

ELECTRON TRANSPORT SYSTEM ACTIVITY OF MICROFOULING MATERIAL: RELATIONSHIPS WITH BIOMASS PARAMETERS

NARAYAN B BHOSLE, AVINASH S TULASKAR and ARUN B WAGH

*Marine Corrosion and Materials Research Division, National Institute of
Oceanography, Dona Paula 403 004 Goa, India*

(Received 6 November 1992; in final form 13 July 1993)

Microfouling material developed on aluminium panels immersed in surface waters of the Dona Paula Bay was analysed for biomass (measured as dry weight, organic carbon, protein and chlorophyll *a*) and electron transport system activity (ETS). The ETS activity ranged from 720 to 1374 $\mu\text{g O}_2\cdot\text{dm}^{-2}\cdot\text{d}^{-1}$. Microfouling biomass and ETS activity of microfouling material increased with the immersion period. ETS activity was significantly correlated with dry weight, organic carbon, protein and chl *a*. At low chl *a* levels microheterotrophs play a major role in the overall respiration, however at high chl *a* concentrations, microalgae can dominate respiration in a microfouling community.

KEYWORDS: ETS, microfouling, aluminium, biomass

INTRODUCTION

Respiration, defined as a biologically controlled oxidation of organic compounds (Burris, 1980), is the major consumption process of photosynthetically fixed carbon in marine environments. Thus, the knowledge of respiration rates is essential for the quantification and modeling of energy and carbon flux within marine microfouling organisms. However, the direct measurement of respiration is difficult (Williams, 1984). To overcome these problems, indirect methods based on biochemical activity rates have been proposed. One of the most widely used of these alternative techniques is the determination of the activity of the enzymes of the respiratory electron transport system (ETS) (Packard, 1971, 1985; Vosjan, 1982). Measured ETS activity rates, which are considered as maximum potential, need to be transformed using appropriate conversion factors, to actual respiration rates. While this is subject to many uncertainties, several studies have shown that the ETS activities and the oxygen consumption rates tend to correlate well, although the values of ratios may vary in different environments (Kenner & Ahmed, 1975; Packard & Williams, 1981).

In oceanographic research ETS measurements have been utilized to estimate the total metabolic activity of marine microorganisms, planktonic communities, benthic organisms and sediments (Packard, 1971; King & Packard, 1975; Christensen *et al.*, 1980; Williams, 1984; Martinez *et al.*, 1990; Cammen *et al.*, 1990; Estrada *et al.*, 1992). Studies on ETS help in understanding the economics of carbon, oxygen and biologically usable energy in organisms. Furthermore, relative comparison of ETS/chl *a* and/or respiration/ETS ratios provide useful data on the structure of micro-planktonic communities. Information about the maturity and efficiency of an ecosystem can be obtained from respiration and productivity data (Packard, 1979).

Solid surfaces immersed in a marine environment adsorb organic matter thereby conditioning the surfaces (Loeb & Neihof, 1977; Characklis & Escher, 1988; Zutic & Tomaic, 1988). Conditioning surfaces are then colonised by living organisms including bacteria, diatoms, fungi and protozoa. Attached organisms grow and develop into a microfouling community. There are numerous studies on microfouling of various types of surfaces placed in polar, temperate and tropical water (Mitchell & Kirchman, 1984; Maki *et al.*, 1990; Bhosle *et al.*, 1990 a,b). Despite these studies little is known about the respiratory activity of microfouling organisms (Bright & Fletcher, 1983; Jeffrey & Paul, 1986).

The aims of the present research are to estimate respiratory metabolic activity, to assess the effect of biomass parameters such as organic carbon, protein and chl *a*, on ETS activity and to differentiate between the heterotrophic and the autotrophic contributions to the total ETS activity of microfouling organisms.

MATERIALS AND METHODS

Chemicals

The chemicals Triton X-100, polyvinyl pyrrolidone (PVP), 2-(p-iodophenyl) -3- (p-nitrophenyl) -5- phenyl tertazolium chloride (INT), NADH, NADPH and sodium succinate were purchased from Sigma Chemical Company, USA. Other chemicals including sodium dihydrogen phosphate were purchased from SD Fine-chemicals, Bombay.

