

Some Aspects of Microfouling and Corrosion of Materials in the Tropical Marine Environment

by

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With 6 Figures, 13 Tables and 1 Plate

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Abstract

Microfouling material developed on aluminium panels after immersion in estuarine, coastal, shelf and oceanic waters was analysed for various biological, chemical and biochemical parameters. A single step scraping with nylon brush removed the highest amount of the microfouling material. Microfouling biomass (as dry weight and/or organic carbon) varied from station to station and was higher in coastal and shelf waters as compared to oceanic waters. Highly significant correlations were observed between bacterial numbers and various chemical and biochemical parameters during short term experiment (< 10 d). The number of fouling diatoms decreased as the immersion depth increased. *Nitzschia*, *Licmophora* and *Navicula* were the most dominant forms. Similarly, microfouling biomass and carbohydrate content generally decreased as the immersion period and the depth of deployment increased. The mannose/xylose ratio (< 5.0), the glucose free weight percentage distribution of ribose and fucose (> 12 %) as well as arabinose and galactose (> 28 %) suggest that phytoplankton and non-woody vascular plant material and grasses appears to be the major sources contributing to carbohydrates of the microfouling material during long term experiments (> 10 d). Amino acid content of the microfouling material, however, increased with the immersion depth and was maximum at 60 m. A relative enrichment of glycine with depth was particularly noteworthy.

A fouling bacterium *Vibrio* sp produced acidic exopolysaccharide containing glucose and galactose as the major (70 mol %) and mannose, rhamnose, arabinose, hexosamine, uronic acid and pyruvate as the minor components.

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The corrosion of mild steel decreased with the immersion depth. Significant positive relationships were observed between the corrosion of mild steel and temperature and dissolved oxygen of seawater. In contrast, corrosion of mild steel was inversely related with organic carbon and water extractable carbohydrates associated with the corrosion products of mild steel.

1. Introduction

Materials immersed in seawater experience a series of discrete chemical and biological events which eventually result in the formation of a complex layer of attached organisms known as biofouling. Solid surfaces immersed in marine environment adsorb organic matter thereby conditioning the surfaces (Loeb and Neihof, 1977; Characklis and Escher, 1988; Zutic and Tomaic, 1988). A number of compounds including glycoproteins (Baier, 1980), humic material (Loeb and Neihof, 1975) and macromolecules (Zaidi et al, 1984) have been implicated as conditioning agents. The presence of these compounds affect surface charge (Loeb and Neihof, 1977; Zutic and Tomaic, 1988), and surface wettability (Dexter et al., 1975). These properties play an important role in the subsequent attachment and development of microfouling organisms including bacteria, fungi, protozoa and diatoms. Furthermore, the formation and succession of fouling organisms in a community are affected by a variety of environmental factors, by biological factors and by the components of surface substratum (Characklis et al., 1984; Fletcher, 1988). Development of microfouling organisms may attract larvae of many animals leading to microfouling (Crisp and Ryland, 1960; Barnes, 1970).

While colonizing the surface, microorganisms may synthesize extracellular polysaccharide (EPS) or other cellular components (Corpe, 1970, 1974; Costerton et al., 1987; Read and Costerton, 1987; Vesper, 1987). Definite evidence for the involvement of EPS bridging in bacterial adhesion was obtained by electron microscopic examination of ultra thin sections of bacteria attached to suitable substrata (Fletcher and Floodgate, 1973; Read and Costerton, 1987). Several functions have been ascribed to EPS in the adherent biofilms, however protection and maintenance appear to be most important (Costerton et al., 1987; Read and Costerton, 1987). Furthermore, EPS may also induce corrosion of metals (Costerton et al., 1987, Ford and Mitchell, 1980). The exact mechanism about the role of EPS in inducing corrosion is not known. It is however suggested that EPS may induce corrosion of metals by developing aeration and ion concentration cells.

Corrosion of metals immersed in seawater is also controlled by several factors such as temperature, dissolved oxygen, salinity, pH and hydrostatic pressure (Chandler, 1985). Furthermore, nitrogen and sulphur containing organic compounds and several water soluble vitamins have been reported to inhibit corrosion of metals in fresh and brackish waters as well as in acidic media (Agarwal and Namboodhiri, 1990; Nigam et al., 1990; Sekine et al., 1988). However, the influence of organic matter associated with the corrosion products on the corrodibility of metals, particularly when immersed in the marine environment has not been considered in earlier studies.

In this paper data on methods to remove microfouling material, distribution of microfouling biomass, biological and chemical composition of microfouling biomass, as well as the corrosion of mild steel immersed in tropical marine environment is presented and discussed.

2. Experimental Methods

Test panels

Aluminium test panels (10 x 15 cm) were used except on one occasion where fiberglass panels were also employed to study microfouling. These panels were cleaned with hydrochloric acid (Bhosle et al., 1989 a, 1980 a, b). Mild steel panels were used for corrosion studies and were cleaned with 10 % H₂SO₄ containing 1 g l⁻¹ thiourea followed by 10 % NaOH for two minutes at 60°C. After the above treatment, all the panels were thoroughly washed with tap water followed by distilled water. Finally, panels were dried in an oven at 80°C for 30 min. and cooled in a desiccator, weighted on a balance (Mettler, model H-80) and stored in a vacuum desiccator until used.

Deployment of panels in surface water

Studies on microfouling material developed on test panels immersed in the surface water were carried out at one station each in the Mandovi estuary (15°30'N, 73°52'E) and Dona Paula Bay (15°31'N, 73°59'E) and at 12 stations in the southern Arabian Sea during the 51st cruise of ORV SAGAR KANYA in March/April, 1989 (Fig. 1). At these stations replicate aluminium and/or fiberglass panels (3 to 6) were fixed vertically on a weight frame. Due to their weight and the attached float, panels remained suspended at 1 m depth.

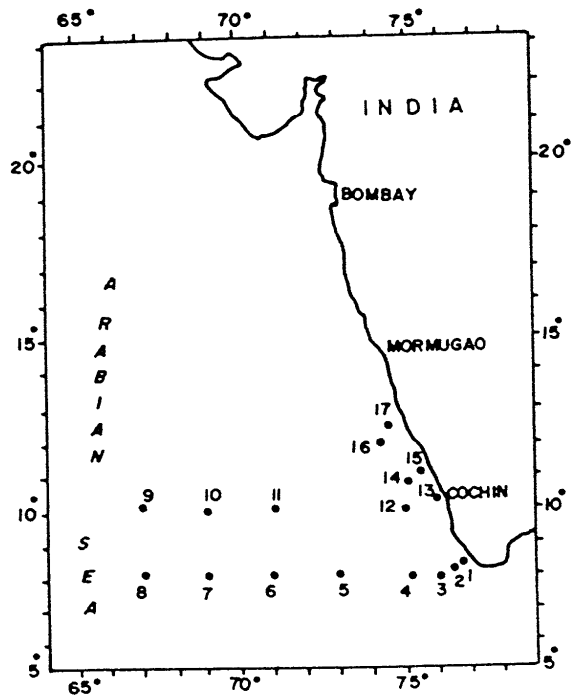


Fig. 1:
Position of stations sampled in the Southern Arabian Sea in March/April, 1989 during 51st cruise of the ORV SAGAR KANYA.

Deployment of panels at various depths

Depth related changes in microfouling on aluminium surface were carried out at two shelf stations. Panels were fixed as above and kept suspended for a period of 18 days at 10, 30 and 50 m at a station (12°16.64' N, 84°27.72' E) in the Bay of Bengal

during the 40th cruise of the ORV SAGAR KANYA (March/April, 1988). Similarly, during the 48th cruise of ORV SAGAR KANYA in the southern Arabian Sea, panels were suspended at 1, 10, 20, 40 and 60 m at a station (19°03.78'N, 72°10.62'E) for 24 hours in December/January, 1988-89, using a mooring system ('l'mooring) used for current measurements.

