

Biological and Biochemical Characterization of Microfouling on Aluminium Panels Placed in the Arabian Sea

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Microfouling biomass was higher in the shelf waters (< 200m) as compared with the slope waters (> 200m). Bacteria were first to colonize on the aluminium surfaces as irregular formations during the initial stages of fouling. The number of fouling diatoms decreased as the depth increased. *Nitzschia*, *Licmophora* and *Navicula* were the most dominant forms. Carbohydrates and lipids were the most abundant constituents in the fouling material as compared with protein.

Key Words: Microfouling, Aluminium panels, Diatoms, Bacteria, Biochemical composition

Introduction

Very little information is available on the development of microfouling on metal surfaces exposed to tropical marine environment (Nair & Pillai 1977). In this paper, preliminary data on the initial stages of microfouling on aluminium metal surfaces have been presented and discussed.

Materials and Methods

Microfouling studies on aluminium panels were carried out in the shelf (<200m) and slope (> 200m) waters of the Arabian Sea during 160th and 165th oceanographic cruises of *R V Gaveshani*. The stations covered during these cruises are shown in figure 1.

Aluminium panels (15 × 10 cm) for the present study were cleaned with concentrated hydrochloric acid (Cameron & Robinson 1968), repeatedly washed with tap water followed by distilled water and then finally, dried in an oven and kept covered until used. For bacterial studies, small panels (2 × 6 cm) were cleaned as above and were sterilized before use.

Spatial (station to station) variation in the microfouling biomass was evaluated at eleven stations by deploying aluminium panels in the surface waters (~ 1m from the ship). The panels were retrieved after 3 to

6 hrs and evaluated for microfouling biomass as organic carbon. At these stations surface water samples were also collected and filtered through GF/C filters to estimate particulate organic carbon (POC). A single station (15° 08'N, 73° 16'E) was selected to study time and depth related variations in the microfouling. Time series experiment was carried out as described above except that the panels were removed at 6 hr intervals during first 24 hr, and thereafter every 24 hr upto 192 hr. The depth-related changes in microfouling were monitored by deploying the panels at 10, 25, 40, 60 and 80mm using 'I' mooring system. The basic aim in these set of experiments was to assess spatial variations and to define the detailed information on the biological and biochemical events during the initial stages of microfouling with time and depth.

Microfouling material formed on the above panels at various stations and with respect to time and depth at a single station (15° 08'N, 73°16'E) was scrapped using nylon brush and filtered seawater. Samples were then diluted to a known volume and subsamples were taken for different biochemical and biological analysis.

In order to know diatom numbers and their composition, subsamples were preserved with Keffer's solution

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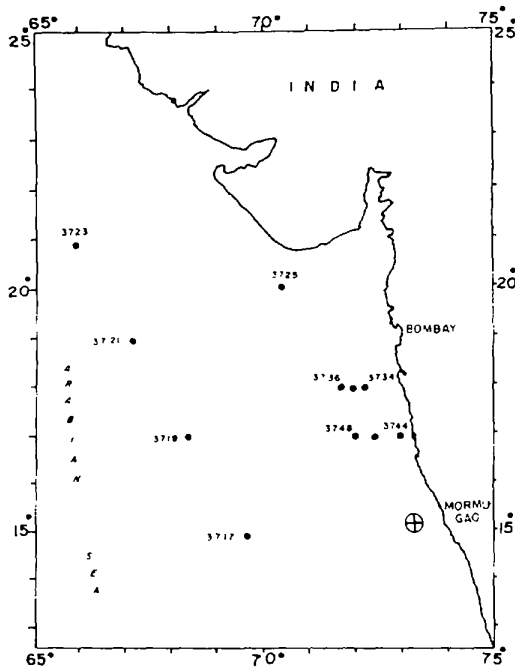


Figure 1 Map showing microfouling survey station (●) and mooring station 15°08'N, 73°16'E (⊕)

Table 1 Microfouling biomass on aluminium panels placed in the surface waters at various stations and particulate organic carbon (POC) of surface waters of these stations

Station No.	Station depth (m)	Sampling depth (m)	A	B
3717	3700	1	55.92	415.00
3719	3500	1	31.48	375.69
3721	3400	1	66.29	416.81
3723	3600	1	68.64	676.72
3725	68	1	346.02	1002.33
3734	68	1	234.65	693.93
3735	90	1	321.36	700.93
3736	80	1	237.64	806.06
3744	45	1	109.97	647.65
3746	85	1	158.34	643.45
3748	93	1	114.55	695.31

A = Microfouling biomass ($\mu\text{g}/100\text{cm}^2/\text{hr}$)

B = POC ($\mu\text{g}/\text{l}$)

