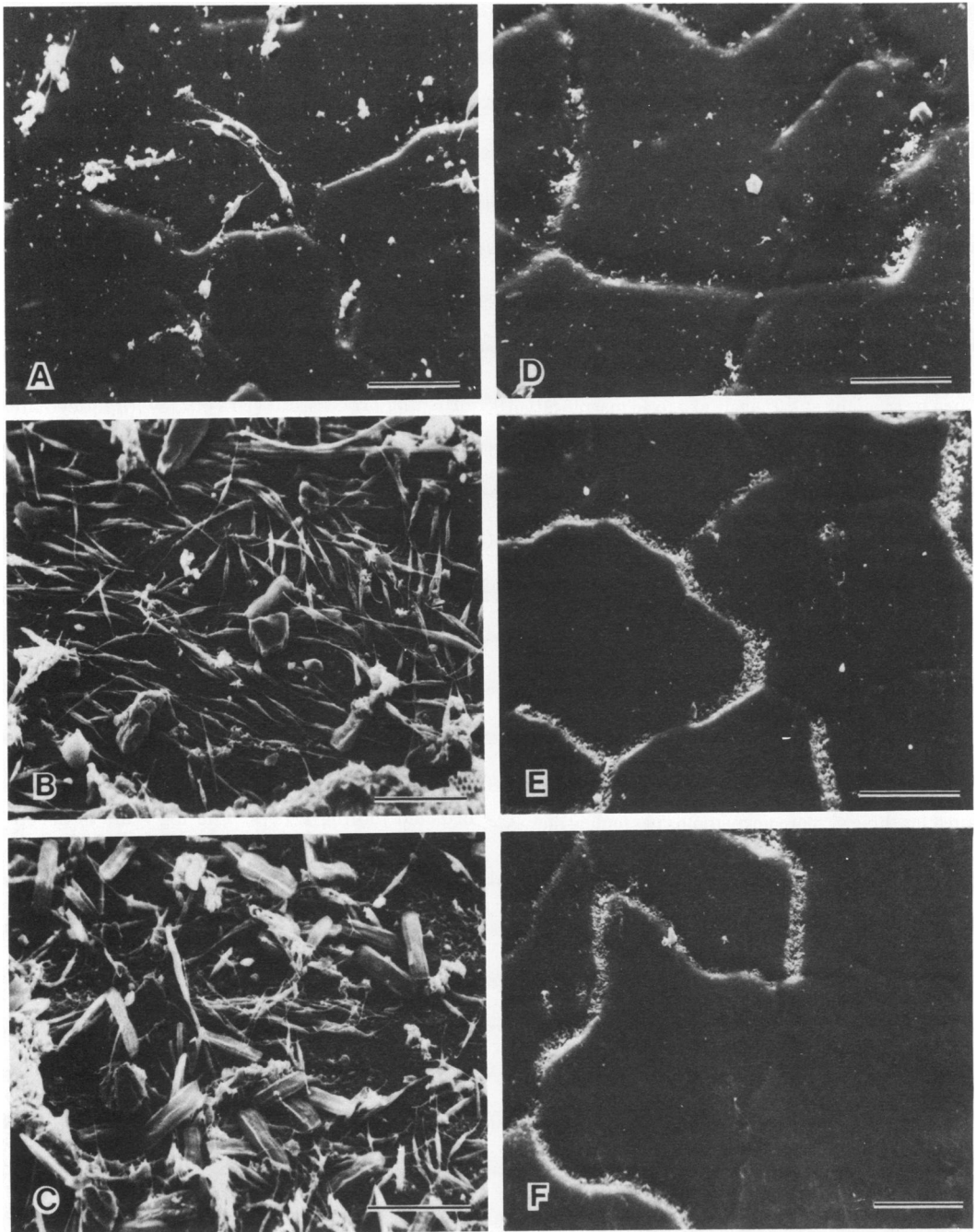
FIG. 1. *Transition of an unrestricted fouling film (A, B, and C) from procaryote to protist domination (fusiform nitzschioid diatoms) occurred between days 3 (A) and 6 (B), whereas larger Nitzschia-like forms appeared by day 16 (C). Twice-weekly brushing of the fiber glass-reinforced plastic substrata (D, E, and F) removed the protists and restricted bacterial colonization to microcrevices where concentrations at days 6 (E) and 16 (F) were similar to those at day 3 (D). Bars, 50 μm.*
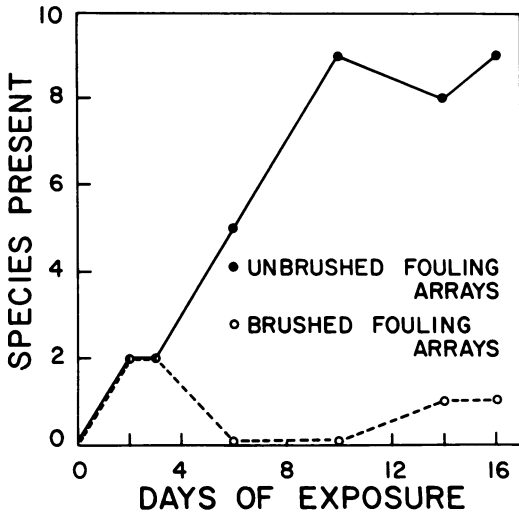
FIG. 2. *Effect of mechanical disruption on the species diversity of protists attached to fiber glass-reinforced plastic fouling arrays submerged in an estuarine mesocosm.*
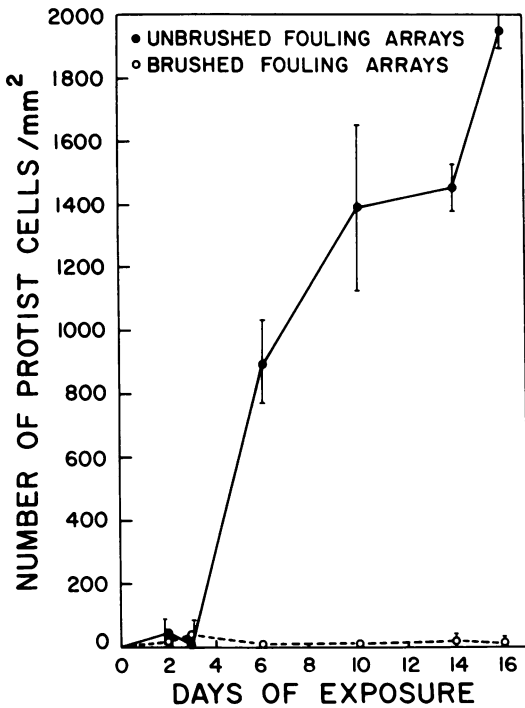
diatoms but also removed many forms with a high affinity for the substratum. Amoebae, sessile choanoflagellates, suctorians, and vorticellid ciliates (21) were also removed by the brushing process, as were large amounts of detritus which accumulated along with the living microorganisms. Stalked bacteria and other bacterial forms possessing attachment organelles such as fimbrae or pilli likewise showed no resistance to mechanical removal.