Test Panels

Aluminium test panels (15 × 10 cm) were cleaned with hydrochloric acid (Camerson & Robinson, 1968; Bhosle *et al.*, 1990 a,b), repeatedly washed with water followed by double distilled water, dried in an oven and kept covered until marine immersion.

Deployment of Panels

Aluminium panels were immersed in the surface waters (~ 1m) of Dona Paula Bay (15.31° N, 73.59° E). Before immersion replicate panels were fixed vertically on a weighted frame using PVC nuts and bolts. A float was attached at about 1 m depth. Panels were deployed on May 9, 1991 and were retrieved at 2 day intervals over a period of 12 d.

Estimation of Microfouling Biomass

After retrieval panels were scraped using a nylon brush and filter-sterilized seawater (0.2 µm) (White & Benson, 1984; Morikawa, 1988; Bhosle *et al.* 1990 a, b). The volume of the scraped material was adjusted to 50 ml and a known volume (2 to 10 ml) was filtered through pre-ignited (450°C 3 h) and pre-weighed glass GF/C filters. Duplicate filters were then analysed for dry weight (DW), organic carbon, protein and chl *a*. DW was determined after drying the filters in an oven at 50°C for 24 h (Bhosle *et al.*, 1990 a,b). The filters were then cooled in a desiccator and weighed on a microbalance (Mettler M3). Organic carbon and chl *a* were estimated following the methods of Parsons *et al.* (1984). Protein content was determined by the method of Lowry *et al.*

(1951) after extracting with 1N NaOH. The precision of these methods was better than ± 5 to 10% (Bhosle & Dhople, 1988; Bhosle *et al.*, 1989a).

Respiratory Electron Transport System Activity (ETS)

ETS determinations were performed following the Kenner and Ahmed (1975) modification of the tetrazolium reduction technique proposed by Packard and Williams (1981). Microfouling material was filtered as above on GF/C filters at a pressure of < 200 mm Hg. For the analysis the filters were ground in 3 ml of phosphate buffer in a ground glass tissue grinder. After homogenization, the crude extract was centrifuged for 5 min. The assay was carried out adding 1 ml of this clarified homogenate to a reaction mixture containing INT, disodium succinate, NADH and NADPH. The incubation was carried out at 22°C for 20 min. The reaction was stopped with 1 ml of a mixture of formalin and phosphoric acid (1:1 v/v) and the quenched mixture was centrifuged to obtain a clear supernatant. Absorbances at 490 nm and 760 nm were read on a spectrophotometer (Beckman DU 64). The 490 nm peak is proportional to INT-formazan production whereas the reading at 760 nm serves as a turbidity blank. Pigment blanks, consisting of an assay run without pyridine nucleotides or succinate were run with every assay. In addition, a reagent blank was run daily by filtering 1 ml of prefiltered seawater and assaying the filter. The ETS activity was calculated using the following Equation,

$$\text{ETS } (\mu\text{l O}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1}) = \frac{0.333 \times 60 \times A \times B \times C \times (\text{COD} - \text{RB})}{1.42 \times f \times V \times t}$$

where A is the crude homogenate volume in milliliters (5 ml), B is the volume of the quenched reaction mixture (6.0 ml), C is the total volume of the microfouling material (50 ml), COD is the corrected absorbance (absorbance of the assay minus turbidity and the pigment blank), RB is the reagent blank, V is the volume of the microfouling material filtered (5 to 10 ml), f is the volume of the homogenate used in the assay (1 ml), and t is the reaction time (20 min). The three constants, 0.333, 60 and 1.42 convert the measurements to units of area, units of hours and oxygen volume (μl), respectively. The 1.42 conversion factor is based on the molar extinction coefficient of the INT-formazan, the stoichiometry of INT-reduction, and the stoichiometry of the O_2 consumption by the respiratory ETS (Packard, 1985). The ETS values thus obtained were multiplied by 34.3 to convert to $\mu\text{g O}_2 \cdot \text{dm}^{-2} \cdot \text{d}^{-1}$.