Table 2 Variation in microfouling and its composition with time at a station (15°08'N, 73°16'E)

Duration (hr)	Organic carbon*	Carbohydrate*	Protein*	Lipid*	No. of bacteria†
6	1139.52	104.24	44.20	112.20	0.91
12	305.28	23.44	17.84	40.10	0.32
18	252.96	21.73	24.21	35.13	1.23
24	163.13	14.77	4.10	12.40	4.20
48	70.80	3.52	1.58	5.84	2.63
72	38.64	3.69	1.15	2.66	0.68
96	43.68	3.72	1.99	1.65	0.42
120	38.88	1.35	0.65	2.13	—
144	48.00	3.19	0.82	5.00	—
168	36.24	1.97	0.54	5.01	—
192	27.12	1.57	0.52	4.82	0.45

* $\mu\text{g}/100\text{cm}^2/\text{day}$; † $\times 10^4/24\text{cm}^2$

and later allowed to settle in a chamber for 24 hr and analyzed quantitatively and qualitatively using inverted microscope.

For the analysis of organic carbon, carbohydrate, protein and lipid, a known amount of subsample was filtered through preignited (450°C for 3 hr) GF/C glass fibre filters. Organic carbon and lipids were estimated as suggested by Parsons et al. (1984). Carbohydrate and protein were analyzed using phenol sulphuric acid (Dubois et al. 1956) and Folin-phenol reagents (Lowry et al. 1951), respectively.

Aseptic techniques were used to scrap and prepare dilutions for bacterial analysis. Two to three dilutions were prepared in sterile seawater. Duplicate aliquots were filtered through Millipore filters ($0.2\ \mu\text{m}$ pore size) and then these filters were placed on ZoBell's marine nutrient agar (No. 2216) plates. The plates were incubated at $20 \pm 2^\circ\text{C}$ to get an estimate of bacterial numbers.

Results

Physical appearance of the test panels changed as the exposure time increased. The material on the panels could be removed easily in the early period of exposure, but as the exposure period increased it became cohesive and more difficult to remove. No visible areas of corrosion could be identified on any of the panels during the sampling period.

Microfouling biomass (as organic carbon) and POC of the surface waters varied from station to station (table 1). In general, stations in shelf waters showed higher microfouling than those in slope waters. Higher

Table 3 Type and number of microfouling diatoms (No./100 cm²/10 days) on aluminium panels placed at various depths at a station (15°08'N, 73°16'E)

Name of Diatom	Depth (m)				
	10	25	40	60	80
<i>Hyalodiscus</i> sp.				6	
<i>Coccinodiscus</i> sp.				2	
<i>Planktoniella</i> sp.					3
<i>Biddulphia</i> sp.				4	
<i>Asterionella</i> sp.				2	
<i>Thalassiothrix</i> sp.	10		10	15	
<i>Grammatophora</i> sp.		18			
<i>Licmophora</i> sp.	112	29	19	4	
<i>Navicula</i> sp.	2	2	10	6	3
<i>Nitzschia</i> sp.	864	709	789	44	10
TOTAL	988	758	828	83	16

microfouling was associated with higher POC content of seawater. There was a highly significant relationship ($R = 0.82$; $P \leq 0.001$) between the concentrations of particulate organic carbon of the water column and organic carbon deposited on the aluminium panels.

In time series experiment, bacteria were the first organisms to colonize the metal surface and they did not show any definite growth pattern (table 2). Diatoms which settle on these panels also showed irregular settlement pattern and very low cell numbers (2 to 3 cells/100 cm²). Diatoms such as *Triceratium*, *Navicula*, *Biddulphia*, *Rhizosolenia* were observed in the microfouling material of the time series experiment.

Ten different diatoms were observed from different depths (table 3). *Nitzschia* was the most abundant group at all depths. Total fouling diatom numbers decreased as the depth increased (four belonging to Centrales and six to pennates). At 10, 25 and 40mm depth four types were recorded, and at 80m only three types were present, whereas, at 60m eight types of diatoms were observed. Bacterial analysis in the microfouling material collected from different depths could not be carried out due to practical difficulties on board the ship.

Microfouling biomass and its biochemical composition varied with time and depth (tables 2 & 4). As observed for bacteria and diatoms, no definite pattern was noticed during initial stages (192 hr) of microfouling. Microfouling was somewhat higher at greater depths than at 10m. There was a highly significant relationship between organic carbon and carbohydrate ($r = 0.99$; $P \leq 0.001$), protein ($r = 0.94$; $P \leq 0.001$) and lipid ($r = 0.98$; $P \leq 0.001$). Lipids and carbohydrates were most abundant in the microfouling material collected during time series experiment and at various depths.