Removal of microfouling material

After retrieval, panels were lightly rinsed with filtered and sterilized estuarine or seawater. During the study carried out at an estuarine station three different tools were employed for removing the microfouling layer from the panels, viz. a nylon brush, 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. In all the subsequent work, the scraping was done only with nylon brush and filtered and sterilized seawater.

Biological and chemical analysis

Subsamples of 1 ml were serially diluted and 0.2 ml of the last 2 dilutions was used for the enumeration of bacterial colonies by pour plate method using ZoBell's marine agar (No. 2116). Enumeration of total bacterial counts was made by the AODC method (Parsons et al., 1984). A subsample was preserved with lugol's iodine for the quantitative microscopic examination of diatoms. Subsamples were filtered through GF/C for the estimation of dry weight (Bhosle et al., 1990a), chlorophyll *a*, organic carbon and lipids (Parsons et al., 1984), organic nitrogen (Smart et al., 1983), proteins (Clayton et al., 1988) and total carbohydrates (Dubois et al., 1956). Monosaccharide composition of the microfouling material was evaluated by capillary gas chromatography as described earlier (Bhosle et al., 1990b). Amino acid composition of microfouling material was determined by high performance liquid chromatography (HPLC). The precision of these methods varied from ± 5 to 10 %. A product moment correlation coefficient was applied to verify the relationships between these parameters (Sokal and Rohlf, 1981).

Exopolysaccharide production by a fouling bacterium

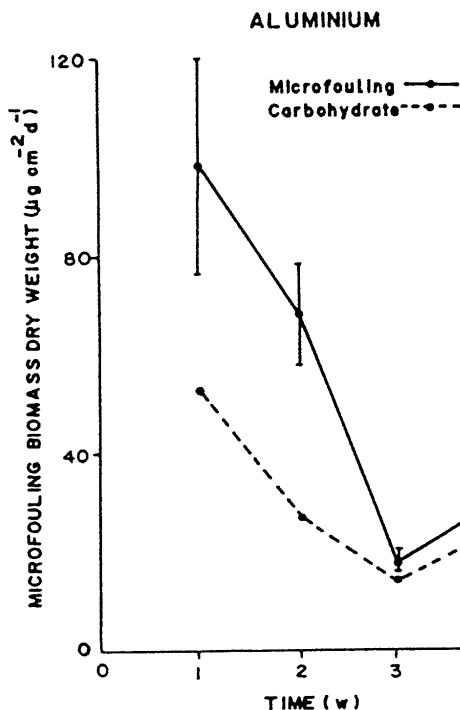
A fouling bacterium *Vibrio* sp was isolated from stainless steel panels placed in seawater. In order to assess the production of exopolysaccharide (EPS), growth curve experiments were conducted. The culture was grown in basal salt solution (BSS) containing trisodium citrate as the sole carbon and energy source (Rodrigues, 1990). Growth was monitored at room temperature ($28 \pm 2^\circ\text{C}$) with shaking at 150 rpm. At regular intervals, three 5 ml aliquots were removed for turbidity measurements (A 420), then centrifuged (6000 rpm for 15 min). The supernatants were collected and analyzed for EPS production by the phenol-sulphuric acid method (Dubois et al., 1956).

In order to isolate EPS, batch cultures were grown in Erlenmeyer flasks using BSS medium containing glucose (1% w/v) to stationary phase (48 h). The cells were removed and supernatant filtered, dialysed and concentrated. The EPS was precipitated using cold ethanol and kept overnight at 4°C. The precipitated EPS was dried at 50°C and used for chemical characterization. The EPS was analysed for sugar and non sugar components (Rodrigues, 1990).

Corrosion of mild steel

Corrosion of mild steel panels was studied at 3 stations in the Arabian Sea during the 213th cruise of RV GAVESHANI in June/July, 1989 (Fig. 2). Panels were fixed as above and were kept suspended at 2, 20, 40 and 50 m at station 1, at 2, 20, 40 and 70 m at station 2 and at 2, 20, 50 and 100 m at station 3 using 'I' mooring. The moorings were kept immersed at the above depths from June 26 to July 5, 1989.

Fig. 2:
Changes in the microfouling biomass and total carbohydrates on aluminium panels during the four week immersion period in the surface waters of the Dona Paula Bay. Bar represent 2 SD (from Bhosle et al., 1990 d).



After retrieval, the corrosion products were removed and stored in a vacuum desiccator until analysis. After the removal of the corrosion products, the panels were cleaned and reweighed as above to estimate weight loss. The corrosion rate ($\text{mg dm}^{-2} \text{d}^{-1}$) were then calculated using the formula

$$C = \frac{W1 - W2}{A t}$$

where C is the corrosion rate ($\text{mg dm}^{-2} \text{d}^{-1}$), W1, and W2 are the weights in grams of mild steel panels before and after immersion, A is the area of panels (cm^2) and t is the duration of immersion in days. Precision of the method based on six replicates was $\pm 7.67\%$.

Organic matter analysis

A known amount of the corrosion products was weighted on a microbalance (Mettler ME) and was analysed for organic carbon (Parsons et al., 1984). Precision of the analytical method based on 6 replicates was $\pm 8.97\%$.

In order to extract water soluble carbohydrates, a known amount of the corrosion products was treated with hot water as suggested by Handa and Yanagi (1969). Carbohydrates were estimated as described earlier (Dubois et al., 1956). Precision of the method based on six replicates was $\pm 11.25\%$.

Scanning electron microscopy

Samples were fixed in 2 % glutaraldehyde and air dried for 24 hours. The dried specimens were mounted on aluminium stubs using pidilite as adhesive and coated with carbon and gold using Balzers sputter coater. Samples were scanned on a Cambax EPMA consisting of two inclined wavelength spectrometers. The operating voltage was 15 to 20 kV and 0.1 to 0.2 mA. Samples were counted for 10 s at each spot and the counts were carried out 5 times. The elemental abundances of Fe, Si, S and P were determined using the intensity of $K\alpha$ line, the strongest line of the elements. The precision of the method was better than $\pm 5\%$.

X-ray diffraction

Bulk sample powders of the corrosion products were analysed on a Phillips X-ray diffractometer using nickel filtered Cu $K\alpha$ radiation at $2^\circ 20/\text{min}$ from 8° to $50^\circ 20$. The minerals were indentified using standard JCPDF cards.

Water sampling

During the immersion period (June to July, 1989) used for corrosion experiment, each station was sampled thrice in order to measure temperature and dissolved oxygen of seawater. Niskin samplers were used for discrete water sampling at these stations. Temperature was recorded with a reversing thermometer. Dissolved oxygen was determined by titration method (Strickland and Parsons, 1972). Precision of the measurements was better than $\pm 1\%$.

3. Results

Five replicate extractions from aluminium panels using the 3 tools described, yielded more or less similar amounts of the microfouling material (Tab. 1). Since the use of nylon brush was found to be relatively easy, this method was followed for further work described below.

Table 1: Comparison of methods for the extraction of the microfouling layer from the aluminium panels immersed in estuarine waters (from Sharma et al., 1990)

Methods	Microfouling biomass ($\text{g cm}^{-2} \text{d}^{-1}$)
Nylon brush	0.04 ± 0.001
Stainless steel razor	0.04 ± 0.002
Sodium pyrophosphate (0.001 M)	0.039 ± 0.004

Microfouling biomass when estimated as dry weight, organic carbon and total bacterial count showed large variation from station to station. Generally, microfouling biomass was higher in shelf waters (< 200 m) as compared with oceanic waters (> 200 m) for aluminium and fiberglass surfaces (Tab. 2). Microfouling biomass generally decreased in the offshore direction, however, no significant relationship between microfouling biomass and distance offshore was observed. Hydrophobic surface (fiberglass) supported higher microfouling biomass as well as microbial populations than that of hydrophilic surface (aluminium).