Chlorophyll a-Specific ETS Activity

Chl *a* specific ETS activity was calculated by dividing ETS values ($\mu\text{g O}_2 \cdot \text{dm}^{-2} \cdot \text{d}^{-1}$) by the chl *a* concentration ($\mu\text{g} \cdot \text{dm}^{-2}$). The chl *a* specific ETS rates were then plotted as a function of chl *a* concentration in order to assess the importance of algae in the total ETS activity.

Water Samples

Surface seawater samples were collected using a clean bottle. Salinity and oxygen were determined as suggested by Strickland and Parsons (1972). A known volume of seawater (200 to 500 ml) was filtered on GF/C filters for the analysis of suspended matter, particulate organic carbon and chl *a*. These parameters were analysed as above.

Statistical Methods

Relationships between different biomass parameters as well as between the various biomass parameters and the ETS activity were assessed using linear regression analysis (Sokal & Rohlf, 1981) using the 123 LOTUS programme.

RESULTS

Variations in some physico-chemical and biological parameters in the surface waters of Dona Paula Bay are shown in Table 1. The temperature of the surface water remained

Table 1 Variation in some physical, chemical and biological parameters in the surface waters of Dona Paula Bay

Date May'91	T °C	S ‰	DO ml.l ⁻¹	SM mg.l ⁻¹	POC µgC.l ⁻¹	Chl a µg.l ⁻¹
9	28.0	29.94	4.49	16.9 ±2.4	375 ±38	2.80 ±0.32
11	28.0	29.94	4.62	16.0 ±1.5	335 ±28	2.66 ±0.61
13	28.0	28.80	4.56	16.7 ±3.5	382 ±61	2.67 ±0.25
15	28.0	29.52	4.42	18.8 ±2.1	420 ±55	3.20 ±0.22
17	28.0	29.80	4.36	15.6 ±3.1	357 ±46	3.51 ±0.45
19	28.0	29.80	4.42	17.2 ±1.6	324 ±23	2.67 ±0.45

T = temperature; S = salinity; DO = dissolved oxygen; SM = suspended matter; POC = particulate organic carbon; Chl a = chlorophyll a.

constant throughout the period of study. Salinity values showed small changes. Dissolved oxygen did not show any appreciable change whereas suspended particulate matter, particulate organic carbon and chl a exhibited some small variations.

Microfouling biomass on the aluminium panels was measured as DW, organic carbon, protein and chl a (Fig. 1). The DW of the microfouling material varied from 7.91 to 15.16 mg·dm⁻²·d⁻¹ over the period of study. When estimated as organic carbon, microfouling biomass exhibited a two fold increase during the period of immersion. The protein content of the microfouling material also showed increasing trends and ranged from 35 to 260 µg·dm⁻²·d⁻¹. Microalgal biomass measured as chl a also increased over the test period and varied from 0.25 to 1.39 µg·dm⁻²·d⁻¹. In general all biomass parameters showed a non-linear increase with the immersion period (Fig. 1).

The ETS technique was used to assess the potential respiration of the microfouling on the aluminium panels. ETS activity varied from 720 to 1374 µg O₂·dm⁻²·d⁻¹, and this increasing trend was similar to those obtained for the various microfouling biomass parameters (Fig. 2).

In order to verify the apparent relationship between the various biomass parameters, as well as between ETS and the biomass indicators, a linear regression analysis was carried out (Sokal & Rohlf, 1981). There were highly significant positive relationships between the various microfouling biomass parameters (Table 2). Similarly, the ETS activity of the

