



The nature of particulate organic matter settled on solid substrata

Substrata
Settled organic matter
Nature
Bacteria
Substrat
Matière organique adsorbée
Composition
Bactérie

Madhumati O. SHARMA, Arun B. WAGH

National Institute of Oceanography, Dona Paula, Goa, 403004, India.

Received 25/01/90, in revised form 16/02/90, accepted 22/03/90.

ABSTRACT

Particulate material settled on aluminium and glass panels during their immersion in estuarine water was analysed for bacteria, chlorophyll *a*, dry weight and various organic constituents such as organic matter, organic carbon, nitrogen, proteins, carbohydrates and lipids. There was no apparent difference in the composition of the organic matter or in the concentrations of the particulate material recovered from these two surfaces. Highly significant correlations were observed between the bacterial numbers and the measured parameters. This probably suggests that bacteria were the major source of the particulate matter settled on these substrata.

Oceanologica Acta, 1990, **13**, 4, 471-474.

RÉSUMÉ

Composition de la matière organique particulaire adsorbée sur un substrat solide

La matière organique particulaire adsorbée sur des panneaux d'aluminium et de verre immergés dans un estuaire a été analysée : bactéries, chlorophylle *a*, poids sec, matière organique, carbone organique, azote, protéines, glucides et lipides. Aucune différence n'a été décelée dans la composition de la matière organique et dans les concentrations des différents constituants de la matière particulaire prélevée à la surface des panneaux. Les corrélations observées entre le nombre des bactéries et les paramètres mesurés sont très significatives et suggèrent que la matière organique pourrait être principalement d'origine bactérienne.

Oceanologica Acta, 1990, **13**, 4, 471-474.

INTRODUCTION

Solid substrata placed in an aquatic environment adsorb organic molecules from the ambient waters onto their surfaces, making them readily available to organisms present in the environment (Zobell, 1943; Marshall, 1981). This adsorption of organic molecules is, however, a selective process and not all organic entities present in seawater are adsorbed on a given surface (Little, Zsolnay, 1983). In natural environments, however, all exposed surfaces rapidly adsorb a proteinaceous film (Horbett, 1982). Besides glycoproteins (Baier, 1980) and humic acids, macromolecules have also been implicated as the conditioning agents which may help in the subsequent attachment of organisms to surfaces (Zutic, Tomaic, 1988). The complex organic layer formed is reported to initiate fouling of exposed structures (Daniel, 1955; Loeb, Neihoff, 1977; Characklis and Escher, 1988). The deleterious phenomenon of fouling thus seems to be significantly influenced

both qualitatively and quantitatively by the adsorbed organic matter.

There are several instances wherein adsorbed material has been characterized either biologically or chemically, (Paul, Loeb, 1983; White, Bensen, 1984 and Paul *et al.*, 1985). For example, adsorbed material has been utilized to study bacteria, diatoms and other microscopic organisms; while the fatty-acid composition of settled particulate material has been used to define microbial community structure of a microfouling layer (White *et al.*, 1979a; b; Morrison, White, 1980). However, no particular attempt has been made to understand the nature of the particulate material present on surfaces. Therefore, in the present paper, we analysed the particulate material from solid substrate for its biological and chemical components. A possible correlation between the particulate material and the biological components, especially bacteria and chlorophyll *a*, was also been sought in order to determine the nature of the settled particulate organic matter.

MATERIAL AND METHOD

The study was carried out at a station in the Mandovi estuary (15°30'N, 73°52'E). The water depth at the sampling station is about 4 m.

Test panels of aluminium and glass were cleaned with hydrochloric and chromic acids respectively (Cameron, Robinson, 1968; Bhosle *et al.*, 1989). Replicate panels (5) were deployed in the surface waters (~1) of the Mandovi estuary, during June 1988, as described earlier (Sharma *et al.*, 1989). Panels were retrieved after 10 days of exposure and transported to the laboratory in an ice box containing estuarine water. Panels were scraped under sterile condition, using a nylon brush and filtered (0.2 µm), sterile estuarine water (FSEW) (Sharma *et al.*, 1989). The volume was adjusted to 125 ml. Aliquots (10 ml) were filtered through pre-ignited (450°C, 3 h) GF/C filters which were analysed for chlorophyll *a* and various organic parameters as described earlier (Sharma *et al.*, 1989). One ml. each was taken for viable and total bacterial counts.

Analyses

Viable counts by pour-plate method were made using Zobell's-Marine Agar (No. 2216) and the colonies were counted after incubating the plates for 72 h at room temperature (28° ± 2°C). The Acridine Orange Direct Count (AODC) method as described by Parsons *et al.* (1984) was employed for total counts of bacteria.