Table 2: Microfouling biomass on aluminium and fiberglass panels immersed in surface waters of the southern Arabian Sea during cruise 51 of ORV SARGAR KANYA in March/April, 1989, (sampling depth was 1 m)

Sta- tion	Station depth (m)	Surface					
		Aluminium		Fiber glass			
		a	b	c	a	b	c
<i>Shelf water</i>							
1	67	1.52±0.30	6.15±1.75	84.24± 3.84	1.55±0.04	9.19±0.31	198.42±12.23
13	28	3.31±0.21	15.31±1.80	254.74±23.22	4.26±0.10	12.20±1.90	353.31±18.81
15	50	3.51±0.08	17.10±2.50	171.37± 1.92	3.96±0.28	29.30±2.20	345.53±59.61
17	87	3.99±0.36	18.62±1.85	188.86± 1.56	3.93±0.47	14.75±0.25	334.12±68.81
<i>Oceanic waters</i>							
2	940	0.64±0.12	4.72±0.42	38.47±13.12	0.72±0.07	7.78±0.80	64.28± 0.17
4	2740	0.54±0.07	2.14±0.10	14.51± 3.25	0.58±0.05	1.96±0.41	33.30± 1.30
6	3000	0.65±0.06	-	31.95± 5.03	0.74±0.08	-	46.19± 0.52
8	4600	0.57±0.01	4.45±0.02	7.23± 1.36	0.54±0.05	2.89±1.08	21.78± 0.44
9	4200	0.58±0.05	1.61±0.10	14.40± 1.66	0.51±0.06	2.36±0.26	41.31± 0.73
12	4176	0.58±0.04	2.25±0.01	31.03± 0.91	0.46±0.02	1.18±0.42	42.64± 3.34
14	1650	0.81±0.07	3.50±0.50	42.88± 7.01	0.80±0.02	4.53±1.77	86.60±12.08
16	1275	0.74±0.07	2.29±0.03	36.92± 4.91	0.81±0.03	2.51±0.11	65.78± 9.92

a = microfouling biomass (mg dm⁻² h⁻¹)

b = total bacterial count (value x 10⁶ dm⁻² h⁻¹)

c = microfouling biomass as carbon (µg C dm⁻² h⁻¹)

- = no data

As the immersion period and the depth of immersion increased microfouling biomass decreased (Fig. 2). However, at a station occupied in the shelf waters of the Arabian Sea, microfouling biomass showed a small increase at intermediate depths (10 to 40 m) which was followed by a decrease at 60 m depth.

3.1. Biological and chemical characteristics of the microfouling material

Chlorophyll *a*, bacterial numbers, chemical and biochemical composition of the microfouling material developed on aluminium panels after ten days exposure in estuarine waters is shown in table 3. The contribution from proteins, carbohydrates and lipid carbon towards the total carbon content was nearly the same (Tab. 4). Abundance of diatoms was, however very less. A very significant correlation was observed between the bacterial numbers and various chemical components (Tab. 5). A fairly high abundance of diatoms was observed in the microfouling material developed on aluminium panels immersed at various depths at a shelf station of the Arabian sea

Table 3: Biological and chemical composition of microfouling material developed on aluminium panels immersed in estuarine waters for a period of 10 days (from Sharma and Wagh, 1990)

Parameters	Aluminium panel (n = 5)
Dry weight ^a	4770 ± 008
Organic matter ^b	1354 ± 130
Organic carbon ^b	677 ± 65.13
Organic nitrogen ^b	192 ± 8.80
C/N ratio (atomic)	4.1 ± 0.20
Total counts	14.2 ± 0.98 x 10 ⁶
Viable counts	5.4 ± 0.14 x 10 ⁵
Chlorophyll <i>a</i> ^b	0.003 ^c

a = mg/plate
b = µg/plate
c = single observation

Table 4: Biochemical composition of the microfouling material settled on aluminium panels immersed in estuarine water (n = 5) (from Sharma and Wagh, 1990)

Parameter	Aluminium		
	a	b	c
Protein	453 ± 76.90	33.24 ± 5.60	31.77 ± 5.40
Carbohydrate	429 ± 38.51	31.75 ± 2.80	25.34 ± 2.27
Lipids	359 ± 17.80	27.22 ± 0.71	27.20 ± 0.70

a = µg/plate
b = % of organic matter
c = contribution as % of total carbon

Table 5: Relationship of microfouling bacteria and chlorophyll *a* with dry weight, organic carbon, nitrogen and biochemical components of microfouling material developed on aluminium panels immersed in estuarine waters (from Sharma and Wagh, 1990)

	Chlorophyll <i>a</i>	Bacterial Counts	
		Viable	Total
Dry weight	0.155	0.993*	0.911*
POC	0.043	0.892*	0.743 [#]
PON	0.254	0.984*	0.973*
Carbohydrates	0.205	0.976*	0.942*
Proteins	0.345	0.725 ⁺	0.850*
Lipids	0.530	0.900*	0.982*
Chlorophyll <i>a</i>	-	0.193	0.296

* = P ≤ 0.001
= P ≤ 0.01
+ = P ≤ 0.05

(Tab. 6). Ten different diatoms were observed from different depths. *Nitzschia* was the most abundant group at all depths. Total fouling diatom numbers decreased as the depth increased. At 10, 25 and 40 m depth four types of diatoms were recorded, and at 80 m only three types were present, whereas, at 60 m 8 types of diatoms were observed (Tab. 6).

Table 6: Type and number of microfouling diatoms (No./dm²/10 days) on aluminium panels placed at various depths at station (15°08'N, 73°16'E) (from Bhosle et al., 1989a)

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

Carbohydrate composition of the microfouling material

As observed for microfouling biomass, the concentration of total carbohydrate (HCHO) also generally decreased with increase in the immersion period (Fig. 2). Capillary gas chromatographic analysis revealed the presence of 8 individual sugars in the microfouling material (Tab. 7). Mannose, glucose and galactose were the most abundant constituents of the microfouling material during the first week of immersion. During the second week of immersion, the concentrations of rhamnose and fucose were relatively high. Galactose and mannose were most abundant during the third week of immersion. During the fourth week of immersion, galactose was the single most abundant sugar.

The relative carbohydrate composition of the microfouling material showed large variations during the period of immersion. From these data it was evident that the glucose content generally decreased and galactose increased with the immersion period. Ribose was a minor constituent especially during the fourth week of immersion.

Carbohydrate concentration and spectral distribution of sugars in the microfouling material also varied with the depth of immersion (Fig. 3; Tab. 8). At 10 m, galactose was the single most abundant sugar followed by mannose and arabinose. Rhamnose and arabinose at 30 m, whereas mannose and arabinose at 50 m were the most abundant sugars. The concentration of glucose and xylose increased with immersion depth. As compared with other sugar components, ribose content was relatively low at 30 and 50 m.

Table 7: Monosaccharide concentration ($\text{ng cm}^{-2} \text{d}^{-1}$) and weight ratios of mannose (MAN)/xylose (XYL) and hexoses (HEX)/pentose (PENT) and glucose free weight percentage of ribose (RIB) plus fucose (FUC) and arabinose (ARB) plus galactose (GAL) of the microfouling material developed on aluminium panels immersed in the surface waters of the Dona Paula Bay, the Arabian Sea (from Bhosle et al., 1990)

Surface Period (W)	Aluminium			
	1	2	3	4
Rhamnose	34.05	48.52	7.25	17.34
Fucose	32.83	25.40	7.46	17.94
Ribose	30.39	8.42	3.43	4.98
Arabinose	26.75	11.41	6.23	11.91
Xylose	30.39	7.72	4.31	6.73
Mannose	104.58	24.91	10.24	29.22
Galactose	66.88	18.49	12.42	44.46
Glucose	105.79	26.13	6.08	14.86
$HCHO^1$ ($\text{ng cm}^{-2} \text{d}^{-1}$)	431.66	171.00	57.42	147.44
$HCHO^2$ ($\text{ngC cm}^{-2} \text{d}^{-1}$)	172.66	68.40	22.96	58.97
RIB+FUC	19.19	23.34	21.21	17.18
MAN/XYL	3.44	3.22	2.37	4.34
ARB+GAL	2 8.73	20.63	36.32	42.51
HEX/PENT	3.93	5.22	3.11	5.24

(2) = values in (1) are multiplied by factor of 0.4 to convert to carbon.