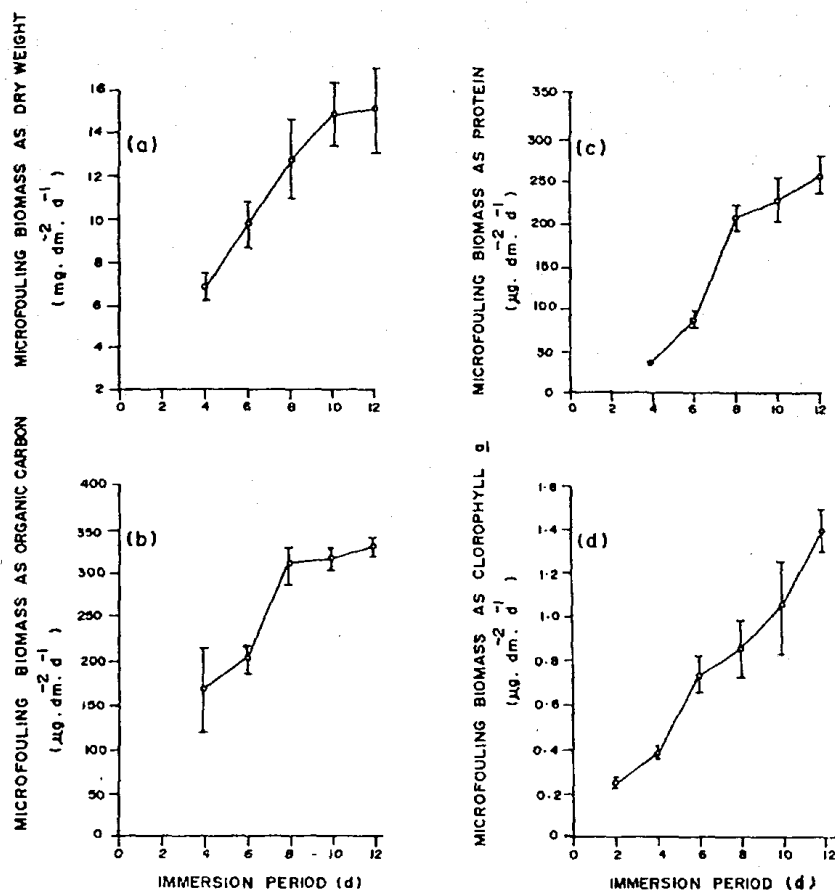


Fig. 1 Changes in microfouling biomass on aluminium panels over the immersion period in the surface waters of Dona Paula Bay. Bars = 2 SD.

microfouling material also showed significant correlations with the microfouling biomass parameters (Table 3).

In order to evaluate the importance of microalgae to the total ETS activity, chl *a* specific ETS activity was plotted as a function of chl *a* concentration (Fig.3). At low chl *a* concentration (i.e. $< 0.74 \mu\text{g}\cdot\text{dm}^{-2}\cdot\text{d}^{-1}$) chl *a* specific ETS activity was very high. In contrast, as the chl *a* concentration increased, chl *a* specific ETS activity showed a decreasing trend. When the chl *a* concentration was relatively high ($> 1.0 \mu\text{g}\cdot\text{dm}^{-2}\cdot\text{d}^{-1}$), chl *a* specific ETS activity did not vary much (Fig.3).

The ETS activity measured in the microfouling samples was due to both autotrophic and heterotrophic organisms. The relative importance of these two components to the total ETS activity was calculated using the following regression equation (Table 3):

$$\text{Microalgal ETS } (\mu\text{g O}_2\cdot\text{dm}^{-2}\cdot\text{d}^{-1}) = 604\text{Chl } a (\mu\text{g O}_2\cdot\text{dm}^{-2}\cdot\text{d}^{-1}) + 0.460.$$

Heterotrophic ETS activity was then calculated using the equation:

$$\text{Heterotrophic ETS } (\mu\text{g O}_2\cdot\text{dm}^{-2}\cdot\text{d}^{-1}) = \text{ETS total} - \text{microalgal ETS}$$