Dry weight of the organic matter was obtained by reweighing the GF/C filter on the microbalance (Mettler M-3). Particulate organic carbon (POC), lipids and chlorophyll *a* were estimated as described by Parsons *et al.* (1984). The method of Smart *et al.* (1983) was used for the estimation of organic nitrogen. Carbohydrates were determined by the method of Dubois *et al.* (1956). Proteins were analysed following the method of Clayton *et al.* (1988).

RESULTS AND DISCUSSION

The biomass of settled particulate material has been estimated by various methods (Holm Hansen, 1969; Hobbie *et al.*, 1977; White *et al.*, 1979a; Moriarity, 1980; Paul *et al.*, 1985; Balkwill *et al.*, 1988). We used dry weight and particulate organic carbon (POC) and

nitrogen (PON) as they provided highly consistent set of data (Bhosle *et al.*, 1989; Sharma *et al.*, 1989).

The values for dry weight, particulate organic carbon and nitrogen obtained from the two test surfaces were more or less identical (Table 1). Nor did biochemical composition of the particulate material developed on these substrata show any appreciable difference (Table 2). The contribution of proteins, carbohydrates and lipid carbon towards the total carbon content was also nearly the same (Table 2). Values for chlorophyll *a* and bacteria (total and viable counts) on either surface were also comparable (Table 1).

Table 1

Dry weight, organic matter, organic carbon and nitrogen, C/N ratios and bacterial numbers of settled particulate material on solid substrata immersed in estuarine waters (n = 5) for a period of 10 days.

	Aluminium	Glass
**		
Dry. weight	4 770 ± 0.008 (1.68%)	4 206 ± 0.015 (3.5%)
*		
Organic matter	1 354 ± 130 (9.61%)	1 072 ± 101 (9.46%)
*		
POC	677 ± 65.13 (9.62%)	536 ± 50.1 (9.42%)
*		
PON	192 ± 8.80 (4.58%)	178 ± 3.2 (1.80%)
C/N ratio		
(atomic)	4.1 ± 0.20 (5.09%)	3.5 ± 0.35 (10.0%)
6		6
Total counts	14.2 × 10 ± 0.98 (6.9%)	15.1 × 10 ± 0.85 (5.6%)
5		5
Viable counts	5.4 × 10 ± 0.14 (2.5%)	6.1 × 10 ± 0.17 (2.7%)

* = mg/plate; ** = µm/plate.

Diatoms and bacteria have been recognized as the primary biological components of the settled material and early colonizers of surfaces placed in aquatic environments (Characklis, 1973; Corpe, 1980; Marshall, 1981a; Cooksey, 1981). A preliminary microscopic observation of the obtained material revealed a very low number of diatoms during the present investigation, probably suggesting their minimal contribution towards the particulate matter settled on these substrata. This is also evident from the low values for pigments (0.003–0.004 µg/plate) and their poor correlation with all the other components analysed (Table 3).

Bacteria were present in large numbers both as total and viable counts on the two surfaces (Table 1). A very significant correlation was shown by the bacterial numbers with the various components of the settled material (Table 3).

Table 2

Biochemical composition of particulate material settled on solid substrata immersed in estuarine water (n = 5).

	Aluminium			Glass		
	a	b	c	a	b	c
Protein	453 ± 76.9 (16.98%)	332.42 ± 5.6 (16.98%)	31.77 ± 5.4 (17.04%)	383 ± 54.62 (14.25%)	35.70 ± 5.0 (14.51%)	33.90 ± 4.8 (14.25%)
Carbohydrates	429 ± 38.51 (8.95%)	31.75 ± 2.8 (8.95%)	5.40 ± 2.27 (8.95%)	369 ± 5.14 (1.39%)	34.41 ± 0.47 (1.39%)	27.52 ± 0.37 (1.37%)
Lipids	359 ± 17.80 (4.95%)	27.22 ± 0.71 (2.62%)	27.20 ± 0.70 (2.58%)	394 ± 31.66 (7.37%)	36.64 ± 7.13 (19.40%)	36.64 ± 7.13 (1.46%)

a = µg/plate; b = % of organic matter; c = contribution as % of carbon; Values in parentheses denote coefficient of variation.

Table 3

Linear relationship of microfouling bacteria and chlorophyll *a* with dry weight, organic carbon, nitrogen and biochemical components of settled particulate material on solid substrata.