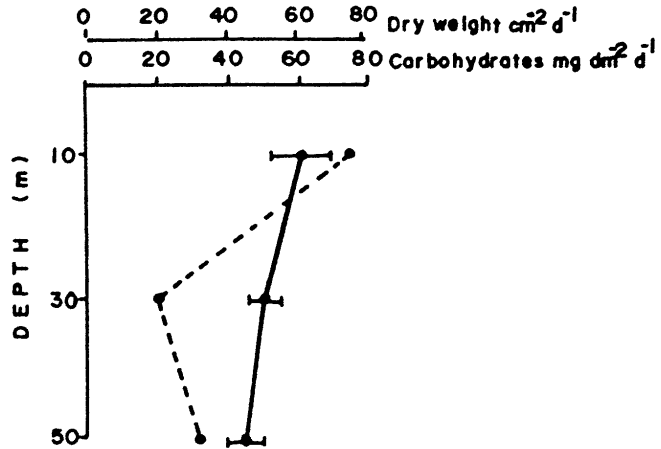
Table 8: Monosaccharide concentration ($\text{ng cm}^{-2} \text{d}^{-1}$) and weight ratios of mannose (MAN)/xylose (XYL), hexoses (HEX)/pentoses (PENT) and glucose free weight percentages of ribose (RIB) plus fucose (FUC) and arabinose (ARB) plus galactose (GAL) of microfouling material developed on aluminium panels placed at various depths at a shelf station of the Bay of Bengal (from Bhosle et al., 1990)

Surface Depth (m)	Aluminium		
	10	30	50
Rhamnose	4.53	6.00	2.93
Fucose	3.76	0.36	2.02
Ribose	3.15	0.26	1.26
Arabinose	11.34	3.49	4.20
Xylose	1.70	0.68	2.16
Mannose	16.34	3.50	6.37
Galactose	28.01	3.40	7.65
Glucose	4.31	2.18	4.92
$HCHO^1$ ($\text{ng cm}^{-2} \text{d}^{-1}$)	73.14	19.87	31.51
$HCHO^2$ ($\text{ngC cm}^{-2} \text{d}^{-1}$)	29.25	7.94	12.60
RIB+FUC	10.03	3.50	12.32
MAN/XYL	9.61	5.15	2.95
ARB+GAL	57.16	38.94	44.56
HEX/PENT	3.51	3.48	3.13

(2) = values in (1) are multiplied by factor of 0.4 to convert to carbon.

Fig. 3:

Vertical distribution of biomass estimated as dry weight (—) and carbohydrates (-----) of microfouling material developed on aluminium panels immersed at 10, 30, and 50 meters for 18 days at a station in the shelf waters of the Bay of Bengal. Bar represent 2 SD (from Bhosle et al. 1990 b).



The mannose/xylose ratio did not vary much during the 4 week immersion period (Tab. 7). A similar pattern was observed for the hexoses/pentoses ratio. Glucose free weight percentages of ribose plus fucose decreased as immersion period increased, especially from the second week onwards. However, the weight percentages (glucose free) of arabinose plus galactose showed reversed trend to those observed for ribose and fucose (Tab. 7).

Table 9: Microfouling biomass and amino acid composition (Mol%) of the microfouling material developed on aluminium panels immersed at various depths at a station in the shelf waters of the Arabian Sea

Amino Acid	Depth (m)				
	1	10	20	40	60
Aspartic acid	3.21	3.91	3.14	3.57	4.13
Glutamic acid	0.36	3.09	2.13	3.25	3.67
Serine	2.40	2.26	0.75	0.10	3.24
Histidine	tra	tra	0.06	tra	4.60
Glycine	17.95	26.23	13.44	25.89	41.67
Threonine	0.07	0.69	3.01	0.17	6.10
Arginine	11.38	25.09	12.31	14.17	1.85
Alanine	6.49	6.05	6.03	8.63	4.42
Tyrosine	0.65	tra	0.06	tra	1.39
Methionine	5.11	tra	tra	tra	0.84
Valine	tra	0.06	tra	0.24	2.08
Phenylalanine	0.07	tra	tra	tra	3.09
Isoleucine	28.97	tra	tra	0.13	4.66
Leucine	5.98	28.24	33.22	22.91	11.17
Lycine	17.29	4.35	25.81	20.86	7.03
<i>Total amino acid</i> (nmol dm ⁻² d ⁻¹)	13.70	15.86	15.92	28.85	34.54
<i>Microfouling biomass</i> mg dm ⁻² d ⁻¹	1.96	2.18	2.88	3.02	1.90

tra = traces

The mannose/xylose ratio decreased, whereas the hexoses/pentoses ratio did not vary much with immersion depth (Tab. 8). On the other hand the glucose free weight percentages of ribose plus fucose and arabinose plus galactose showed a similar pattern with respect to the depth of immersion.

Amino acid composition of the microfouling material

Fifteen amino acids were detected in the microfouling material obtained from various depths (Tab. 9). Total amino acid content varied from 13.70 to 34.50 nmol dm⁻² d⁻¹. Total amino acid concentration increased with depth. Glycine, aspartic acid leucine and lysine were the most abundant amino acids of the microfouling material. Glycine contribution to the total amino acids increased with depth and was about 42 mol % at 60 m. Higher content of glycine was associated with lower microfouling biomass.

3.2. Production of EPS by a fouling bacterium

To study the production of EPS by *Vibrio* sp, a fouling bacterium, growth curve experiment was performed wherein EPS synthesis was measured. Growth ceased after about 36 h, at a final optical density of 1.6. Synthesis of EPS was detected during early exponential phase and continued into stationary phase (Fig. 4).

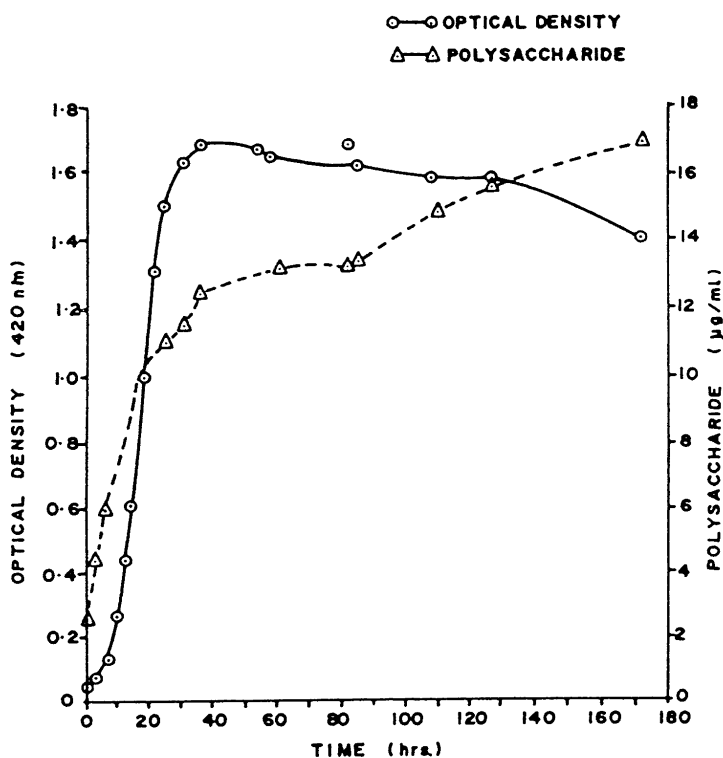


Fig. 4: Growth of a fouling bacterium *vibrio* sp and the production of polysaccharide (from Rodrigues, 1990)

The chemical composition of the isolated EPS is shown in table 10. The EPS was composed of glucose and galactose as the major and mannose, rhamnose and arabinose as the minor components. Hexosamine, uronic acid and pyruvate were detected as non-sugar components of the EPS.