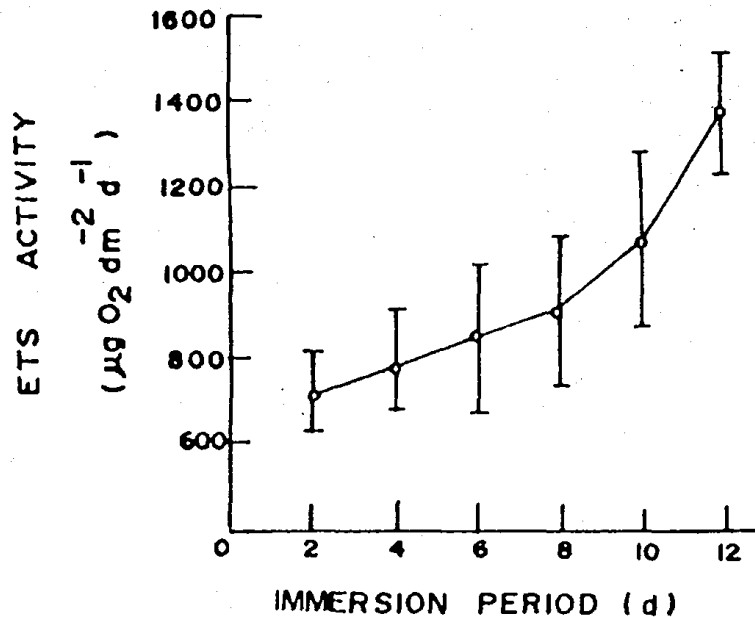


Fig. 2 ETS activity of the microfouling organisms over the period of immersion. Bars = 2 SD.

Table 2 Correlation coefficients (r) among the various microfouling biomass parameters such as dry weight (DW), organic carbon (OC), protein (PR) and chlorophyll a (Chl a)

Parameter	DW	OC	PR	Chl a
DW	1.00	0.975*	0.981*	0.936'
OC		1.00	0.994*	0.879'
PR			1.00	0.916'
Chl a				1.00

* = $P \leq 0.01$; # = $P \leq 0.001$; number of samples 5

Table 3 Linear regression equation between ETS and DW, organic carbon (OC), protein (PR) and chlorophyll a (Chl a) of microfouling material.

Equation	n	r
ETS = 0.063 DW + 0.230	5	0.846'
ETS = 2.415 OC + 0.358	5	0.779 ⁱ
ETS = 1.980 PR + 0.660	5	0.802 ⁱ
ETS = 604 Chl a + 0.460	6	0.949*

ⁱ = $P \leq 0.05$; * = $P \leq 0.02$; # = $P \leq 0.001$; n = number of samples; r = correlation coefficient

The results (Table 4) suggest that the heterotrophic potential was relatively high (69% to 79% of total ETS) during the first 4 d of immersion. Thereafter the heterotrophic contribution decreased and the microalgal contribution to the total ETS activity increased. When the chl a concentration was relatively high, autotrophic and heterotrophic organisms contributed about 61% and 39% respectively to the total ETS activity (Table 4).

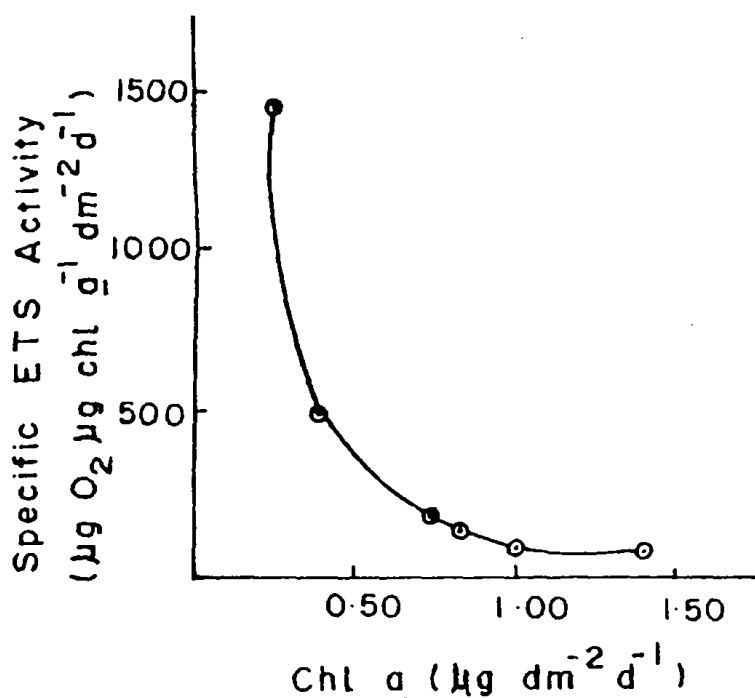


Fig. 3 Chl *a* specific ETS activity of the microfouling material as a function of chl *a* concentration.