Discussion

Method of removal, perhaps, is the most critical step in assessing the microfouling biomass formed on the solid surfaces placed in seawater. Extraction of microfouling layer by brushing is one of the most commonly employed method (White & Benson 1984, Adamson & Liberatore 1984). Therefore, we used nylon brush to remove microfouling formed on aluminium surfaces. Although this method is easy to operate and is routinely used by many workers including ourselves to monitor microfouling biomass, it has got certain limitations. For example, the removal of the microfouling from the surfaces may not be 100%. It is therefore possible that we might have slightly underestimated the microfouling biomass formed on aluminium surfaces at these stations. Under the circumstances, microfouling biomass values reported here are perhaps conservative.

Several parameters such as carbon, nitrogen, ATP and carbonate content (Afring & Taylor 1979, Mayack et al. 1984), heat transfer resistance (Characklis 1973) and total dry weight of the fouling material (Burton & Margrey 1979) have been used to quantify the extent of biofouling. We used organic carbon content to assess the extent of microfouling on aluminium metal surfaces with space (stations), time and depth because it gave highly reproducible results and was less time-consuming (Sharma & Bhosle; in preparation).

Highly significant relationship was associated between organic carbon of the surface water and organic carbon deposited on the aluminium surface. This implies that organic matter content plays an important role in controlling the magnitude of microfouling. As it is relatively simple to collect and analyze organic carbon of seawater, perhaps an indirect estimate of microfouling potential of seawater can be made by estimating the organic matter of seawater. Microfouling potential of slope waters was lower by an order of magnitude as compared with shelf waters. This may be due to differences in the physico-chemical and biological parameters in these two environments. It is suggested that the sea area, exposure time and depth played important roles in the development of microfouling (Yanshun et al. 1984). This is evident in the data presented here (tables 1-4). Microfouling was

Table 4 Microfouling biomass and its biochemical composition at different depths at a station (15°08'N, 73°16'E)

Depth (m)	Organic carbon*	Carbohydrate†	Protein†	Lipid†
10	80.44	16.62	9.81	21.52
25	132.63	12.63	11.50	21.99
40	110.51	27.73	10.95	19.83
60	105.23	10.08	8.89	20.59
80	110.38	15.12	8.21	22.00

* $\mu\text{gC}/100\text{cm}^2/\text{day}$; † $\mu\text{g}/100\text{cm}^2/\text{day}$

maximum at 25m (table 4). This is in agreement with our earlier observations carried out in the nearby area of Bombay High region wherein maximum macrofouling was obtained at 22m over a period of three years (Wagh, unpublished results).

Various workers have described the developing stages of the primary slime film and ecological succession of complex microfouling communities in the temperate and subtropical environments (Mitchell & Krichman 1984, White & Benson 1984, Yanshun et al. 1984). These studies suggest that bacteria were the first organisms to appear on the various surfaces placed in the seawater. Our observation on the microfouling material formed on the aluminium panels is in agreement with the above findings. Irregular growth pattern seems to be a regular phenomenon on many other surfaces (Corpe 1972, Yanshun et al. 1984). This irregular growth pattern may be a result of reversible attachment of bacteria to surfaces which are removed due to small stress and/or due to grazing by protozoa (Marshall et al. 1971, Marshall 1976).

Diatoms which foul man-made surfaces belong to restricted group of organisms and are usually of the Pennate type (Characklis & Cooksey 1983, Cooksey et al. 1984). In the present study, in addition to pennates (60%), centrales (40%) were also present. Of the various diatoms observed, *Triceratium* was the first to appear after 48 hr on aluminium panels followed by other diatoms. The predominance of *Nitzschia* and *Licmophora* in the microfouling material developed on nontoxic surfaces have been reported from temperate

waters (Cooksey et al. 1984). Our data on these species suggest that they are also predominant in the microfouling material developed on aluminium panels placed in tropical seawater thus, indicating common occurrence of these fouling diatoms in various environments.

Biochemical analysis of the micro film showed large amount of lipid, carbohydrate and protein which may serve as food source for the settlement of the larvae of many macrofouling organisms. Significant correlations observed between organic carbon and carbohydrates, proteins and lipids suggest common origin of these compounds. Amongst the constituents of organic matter studied, lipids and carbohydrates were the most abundant. This probably implies that lipopolysaccharide type compounds were produced by microfouling organisms. The observed irregularity in these compounds with the time and depth perhaps indicates utilization by zooplankton and/or fishes. The migratory zooplankton play an important role in the removal of organic matter (Harding et al. 1987). Microfouling material formed on the aluminium surface can attract these animals, thus, resulting in irregular pattern of these compounds with the time and depth.

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