The fouling process is initiated by bacteria free in the water and adsorbed onto particulates (2, 4) with the subsequent appearance of protists and macroorganisms (20, 21). The smooth nature of the resin surface is undoubtedly a large factor in the high efficiency of removal by the brushing procedure. The microorganisms are afforded little protection from the abrasive action of the brush due to the lack of deep furrows or fissures. The surface is not completely smooth at the microscopic level, however, and the shallow microcrevices are the usual location of sedimented debris and microorganisms which re-
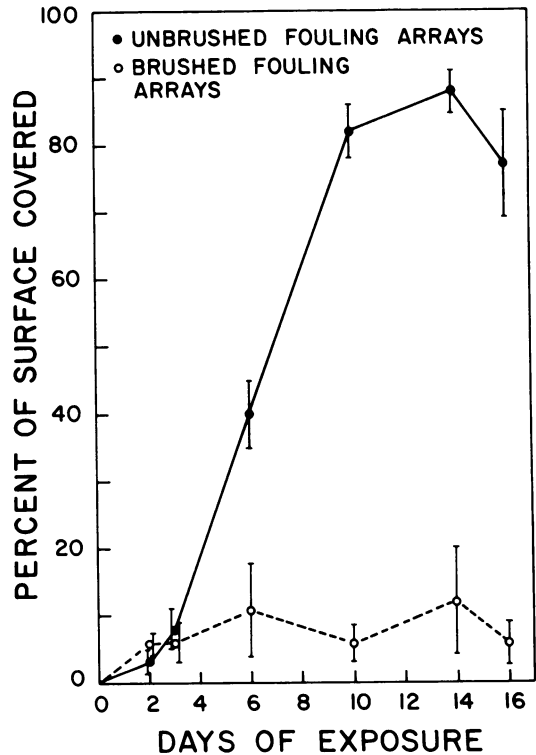

FIG. 3. *Effect of mechanical disruption on the population density of protist assemblages, dominated by pennate diatoms, accumulating on fiber glass-reinforced plastic fouling arrays submerged in an estuarine mesocosm. Error bars indicate ± 1 standard deviation.*

