

Methods for removal and estimation of microfouling biomass

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A comparative evaluation of various methods for the removal and estimation of microfouling material and biomass respectively was carried out. Efficiencies of removal of the microfouling layer were almost the same with a nylon brush, a stainless steel razor and a combination of nylon brush with sodium pyrophosphate. A single step scraping using a nylon brush removed the highest amount of the microfouling material (as dry weight and organic carbon). Most of the methods used for estimating microfouling biomass produced highly quantitative data. Estimation of microfouling biomass as dry weight and/or organic carbon is recommended, especially during oceanographic cruises, since these methods are quantitative and simple to use.

Microbial biomass is estimated¹⁻⁴ by measuring adenosine triphosphate, lipid phosphate, nucleic acids and muramic acid and viable and total bacterial numbers. Some of these methods⁴⁻⁷ are also utilized for the estimation of microfouling biomass. Although these methods are sensitive, they are relatively time consuming and uneconomical. Probably, because of this, their usefulness for routine monitoring of microfouling biomass is limited¹⁻⁴. Microfouling studies in the nearshore waters have also been carried out by deploying the test panels for various periods ranging from days to months. Such prolonged exposures are not always possible while working in the oceanic waters. The aim of the present study is therefore to develop a simple method for reliable estimation of microfouling biomass even during short exposures. The field applicability of the suggested method for the estimation of microfouling biomass is also evaluated during a cruise in the Arabian Sea.

Materials and Methods

The study was carried out at a station (water depth 4 m) in the Mandovi estuary (73°52'E; 15°30'N). Aluminium (14.5 × 13 cm) and glass (9 × 10 cm) panels were cleaned with hydrochloric and chromic acids respectively, washed under running water, rinsed with distilled water, dried in an oven (100°C, 24 h) and finally kept in polythene bags. Five replicate panels each of glass and aluminium were fixed into a bar with PVC nuts and bolts. The assembly was maintained in surface water with the help of a float. In order to avoid any contamination, the polythene bags were removed only after the deployment of the panels in water. Test panels were retrieved after 4-10 d exposure and transported to the laboratory in an ice box containing

in situ water. For each of the experiments described below, 4-5 replicates were used.

Three different tools were employed for removing the microfouling layer from the panels, viz. a nylon brush, a stainless steel razor and a combination of nylon brush and sodium pyrophosphate solution (0.001 M). The panels were scraped in a chamber with laminar flow. The scraped material was collected separately and made to a known volume (125 ml). A subsample of 10 ml was filtered through preignited (450°C, 3 h) and preweighed GF/C filters. Filters were then dried (50°C, 4 h) and reweighed to get the dry weight of the residue (microfouling material). In all the subsequent experiments, the scraping was done only by nylon brush using filtered (0.2 µm) sterile estuarine water (FSEW).

In a second set of experiment, aluminium and glass panels were retrieved after 4 days exposure and each panel was scraped thrice with nylon brush and a known volume of FSEW. Scraped material obtained at each step was collected separately for dry weight as described earlier.

In the third set of experiment, scraping of the microfouling material was evaluated using glass slides. Clean glass slides (7.5 cm × 2.2 cm × 2 mm) exposed to estuarine waters were scraped and the material obtained was filtered as described earlier and used for the analysis⁸ of particulate organic carbon (POC). The slides were then rinsed with distilled water, dried in an oven and powdered in a mortar. Organic carbon analysis⁸ of the powdered material was then carried out as above for the estimation of any residual carbon on the scraped slides.

Aluminium and glass panels were likewise retrieved after an exposure period of 10 days and were scra-

ped with nylon brush. The scraped material was made to a known volume of 125 ml. Subsample of 1 ml was serially diluted and 0.2 ml of the last 2 dilutions was used for the enumeration of bacterial colonies by the pour plate method using Zobell's Marine Agar (No. 2216). Total bacterial counts were made by the AODC method⁸. A subsample of 1 ml was preserved in Lugol's iodine for the qualitative microscopic examination of diatoms. Subsamples of 10 ml each were filtered through GF/C as described above for the estimation of dry weight, chlorophyll *a*⁸, organic carbon⁸, organic nitrogen⁹ and proteins¹⁰. Product moment correlation coefficient was applied to verify the relationship between these different methods.

Results and Discussion

During the period of study (June 1988) the temperature (29.8°C) and salinity (20.56×10^{-3}) did not vary much. The nutrients phosphate ($1.52 \mu\text{m dm}^{-3}$) and nitrate ($6.85 \mu\text{m dm}^{-3}$) were abundant.

Removal of microfouling is perhaps the most critical step in evaluating microfouling biomass developed on solid substratum immersed in aqueous environments. Five replicate extractions (Table 1) from aluminium and glass surfaces using the 3 tools described, yielded more or less similar amounts of the microfouling material. Sodium pyrophosphate which has been utilised earlier to release adsorbed microorganisms from surfaces, did not show any appreciable advantage over FSEW^{7,11}. Since the use of nylon brush was found to be relatively easy, this method was followed for the further work described below.