Table 4 Contribution of autotrophic and heterotrophic ETS activity ($\mu\text{gO}_2 \cdot \text{dm}^{-2} \cdot \text{d}^{-1}$) to the total ETS activity ($\mu\text{gO}_2 \cdot \text{dm}^{-2} \cdot \text{d}^{-1}$) of the microfouling organisms on aluminium panels immersed in the surface waters of Dona Paula Bay for 12 d.

Immersion period (d)	Total ETS	Autotrophic ETS		Heterotrophic ETS	
		a	b	a	b
2	720	151.46	21.03	568.54	78.96
4	784	236.02	30.10	547.98	69.89
6	845	447.42	52.95	397.58	47.05
8	906	513.86	56.71	392.14	43.28
10	1072	640.70	59.76	431.30	40.23
12	1374	839.56	61.10	534.44	38.89

a = ETS ($\mu\text{g O}_2 \cdot \text{dm}^{-2} \cdot \text{d}^{-1}$), b = % of the total ETS.

DISCUSSION

Removal of microfouling material is perhaps the most critical step in evaluating biomass and the activity of microfouling organisms on a solid substratum immersed in aqueous environments. Extraction of microfouling by brushing is one of the commonly employed technique (Adamson & Liberatore, 1984; White & Benson, 1984; Bhosle *et al.*, 1989 b; 1990 a,b). A nylon brush has been found to remove about $89 \pm 2.19\%$ of the microfouling material on aluminium and glass panels immersed in estuarine waters for a period of 10 d (Sharma *et al.*, 1990). A brush was therefore efficient in removing fouling material from aluminium panels.

DW, organic carbon and chl *a* were used to estimate microfouling biomass as it is reported that these are good indicators (Afring & Taylor, 1979; Bhosle *et al.*, 1989 b, 1990 a,b; Sharma *et al.*; 1990). The microfouling biomass on aluminium panels increased over the period of immersion in the surface seawaters of Dona Paula Bay. This corroborated with earlier studies suggesting a non-linear increase in microfouling biomass as a function of immersion period (Martinez *et al.*, 1984; White & Benson, 1984; Chamberlain & Garner, 1988). The suspended particulate matter, particulate organic carbon and chl *a* content of the surface seawater did not show any appreciable variation. The observed increase in the microfouling biomass over the period of study was therefore due to enhanced settlement and/or growth of microfouling organisms on the aluminium panels.

Chl *a* has been widely used as a measure of plant biomass, whereas, DW, organic carbon and protein are used to estimate plant and animal biomass with a possible detrital component. Chl *a* showed significant positive correlations with DW, organic carbon and protein, indicating that plant biomass comprised a fairly constant percentage of the total biomass (the detrital component was not estimated).

The activity of the ETS, the system of enzymes and cytochromes involved in respiratory energy production, has been used to estimate the oxygen consumption rate of marine plankton, algae, bacteria, zooplankton, benthos and sediments (Packard, 1971; King & Packard, 1975; Christensen *et al.*, 1980; Williams, 1984; Martinez *et al.*, 1990; Cammen *et al.*, 1990; Estrada *et al.*, 1992). However, it is possible that not all species of microorganism can reduce INT. Moreover, INT may inhibit or suppress respiratory activity in some species of microorganisms (Trevors, 1984). The ETS method, therefore, gives overall metabolic rates as it represents the catch all approach. Despite these limitations, the method has been routinely used to assess the respiratory potential of various samples because of its simplicity and high sensitivity. The application of the ETS method to microfouling studies may provide useful information on biologically usable energy, metabolic rates, and the respiratory potential of the various components of the microfouling community.

A possible problem with ETS measurements in the present study is in the use of GF/C filters for concentrating samples. It could be argued that a significant part of the heterotrophic ETS activity might have passed through these filters thereby underestimating the ETS activity of the microfouling organisms. It is well documented that microfouling organisms are attached to solid surfaces and not free floating as generally observed in seawater samples. Moreover, for microfouling samples obtained from aluminium panels highly significant correlations ($r=0.920$ to 0.993 ; $P \leq 0.001$) between bacteria (total and viable count) and the DW GF/C filtered particulate matter, particulate organic carbon and nitrogen, carbohydrate and proteins have been reported earlier (Sharma *et al.*, 1990; Sharma & Wagh, 1990). The authors consider, therefore, that most of the bacterial biomass was retained on the filters in their microfouling samples.