Table 10: Chemical composition of the exopolymer produced by a fouling bacterium *Vibrio* sp (from Rodrigues, 1990)

Component	Concentration (Mol %)
Glucose	34.41 ± 1.37
Galactose	37.41 ± 1.91
Mannose	15.10 ± 0.30
Arabinose	5.60 ± 0.60
Rhamnose	6.78 ± 0.25
Uronic acid	+
Pyruvate	+
Methyl pentoses	+
Hexosamines	+
Sulphate	-

+ = present
 - = absent

3.3. Corrosion of mild steel in the shelf waters of the Arabian Sea

Corrosion of mild steel immersed at various depths (2 to 100 m) from 3 shelf stations of the Arabian Sea was investigated (Fig. 5). Generally, temperature of seawater decreased with increasing water depth at all the three stations (Tab. 11). Temperature profiles imply that the upper 20 m water column was well mixed. Dissolved oxygen showed a pattern similar to that of temperature.

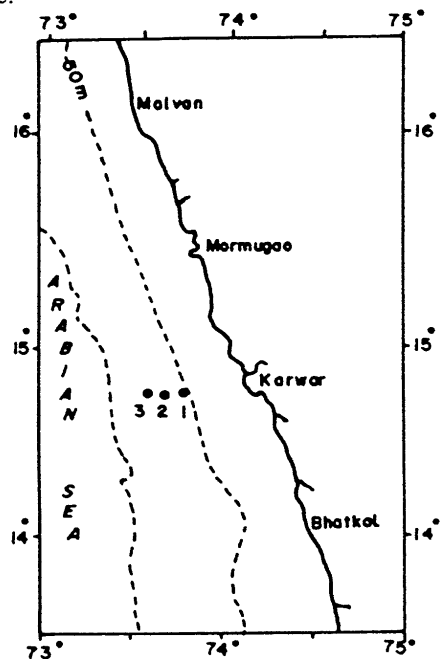


Fig. 5: Location of stations sampled in the Arabian Sea in June/July, 1989 during the 213th cruise of the RV GAVESHANI.

Corrosion rates were maximum at 2 m and thereafter generally decreased with increasing water depth with vertical profiles similar to those observed for temperature and dissolved oxygen (Fig. 6; Tab. 11). Spatial variations in the corrosion rate of mild

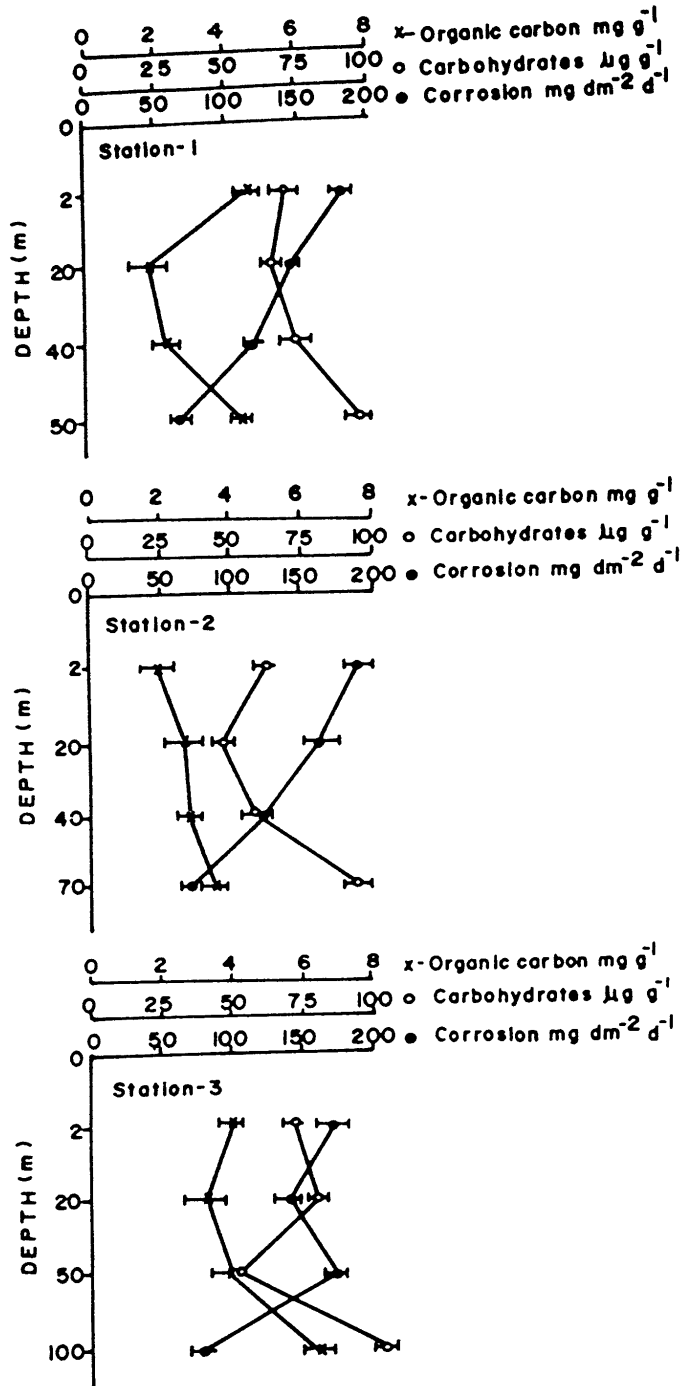


Fig. 6: Depth related variation in the corrosion of mild steel and organic carbon and water extractable carbohydrates of the corrosion products of the mild steel at station 1, 2 and 3 of the Arabian Sea during the 213th cruise of the RV GAVESHANI in June/July, 1989.

steel were conspicuously absent. The concentration of organic matter viz. organic carbon and water extractable carbohydrates associated with the corrosion products of mild steel, on the other hand, increased with the immersion depth (Fig. 6).

Table 11: The corrosion rate of mild steel and distribution of temperature and oxygen at three stations of the Arabian Sea during the cruise 213 of the RV GAVESHANI (from Bhosle and Wagh, 1990)

Depth (m)	Temperature ^a (°C)	Dissolved oxygen ^a (ml l ⁻¹)	Corrosion rate ^b (mg dm ⁻² d ⁻¹)	
<i>STATION 1</i>				
2	29.00	3.96	179 ± 11	(6.14 %)
20	28.80	3.80	146 ± 05	(3.56 %)
40	24.61	1.53	120 ± 04	(3.33 %)
50	24.31	0.83	67 ± 06	(3.33 %)
<i>STATION 2</i>				
2	28.32	3.89	187 ± 11	(5.88 %)
20	28.30	3.38	163 ± 12	(7.36 %)
40	25.24	2.02	119 ± 03	(4.18 %)
70	22.76	1.22	71 ± 04	(5.13 %)
<i>STATION 3</i>				
2	28.56	3.74	169 ± 14	(8.28 %)
20	28.56	3.72	143 ± 13	(9.10 %)
50	27.81	3.48	173 ± 08	(4.62 %)
100	21.49	0.98	77 ± 08	(10.39 %)

a = mean of three sampling

b = mean of six replicates

± = standard deviation (coefficient of variation)

Scanning electron microscopic observation of the corroded mild steel panels showed the presence of several microorganisms including individual bacteria, chains of bacteria, filamentous bacteria and diatoms (Plate 1).

Electron probe microanalysis of corroded mild steel panels suggest that the content of iron decreased, whereas silica phosphate and sulfide increased in the corrosion products with immersion depth (Tab. 12).