	Chlorophyll <i>a</i>	Bacterial counts	
		Viable	Total
Dry Weight	0.155	0.993	0.911
POC	0.043	0.092	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

... = $p \leq 0.001$; .. = $p \leq 0.01$; . = $p \leq 0.05$.

Thus, from the total and viable count as well as the microscopic examination of the settled particulate matter, it appears that bacteria and not the diatoms were the dominant initial colonisers on these substrata and hence also the possible major contributors the organic matter obtained from these surfaces.

REFERENCES

- Baier R. E. (1980). Substrate influence in adhesion of microorganisms and their resultant new surface properties, in: *Adsorption of microorganisms to surfaces*, edited by G. Bitton and K. C. Marshall, John Wiley and Sons, Inc. N. Y., 59-104.
- Balkwill D. L., F. R. Leach, J. T. Wilson, J. F. McNabb, D. C. White (1988). Equivalence of microbial biomass measured based on membrane lipid and cell wall components, adenosinetriphosphate and direct counts in subsurface aquifer sediments, *Microbial. Ecol.*, 16, 73-84.
- Bhosle N. B., S. S. Sawant, P. D. Sankaran, A. B. Wagh (1989). Sedimentation of particulate material in stratified water column in the Bombay High area of the Arabian Sea, *Mar. Ecol.-Prog. Ser.*, 57, 225-236.
- Bhosle N. B., K. Nandakumar, A. B. Wagh (1989b). Influence of particulate matter on microfouling biomass in the Arabian Sea, *Biofouling*, Vol. 2, 65-74.
- Cameron D. B., A. V. Robinson (1968). Prevention of bacterial corrosion of mild steel with paint films, in: *Second International Congress on Marine Corrosion and Fouling*, Tech. Chamber, Greece, Verlag, Athens, 537-543.
- Characklis W. G. (1973). Attached microbial growths-II Frictional resistance due to microbial slimes, *Wat. Res.*, 7, 1249-1258.
- Characklis W. G., A. R. Escher (1988). Microbial Fouling: Initial Events, in: *Marine Biodeterioration*, edited by Thompson M. F., R. Sarojini and R. Nagabhushanam, Oxford and I.B.H. Publ. Co. Ltd, New Delhi, Bombay, Calcutta, 249-260.
- Clayton J. R., Q. Dortch, S. S. Thoresen, S. I. Ahmed (1988). Evaluation of methods for the separation and analysis of proteins and free amino acids in phytoplankton samples, *J. Plankt. Res.*, 10, 341-358.
- Cooksey K. E. (1981). Requirement of calcium in adhesion of a fouling diatom to glass, *Appl. Environ. Microbiol.*, 1378-1382.
- Corpe W. A. (1980). Microbial surface components involved in the adsorption of microorganisms to surfaces: in *Adsorption of microorganisms to surfaces* edited by G. Bitton & K. C. Marshall. John Wiley & Sons, Inc. N.Y., 105-144.
- Cuhel R. L., C. D. Taylor, H. W. Jannasch (1982). Assimilatory sulphur metabolism in marine microorganisms, considerations for the application of sulphate incorporation into protein as a measurement of natural population protein synthesis. *Appl. Environ. Microbiol.*, 43, 160-168.
- Daniel A. (1955). The primary film as a factor in settlement of marine foulers, *J. Madras. Univ. B XXV*, 2, 189-200.
- Dubois M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, 1956. Colometric method for determination of sugars, *Analyt. Chem.*, 28, 350-356.
- Fenchel T., T. H. Blackburn (1979). Bacteria and mineral cycling. Acad. Press. London, 225.
- Hobbie J. E., R. J. Daley, S. Jasper (1977). Use of nucleopore filters for counting bacteria by fluorescence microscopy *Appl. Environ. Microbiol.*, 33, 1225-1228.
- Holm Hansen O. (1969). Determination of microbial mass in ocean profiles, *Limnol. Oceanogr.*, 14, 740-747.
- Horbett T. A. (1982). Protein adsorption on biomaterial, *Adv. Chem. Ser.*, 199, 233-244.
- Little B. J., A. Zsolnay (1983). Chemical fingerprinting of adsorbed organic materials on surfaces, *J. Colloid Interface. Sci.*, 104, 79-85.
- Linley E. A. S., R. C. Newell (1984). Estimates of bacterial growth yields based on plant detritus, *Bull. mar. Sci.*, 35, 409-425.
- Lipinsky E. S., J. H. Litchfield (1970). Algae, bacteria and yeast as food or feed, *Chem. Rubber Co. Critical Review. Food Tech.*, 1, 581-618.
- Loeb G. L., R. A. Neihoff (1977). Adsorption of an organic film at the platinum-seawater interface, *J. mar. es.*, 35, 283-291.
- Luria S. E. (1960). The bacterial protoplasm, composition and organization, in: *The Bacteria*, edited by I.C. Gunsalus and R.K. Stainer, vol. 1, N-Y, 1-34.
- Marshall K. C. (1981). Bacterial behaviour at solid surfaces: a prelude to microbial fouling, in: *Fouling of heat transfer equipment*, edited by E. F. C. Somerscales and J. G. Knudsen. N.-Y., 305-312.
- Moriarty D. M. (1980). Problems in the measurement of bacterial biomass in sandy sediment, in: *Biogeochemistry of ancient and modern environments*, edited by P. A. Trudinger, M. R. Walter and B. J. Ralph. Aust. Acad. Sci., Canberra, 131-139.
- Morrison S. J., D. C. White (1980). Effects of grazing by estuarine gammaridean amphipod on the microbiota of allochthonous detritus, *Appl. environ. Microbiol.*, 40, 569-671.
- Parsons T. R. (1975). Particulate organic carbon in the sea. *Int. Chemical Oceanography*, edited by J. P. Riley and G. Skirrow, Academy Press, London, 338-425.