FIG. 4. *Estimates of percent surface cover for brushed and unbrushed fiber glass-reinforced plastic fouling arrays submerged in an estuarine mesocosm. Error bars indicate ± 1 standard deviation.*
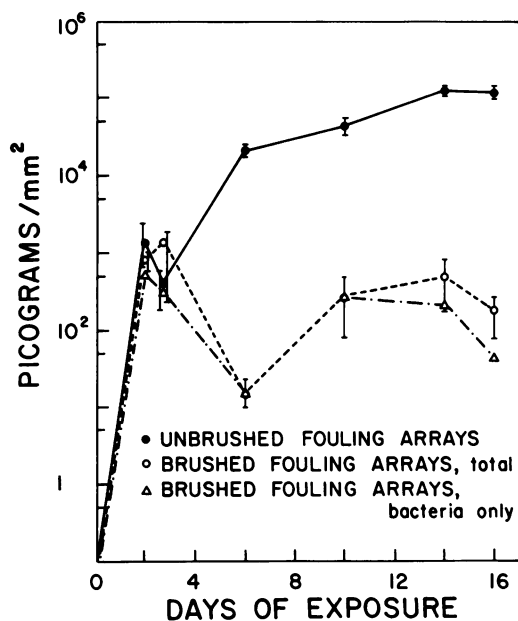
FIG. 5. *Effect of mechanical disruption on the living biomass of the fouling film accumulating on fiber glass-reinforced plastic fouling arrays submerged in an estuarine mesocosm. Biomass is expressed as wet weight; error bars indicate ± 1 standard deviation.*

main after the brushing procedure (Fig. 1, D, E, and F).

Although the study period was relatively short, colonization of microorganisms on the unbrushed fouling disks appeared to have reached a maximum by day 16. Biomass at this time was approximately 150 ng mm^{-2}. Protist species diversity also appeared to be maximal by day 16 (Fig. 2) and may be due to the sloughing of part of the film from the surface at this time. This hypothesis is also supported by the percent cover values (Fig. 4) for these disks which show a maximum at day 14 for unrestricted (unbrushed) colonization and a drop at day 16. There is also a shift in species dominance at this time.

The gross effect of the brushing procedure employed at MERL is to effectively remove the faster-accumulating protists which are secondary colonizers and leave a primary fouling film of more slowly accumulating bacteria equal to a "day 3" stage of colonization. It is possible that this mechanical disruption of the microbial fouling process may also substantially reduce the attachment of larval invertebrates, since this process has been suggested as an important factor in the conditioning of the substratum for invertebrate settlement (2, 3, 5, 6, 15, 24). Furthermore, the removal of the protists at 3-day

intervals prevented a major film buildup. Using the apparent rate of biomass accumulation shown in Fig. 5, we calculated that by day 15 unrestricted fouling produced 150 ng (wet weight) mm^{-2} of wall. Protist removal every 3 days maintained a fouling rate of 5.5 ng mm^{-2} day^{-1} (rate calculated for days 2 to 6) through day 15, with protists contributing 66 ng to the plankton for each mm^2 of wall by day 15. This is less than half of the contribution of protists in the unrestricted film. Also, these estimates do not take into account additional contributions of unrestricted fouling due to sloughing of film material into the water and inducement of larval settlement. The restricted bacterial film on brushed surfaces would have a minimal wall effect, affecting only heterotrophic utilization of organic matter, whereas the mature protist film dominated by pennate diatoms would not only contribute to primary productivity but also would falsely influence the measurements used to determine the activity of planktonic phototrophs.

Minimization of the biomass of attached microorganisms is essential to the validity of research obtained from contained ecosystem projects such as MERL. Unlike controlled ecosystem pollution experiments (CEPEX) (16) and other bag ecosystems anchored in the water column, the placement of MERL tanks on shore not only allows greater safety and sampling flexibility but permits antifouling measures which can continue over extended periods.

Mechanical removal of attached microorganisms does have the complication of causing a small biomass input to the contained water mass. However, fouling organisms affect the enclosed system whether or not they are attached. Periodic removal of the fouling film minimizes the effect of wall growth to processes within the experimental system by maintaining a slow fouling rate characteristic of the initial fouling sequence.

Mechanical disruption of the fouling film at regular intervals before extensive fouling takes place is a novel idea and merits further investigation. It should be directly applicable to any smooth surface such as the fiber glass-reinforced plastic hulls of pleasure and working craft, the sensing surfaces of submerged instruments, and the heat-exchanging surfaces of ocean thermal energy conversion systems.

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