Table 12: EPMA analysis of the corrosion products of mild steel immersed at various depths at a station in the shelf waters of the Arabian Sea

Depth (m)	Metal (%)			
	Iron	Silica	Phosphate	Sulphide
2	97.41	1.85	0.22	0.51
20	96.55	1.57	0.45	1.41
40	93.06	2.43	1.96	2.54
70	90.46	3.03	4.24	2.26

The XRD data showed the presence of ferrihydrite ($\text{Fe}_2\text{O}_3 \cdot \text{OH} \cdot 4 \text{H}_2\text{O}$), halite (NaCl), calcite (CaCO_3), lepidocrocite ($\gamma\text{-FeOOH}$), pyrite (FeS_2) and magnesite (MgCO_3).

There were positive and highly significant relationships between corrosion rates and temperature ($r = 0.901$; $P < 0.001$; $n = 12$) and dissolved oxygen ($r = 0.910$; $P < 0.001$; $n = 12$) of seawater. Surprisingly, however, inverse relationships were observed between corrosion rates and organic carbon and water extractable carbohydrates associated with the corrosion products of mild steel (Tab. 13).

Table 13: Linear correlation coefficients (r) between the corrosion rate (CR) of mild steel and organic carbon (OC) and water extractable carbohydrates (CHO) of the corrosion products (from Bhosle and Wagh, 1990)

Station	Parameters	Correlation Coefficient (r)	
1	OC VS CR	- 0.965	($P = < 0.001$; $n = 4$)
2	OC VS CR	- 0.846	($P = < 0.02$; $n = 4$)
3	OC VS CR	- 0.846	($P = < 0.02$; $n = 4$)
1	CHO VS CR	- 0.894	($P = < 0.01$; $n = 4$)
2	CHO VS CR	- 0.781	($P = < 0.05$; $n = 4$)
3	CHO VS CR	- 0.920	($P = < 0.01$; $n = 4$)

4. Discussion

4.1. Biology and chemistry of microfouling

Removal of microfouling material from surface is the most critical step in estimating microfouling biomass developed on solid substrata placed in aqueous environment. Five replicate extraction from aluminium surfaces using 3 tools described yielded more or less similar amounts of the microfouling material (Tab. 1). Sodium pyrophosphate which has been utilized earlier to release adsorbed microorganisms from surfaces, did not show any appreciable advantage over other methods (Afring and Taylor, 1979; Jeffrey and Paul, 1986). Since the use of nylon brush was found to be relatively easy, this method was used for the removal of microfouling material from solid substrate.

Several parameters such as carbon, nitrogen, ATP, carbonate content and total dry weight have been used to quantify the extent of biofouling (Afring and Taylor, 1979; Burton and Margrey, 1979; Mayack et al., 1984). We used dry weight and/or organic carbon content to assess the extent of microfouling on aluminium and fiberglass surfaces with space, time and depth because these methods gave highly reproducible results and was less time consuming (Sharma et al., 1990).

Microfouling biomass developed on aluminium and fiberglass surfaces varied from station to station in the southern Arabian Sea (Tab. 2). Microfouling in general was higher in shelf water as compared with slope and oceanic waters. This observation agrees well with earlier studies. For example, Bhosle et al., (1990 a) reported an order of magnitude reduction in the microfouling biomass in the oceanic waters as compared

with shelf waters of the central Arabian Sea. Similarly, De Palma (1984) observed considerable reduction in fouling biomass in slope waters. Microfouling biomass is influenced by environmental and biological factors and by the nature of the surface substratum (Characklis and Escher, 1988; McEldowney and Fletcher, 1986, 1988). These factors are known to vary from station to station and thus might have played some role in controlling the microfouling biomass developed on these substrata.

Amongst the two surfaces used, one was hydrophilic (aluminium) and the other was hydrophobic (fiberglass). In general the hydrophobic surface (fiberglass) supported higher amount of microfouling biomass and bacterial numbers. This implies that the nature of surface substrata played an important role in the development of bacterial biofilm. It is suggested that different numbers of bacteria initially attach to hydrophobic and hydrophilic surfaces (Pringle and Fletcher, 1983; McEldowney and Fletcher, 1986; 1988; Pedersen, 1990). This effect, however, was not evident at some stations (Tab. 2). The reason might be that the biofilm processes of exponential growth, product formation and debris entrapment dominated (Characklis, 1984; Characklis et al., 1986) over the initial, surface energy dependent processes (Bitton and Marshall, 1980; Fletcher and Marshall, 1982).

Various workers have described the developing stages of the primary film and ecological succession of complex microfouling communities in the temperate and subtropical environments (Mitchell and Krichman, 1984; White and Benson, 1984). These studies suggest that bacteria and diatoms were the first organisms to appear on various surfaces placed in aquatic environment. Our observation on the microfouling material formed on aluminium and fiberglass panels is in agreement with the above findings. Bacteria were abundant in the microfouling material developed on aluminium panels placed in estuarine waters. Bacterial numbers were found to be significantly related with various parameters implying that bacteria were the major contributor to microfouling material (Tab. 5).

A number of diatoms were also observed in the microfouling (Tab. 6). Diatom which fouled manmade surfaces belong to restricted group of organisms and are usually of the pennate type (Characklis and Cooksey, 1983, Cooksey et al., 1984). In the present case, in addition to pennates (60 %), centrals (40 %) were also present. Of the various diatoms observed, *Triceratium* was the first to occur 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.

The abundance of bacteria and diatoms was also evident from C/N ratio of the microfouling material. The C/N ratio is an important indicator of the origin of organic matter in marine environment (Pocklington and Leonard, 1979). The obtained C/N ratio was low and ranged between 3.5 and 4.1. These values are similar to those reported for the organic matter originated from the biogenic material (Fenchel and Blackburn, 1979; Cubell et al., 1982; Linley and Newell, 1984). Biochemical analysis of the microfouling material showed large amount of protein, carbohydrate and lipid which may serve as a food source for the settlement of many other organisms (Tab. 4).

Microfouling biomass generally decreased as the period and depth of immersion

increased (Fig. 3 & 4). This was in contrast with earlier studies suggesting a non linear increase in microfouling biomass with immersion period (Martinez et al. 1984; White and Benson, 1984; Chamberlain and Garner, 1988). The observed decrease in the microfouling biomass suggests the absence of growth of adsorbed organisms, decrease in production rates, desorption of adsorbed organisms, shearing and/or sloughing of adsorbed material and grazing by aquatic organisms.

Eight natural sugars rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose were indentified in acid hydrolysate when separated by capillary gas chromatography (Tab. 7 & 8). The contribution of these sugars to total sugars varied with the period and depth of immersion. The contribution of glucose to total sugars was relatively less. This was surprising since it is the dominant sugar in all forms of terrestrial and aquatic matter (Degens and Mopper, 1976). Thus a low content of glucose either points to a selective enrichment in other sugar fractions or to a preferential degradation of glucose or to an increased contribution from sources poor in glucose (Liebezeit, 1986; Tanoue and Handa, 1987).

Labile compounds such as storage polysaccharide, lipids and proteins of suspended particles and settling phytoplankton are biodegraded during their transport to greater depths (Handa and Tominaga, 1969; Handa and Yanagi, 1969; Ittekkot et al., 1982; Tanoue and Handa, 1987; Bhosle and Wagh, 1989; Bhosle et al., 1989 b). The selective removal of these compounds leaves the original material relatively rich in structural carbohydrates containing higher concentration of glucose. The relative increase in the abundance of glucose in the microfouling material with immersion depth suggest an increased contribution by such glucose rich particles.

Generally, ribose was a minor constituent of the microfouling material during the 4 week immersion period and also from various depth of immersion. This implies that the contribution from bacteria and calcareous planktonic organisms generally decreased with increase in the immersion period and depth (Klok et al., 1984; Ittekkot et al., 1984; Ittekkot and Arain, 1986).

The contribution of ribose plus fucose (calculated without glucose) varied from 17.28 to 23.34 %, suggesting the presence in varying amounts of biogenic organic material (Cowie and Hedges, 1984). The contribution of these two sugars, on the other hand, was relatively less (< 12 %) in the microfouling material obtained from aluminium test panels immersed at various depths, thus implying the predominance of organic material derived from terrestrial sources.