The ETS activity of the microfouling material varied from 720 to 1374 $\mu\text{g O}_2\text{-dm}^{-2}\text{d}^{-1}$ over the 12 d period of immersion. These values cannot be compared with other ETS measurements on biofilms due to differences in analytical approach (Jeffrey & Paul, 1986). Nevertheless, the increasing trends observed for ETS activity in the present study are similar to those reported for biofilms on polystyrene panels immersed in a temperate eutrophic estuary (Jeffrey & Paul, 1986).

Generally, the trends observed for various microfouling biomass parameters were also reflected in the ETS activity of the microfouling organisms. This was evident from the reasonably good correlations obtained between ETS activities and the various biomass

parameters of the microfouling organisms (Table 3). The significant positive relationship between DW and the ETS activity indicates that the measured ETS activity varied directly (but not linearly) with the quantity of biomass. This was also the case with respect to the relationship between ETS activity and the organic carbon of microfouling organisms. Similar results have been reported for planktonic bacteria, phytoplankton, zooplankton, benthic organisms and sediments (King & Packard, 1975; Christensen *et al.*, 1980; Cammen *et al.*, 1990; Estrada *et al.*, 1992).

There was a significant relationship between the ETS activity and the protein content of the microfouling material. This observation corroborates earlier studies suggesting significant correlations between these two parameters in marine suspended particles and sediments (Christensen & Packard, 1977; Garfield *et al.*, 1979; Estrada *et al.*, 1992). Since ETS activity is only associated with living organisms, this relationship suggests that the proteinaceous material was associated with the organisms' metabolism.

ETS activity measured in the microfouling samples included two biological components, i.e. the microalgal part (the biomass related to the amount of chl *a*), and the micro-heterotrophic part (including bacteria, fungi and protozoa *etc.*). The most useful criterion to differentiate the microalgal part from the total activity is the amount of chl *a*. Thus, the greater the importance of the microalgae in the living component, the lower will be the values of chl *a* specific ETS activity. In other words, the chl *a* specific ETS activity and chl *a* (as an index of the relative importance of microalgae) have opposite variations (Packard, 1979; Iriarte *et al.*, 1991). Few data points are available in this study, nevertheless, the results support this observation because chl *a* specific ETS activity plotted against chl *a* concentration significantly deviates from a constant relationship at low chl *a* levels i.e. below $0.74 \mu\text{g}\cdot\text{dm}^{-2}\cdot\text{d}^{-1}$ (Fig.3). Natural plankton populations have been observed to exhibit similar trends (Martinez *et al.*, 1990; Iriarte *et al.*, 1991).

The linear regression analysis between total ETS activities and the chl *a* concentrations of the microfouling samples showed significant positive relationship (Table 3). These data support earlier studies wherein microplankton ETS activities have been shown to correlate with chl *a* concentrations, although the corresponding regression coefficient may vary widely (e.g. Packard, 1979; Setchell & Packard, 1979; Packard & Williams, 1981; Iriarte *et al.*, 1991; Estrada *et al.*, 1992). A close association between ETS activities and chl *a* concentrations suggests two possibilities. Either photosynthetic organisms are the major contributor to the ETS activities of the microfouling community (Packard, 1979; Iriarte *et al.*, 1991), or, the standing stock and growth of the microalgal population significantly enhances the presence and/or activity of bacteria or microheterotrophs. The latter are the most important contributor to overall respiration even under conditions of high chl *a* concentrations (Iriarte *et al.*, 1991; Estrada *et al.*, 1992). The relative importance of the heterotrophic and autotrophic components to the total ETS activity was calculated using the regression equation between ETS activities and chl *a* concentrations (Table 3). Such analysis can only be approximate because the different living compartments of microfouling organisms probably have various ETS/biomass ratios, as is the case for planktonic samples. Nevertheless it is a useful tool in differentiating these different compartments. The heterotrophic potential was relatively high, especially during the first 4 d of immersion where it ranged from 69 to 79% of the total ETS activity (Table 4). Thereafter, the heterotrophic contribution decreased and the microalgal ETS contribution to the total ETS increased. Under the conditions of high algal biomass, autotrophic and heterotrophic organisms contributed about 61% and 39%, respectively to the total ETS activity. This implies that the ETS contribution of microheterotrophs to the total ETS was still fairly high even under conditions of high chl *a* concentration (Iriarte *et al.*, 1991; Estrada *et al.*, 1992). Thus a close coupling between