The C/N ratio is an important indicator of the origin of organic matter in the marine environment (Trask, 1955; Pocklington, Leonard, 1979). The obtained C/N ratios were low and ranged between 3.5 and 4.1. These values are similar to those reported for bacteria (Luria, 1960; Lipinsky, Litchfield, 1970; Fenchel, Blackburn, 1979; Cuhel *et al.*, 1982; Linley, Newell, 1984) and support the earlier conclusion that the settled material originated mainly from bacteria.

In summary, it may be concluded that the particulate material settled on these substrata was of bacterial origin.

Acknowledgements

The authors are grateful to Dr. B. N. Desai, Director, N.I.O., for his constant encouragement. Thanks are due to Dr. N. B. Bhosle, Scientist, N.I.O., for suggesting the problem and his valuable comments during the preparation of the manuscript. We also thank the authorities of the River Navigation Department, Govt. of Goa, for permitting the use of their jetty for carrying out the present work.

- Parsons T. R., Y. Matali, C. M. Lalli (1984). *A Manual of Sea Water Analysis*, Pergamon Press, N. Y.
- Paul J. H., G. I. Loeb (1983). Improved microfouling assay employing a DNA specific flouochrome and polystyrene as substratum, *Appl. environ. Microb.*, **46**, 338-343.
- Paul J. H., W. H. Jeffrey, M. DeFlaun (1985). Particulate DNA in Subtropical oceanic and estuarine planktonic environments, *Mar. Biol.*, **90**, 95-101.
- Pocklington R., J. D. Leonard (1979). Terrigenous organic matter in sediments of the St. Lawrence estuary and the Saguenay fjord, *J. Fish. Res. Bd. Can.*, **36**, 1250-1255.
- Sharma M. O., N. B. Bhosle, A. B. Wagh (1989). Methods for the removal and estimation of microfouling biomass, *Indian J. mar. Sci.*, (in press).
- Smart M. M., G. Rada, G. N. Donnermeyer (1983). Determination of total nitrogen in sediments and plants using persulfate digestion, *Wat. Res.*, **17**, 1207-1211.
- Trask P. D. (1955). Organic content of recent marine sediments, in: *Recent Marine Sediments*, edited by P. D. Trask, Dover, N.Y., 736.
- White . C., R. J. Bobbie, J. S. Herron, J. D. King, S. J. Morrison, (1979 a). Biochemical measurements of microbial mass and activity from environmental samples. in: *Native aquatic bacteria: enumeration, activity and ecology*, edited by J. W. Costlow and R. R. Colwell, Philadelphia: American Society for testing of materials, 695.
- White D. C., W. M. Davis, J. S. Nickels, R. J. Bobbie (1979 b). Determinatin of sedimentary microbial biomass by extractable lipid phosphate, *Oecologia*, **40**, 51-62.
- White D. C., P. H. Bensen (1984). Determination of biomass, physiological status, community structure and extracellular plaque of the microfouling film, in: *Marine Biodeterioration: An Interdisciplinary Study*, edited by J. D. Costlow and R. C. Tipper, 68-72.
- Zobell C. E. (1943). The effect of solid surfaces upon bacterial activity, *J. Bact.*, **46**, 39-56.
- Zutic V., J. Tomaic (1988). On the formation of organic coatings on marine particles: interactions of organic matter at hydrous alumina/seawater interfaces, *Mar. Chem.*, **23**, 51-67.