During the time series experiments the mannose/xylose ratio (Tab. 7) was similar to those observed for phytoplankton, angiosperm tissues and grasses (Cowie and Hedges, 1984; Ittekkot and Arain, 1986), whereas this ratio was about 9.61 and 5.15 for microfouling material collected at 10 and 30 m (Tab. 8), respectively. This suggests the predominance of gymnosperm tissues in microfouling material obtained from 10 and 30 m depths. In contrast to this, at 50 m the ratio was about 2.95 indicating the predominance of phytoplankton, angiosperm tissues and grasses. From the percentage distribution of fucose and ribose as well as mannose/xylose ratio it was evident that microfouling material had a marine and terrestrial origin.

Pectin, a polysaccharide containing arabinose and galactose as predominant sugars is rich in non-woody tissues as compared with woods (Sjostrom, 1981). The

contribution of woody and non-woody tissues to the microfouling material can be calculated from the weight percentage distribution (without glucose) of these sugars (Cowie and Hedges, 1984). Arabinose and galactose together contributed between 20 to 57 % (Tab. 7 & 8). These values are similar to those observed for non woody tissues. Thus it is most likely that non-woody tissues derived from phytoplankton, angiosperm and gymnosperm tissues and grasses are the most important source materials of carbohydrates in the microfouling material.

Amongst the amino acids detected in the microfouling material, glycine was the only amino acid which was enriched with increasing depth (Tab. 9). This amino acid was found to be relatively abundant in diatom cell walls (Hecky et al., 1973). Microfouling material was characterized by a predominance of glycine (18 to 41 %) and a low aspartic acid/glycine ratio (0.10 to 0.23). This implies that the microfouling material was relatively abundant in diatomaceous material, especially at greater depth during short term experiment (24 h) at a shelf station in the Arabian Sea.

During the stages of biofilm development, EPS begins to accumulate and the biofilm takes on the microscopic appearance of a gel (Costerton et al., 1981; McFeters, 1984). This gel appears amorphous in the microscope as it acts as a trap for debris, corrosion products and other materials, sometimes including nutrients from the bulk phase. A fouling bacterium *Vibrio* sp isolated from biofilm developed on stainless steel panels was used to assess the production of EPS. The EPS produced by *Vibrio* sp resembles in many aspects the most common exopolymers of other bacteria. For example, the synthesis was first detected in early exponential phase and continued in stationary phase (Fig. 4) as observed in several other bacteria (Read and Costerton, 1987; Sar and Rosenberg, 1989). The production of EPS while growing seems to be an advantage for a bacterium fouling a surface as it may help to increase the strength of the adhesive bond and also to build a protective glycocalyx (Red and Costerton, 1987).

The EPS was retained by 10000 ultrafilters as well as dialysis bags with MW cut off of 13,000, indicating a molecular weight in excess of this value. Glucose and galactose were the most abundant sugars contributing more than 75 mol% of the total carbohydrate (Tab. 10). Furthermore, these sugars were present in nearly the same molar ratio (0.9 : 1.0). The adhesive EPS produced by two strains of *Pseudomonas* also showed similar ratio (Read and Costerton, 1987). Mannose, rhamnose and arabinose were also present as minor constituents of the EPS. Presence of these sugars suggests that the EPS is a heteropolysaccharide. The occurrence of non-sugar components such as uronic acid, pyruvate and hexosamine indicates the acidic nature of the EPS. The presence of these constituents gives negative charge to EPS as a result of which EPS can react with positively charged molecules such as metals and thus may play a major role in biofouling and corrosion of various metals (Ford and Mitchell, 1990).

4.2. Corrosion of mild Steel

The corrosion of mild steel decreased with increasing water depth at all the stations. The observed decreasing trends of the corrosion rate of mild steel in the Arabian Sea are similar to those reported earlier (Sawant et al., 1989). It appears that temperature and dissolved oxygen of seawater were the two important parameters controlling the corrodibility of mild steel in the Arabian Sea. This was evident from highly significant positive correlation coefficients obtained for these two parameters. with the corrosion rate of mild steel.

Biofilm of organisms may influence the corrosion of metals. The development of microbial biofilm on the metal surface is also known to be influenced by several factors including temperature and dissolved oxygen of seawater (Dexter et al., 1986; Gaylarde and Jonston, 1986; Bhosle et al., 1990 a). Scanning electron microscope observation of the corroded mild steel panels obtained from various depths showed noticeable variations in the composition of biofilm (Plate 1). The observed depth related changes in the corrosion of mild steel could therefore be attributed to variations in the composition of biofilm.

Biomass of fouling microorganisms generally decreases with increasing water depth (Bhosle et al., 1990 b). Furthermore, these organisms are known to produce metabolic products for their adhesion to metal surfaces (Costerton and Geesey, 1986; Little et al., 1986; White et al., 1986). In view of this higher amount of organic matter associated with the corrosion product at surface and a progressive decrease would be expected with increasing water depth. Surprisingly, however increasing abundance of organic matter associated with the corrosion products of mild steel was noticed with increasing depth (Fig. 6). The X-ray diffraction studies indicated the presence of several minerals including ferrihydrite, lepidocrocite, pyrite, halite and magnesite in the corrosion products of mild steel. Moreover, microfouling organisms were also observed on the corroded test panels (Plate 1). The increased adsorption of dissolved organic matter by these minerals and/or increased production of organic matter by fouling microorganisms at greater depths might have been responsible for the increase in the concentration of organic matter associated with the corrosion products of mild steel. This increase in the concentration of organic matter of the corrosion products was associated with a decrease in the corrodibility of mild steel. This was surprising because several organic compounds, especially exopolymers (carbohydrates) are known to accelerate corrosion rate of metals (White et al., 1986; Ford et al., 1988).

Polysaccharide may provide sites for aeration cells, ion concentration cells and metal binding (Geesey et al., 1986; Little et al., 1986; Ford et al., 1988; Ford and Mitchell, 1990). The ability of polysaccharide to bind specific metal ions strongly influences its adhesion to metal surface and its ability to concentrate metal ions from these surfaces and bulk media. Binding of metals may be important in both passivation and activation reaction. The observed inverse relationship between the water extractable carbohydrates of the corrosion products and the corrosion rates of mild steel immersed in seawater suggests that similar reactions may be occurring in the natural environment in order to form a protective film on the metal surface. In this connection it is interesting to compare our data obtained from natural environment with a recently published laboratory study. Biofilm of a polysaccharide producing culture *Delya marina* was found to act as a strong corrosion inhibitor with almost complete passivation of mild steel, reducing the corrosion rate by 95 %. From this, it is evident that some microorganisms and/or their polysaccharide may act as a strong corrosion inhibitor.

The corrosion of mild steel starts with generation of ferrous ions by anodic oxidation at the surface because of the reaction ($\text{Fe} \rightarrow \text{Fe}^{2+} + 2 \text{e}^-$) which may undergo further oxidation producing Fe^{3+} species ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{e}^-$). Ferric ions are particularly deleterious for mild steel as they tend to accelerate the corrosion by the reaction ($\text{Fe} + 2 \text{Fe}^{3+} \rightarrow 3 \text{Fe}^{2+}$). If ferric ions are immobilized then it may be possible to control the corrosion of mild steel. Some polysaccharides are reported to exhibit strongest stability constant for Fe^{3+} ions (Ford et al., 1988). Such a complex may serve as a

corrosion inhibitor. The observed inverse relationship between the organic matter and the corrosion rate of mild steel suggest that such a metal-polysaccharide complex was probably involved in developing a protective film on the metal surface in natural seawater which was perhaps more stable at lower temperature and dissolved oxygen in these tropical marine waters.