Three sequential extractions of each panel were carried out in order to quantify the efficiency of the removal of microfouling layer in each extraction step. A major portion of the microfouling layer was removed from the 2 test surfaces in the very first step when estimated as dry weight (Table 2). In terms of organic carbon the removal was found to be 89% (Table 3) when glass slides were used. This implies that the method of removal using a brush was fairly quantitative.

Although several methods have been employed to assess microfouling biomass^{7,12-14}, their usefulness during short term exposures and the interrelationship between the various methods was however not considered in the earlier studies. Some biochemical parameters were analysed as means to assess microfouling biomass developed on aluminium and glass test panels. Generally, all the parameters employed showed small variations and thus gave highly quantitative data (Table 4). Variations were relatively high among individual panels with respect to proteins and Chl *a* estimates.

Table 1—Comparison of methods for the extraction of microfouling layer from the 2 test panels as dry weight (Values in parentheses denote coefficient of variation)

Method	Panels ($\text{mg.cm}^{-2} \text{d}^{-1}$)	
	Aluminium (n=5)	Glass (n=4)
Nylon brush	0.040 ± 1.83 (4.58%)	0.086 ± 1.63 (1.89%)
Stainless steel razor	0.040 ± 2.30 (5.78%)	0.085 ± 7.13 (8.39%)
Sodium-pyrophosphate and nylon brush	0.039 ± 0.066 (1.20%)	0.084 ± 1.69 (2.02%)

Table 2—Efficiency of nylon brush scraping in the removal of microfouling layer, expressed in terms of dry weight (Values in parentheses denote coefficient of variation)

Extraction assay	Panels ($\text{mg.cm}^{-2} \text{d}^{-1}$)	
	Aluminium	Glass
First	0.232 ± 0.010 (12.3%)	0.475 ± 0.079 (16.70%)
Second	0.011 ± 0.001 (9.09%)	ND
Third	ND	ND

ND = Not detectable

Table 3—Efficiency of nylon brush¹ scraping in the removal of microfouling layer, expressed in terms of organic carbon ($\text{mg.cm}^{-2} \text{d}^{-1}$) using glass slides (Values in parentheses show % of total)

Extraction step	Carbon	Residual
1	0.385(88.50)	0.050(11.49)
2	0.382(87.21)	0.056(12.78)
3	0.388(92.38)	0.032(7.60)
Mean	0.385(89.36)	0.046(10.60)
SD	$\pm 0.002(\pm 2.19)$	$\pm 0.010(\pm 2.20)$
CV (%)	0.05(2.45)	22.16(20.77)

SD = Standard deviation
CV = Coefficient of variation

In general, highly significant correlation was observed between the parameters, except for Chl *a*. Microscopic examination of the microfouling material did show the presence of a few diatoms, such as *Navicula* sp., *Amphora* sp., *Coscinodiscus* sp. and *Rhizosolenia* sp. Poor settlement of these diatoms and grazing by other organisms were perhaps responsible for the observed poor correlation between Chl *a* and other parameters estimated. Among the parameters analysed for the estimation of microfouling biomass, dry weight and organic carbon content of scraped mate-

Table 4—Estimation of different parameters of scraped material for estimation of microfouling biomass, on panels

Surface	Dry weight ^a	Chl- <i>a</i> ^b	Carbon ^b	Nitrogen ^b	Proteins ^b	Bacterial count ^c	
						Total (× 10 ³)	Viable (× 10 ²)
Aluminium Panels							
Mean (n=5)	1.26	1.22	179.60	50.25	119.20	3.78	1.44
SD	±0.02	±0.12	±17.14	±2.27	±19.20	±0.25	±0.03
CV (%)	1.58	10.60	9.59	4.53	16.13	6.86	2.75
Glass Panels							
Mean (n=4)	2.28	2.00	302.20	98.60	212.00	8.45	3.42
SD	±0.09	±0.49	±30.00	±1.49	±30.10	±0.52	±0.10
CV (%)	4.29	14.14	9.95	1.51	14.20	6.17	3.00

a = mg.cm⁻² d⁻¹; b = ng.cm⁻² d⁻¹; c = no.cm⁻² d⁻¹

rial may offer advantage over others as they are not only quantitative, but also relatively less time consuming. These two parameters have also shown significant relationship with the other parameters analysed.

Therefore, estimations of dry weight and organic carbon are recommended for the evaluation of microfouling biomass. Field applicability of these methods has been tested during a cruise of *R V Gaveshani* (cruise No. 160). Despite short exposures, the significant difference in estimated values for microfouling biomass in the shelf, slope and oceanic waters suggest these estimations to be quantitative and also reliable for routine studies.

It is concluded that nylon brush can be successfully used for the removal of microfouling layer from test panels during routine oceanographic studies and organic carbon estimates can be reliably be adopted for the assessment of microfouling biomass of surface waters.

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