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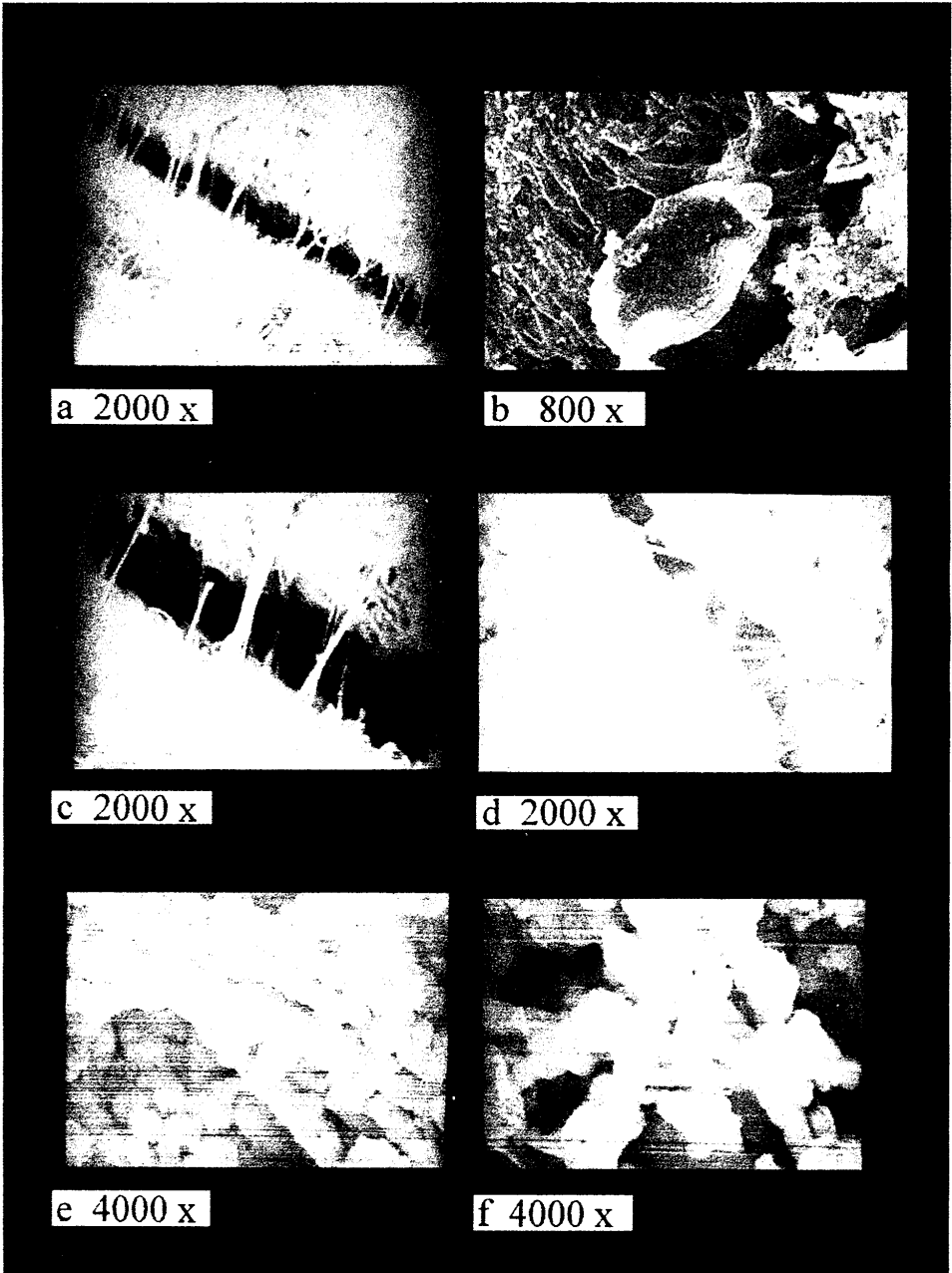


Plate 1

Microfouling organisms on mild steel panels placed at various depths at a station in shelf waters of the Arabian Sea; Filamentous bacteria at 2 m (a, c); Filamentous bacteria, rods in chains and diatom at 20 m (b, d); Cocci in chains and individual Cocci at 50 m (e to h). Cocci and rod shape bacteria single and in chains (i to k) and Foraminifera and fecal pellets (l) at 100 m depth. Presence of exopolysaccharide is evident in plate 1 a to i.

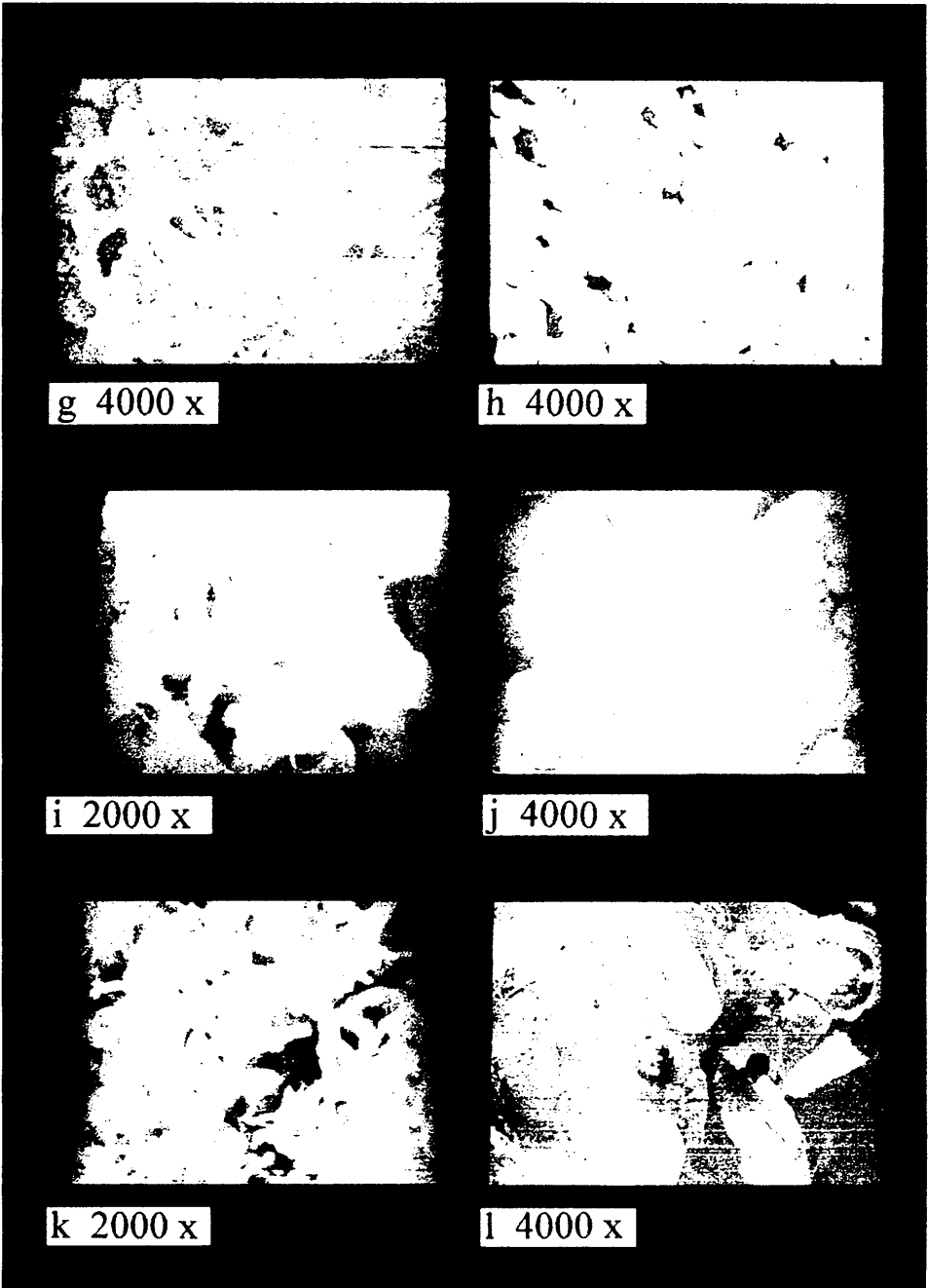


Plate 1 (continued)