ETS activity and chl *a* concentration does not exclude an important role for bacteria or microheterotrophs in the overall ETS activity or oxygen consumption. The authors have not estimated bacterial abundance in their samples, nevertheless, it is well documented that bacterial numbers are often positively correlated with microalgal standing stock for natural plankton samples (Fuhrman *et al.*, 1980; Linely *et al.*, 1983; Antai, 1990).

ETS measurements can be converted to carbon dioxide production rates using a suitable conversion factor in order to understand carbon cycling in an ecosystem. Conversion factors are available for planktonic samples and their application to microfouling samples is doubtful. In view of these uncertainties ETS activity was not converted to carbon.

Acknowledgments

We are grateful to the Director, The National Institute of Oceanography, Dona Paula, for his encouragement. We are also grateful to Professor Len Evans and the two anonymous referees for useful suggestions and criticism on the earlier draft of the manuscript. The authors also thank Miss Subhashini for help in typing the manuscript.

References

- Adamson W L, Liberatore G L (1984) Control of microfouling in ship piping and heat exchanger systems. In: Costlow J D, Tipper R (eds) *Marine Biodeterioration: An Interdisciplinary Study*. US Naval Institute, Annapolis MD, pp 95–102
- Afring R P, Taylor B F (1979) Assessment of microbial fouling in an ocean energy conversion experiment. *Appl Environ Microbiol*, **38**: 734–739
- Antai E E (1990) Seasonal trophodynamics of bacterioplankton and heterotrophic microflagellates in Southampton water. *Ph D Thesis*, University of Southampton, UK
- Bhosle N B, Dhople V M (1988) Distribution of some biochemical compounds in sediments of the Bay of Bengal. *Chem Geo* **67**: 341–352
- Bhosle N B, Sawant S S, Prabha D S, Wagh A B (1989 a) Sedimentation of particulate material in stratified and non stratified water column in the Bombay High area of the Arabian Sea. *Mar Ecol Prog Ser* **57**: 225–236
- Bhosle N B, Nandakumar K, Venkat K, Dhople V M, Sawant S S, Wagh A B, Kelkar P G (1989 b) Biological and biochemical characterisation of microfouling on aluminium panels placed in the Arabian Sea. *Proc Indian Natl Sci Acad* **55**: 51–56
- Bhosle N B, Nandakumar K, Wagh A B (1990 a) Influence of particulate material on microfouling biomass in the Arabian Sea. *Biofouling* **2**: 65–74
- Bhosle N B, Prabha D S, Wagh A B (1990 b) Carbohydrate sources of microfouling material developed on aluminium and stainless steel panels. *Biofouling* **2**: 151–164
- Bright J J, Fletcher M (1983) Amino acid assimilation and electron transport system activity in attached and free-living marine bacteria. *Appl Environ Microbiol* **45**: 818–825
- Burris J E (1980) Respiration and photorespiration in marine algae. In: Falkowski P G (ed) *Primary Productivity in the Sea*. Plenum Press, pp 411–431
- Camerson D B, Robinson A V (1968) Prevention of bacterial corrosion of mild steel with paint films. In: *Second Int Congr Mar Corr Foul*. Tech Chamber Greece, Verlag, Athens, Greece, pp 537–534
- Cammen L M, Corwin S, Christensen J P (1990) Electron transport system (ETS) activity as a measure of benthic macrofouling metabolism. *Mar Ecol Prog Ser* **65**: 171–182
- Chamberlain A H L, Garner B J (1988) The influence of iron content on the biofouling resistance of 90/10 copper-nickel alloys. *Biofouling* **1**: 79–96
- Characklis W G, Escher A R (1988) Microfouling in initial events. In: M F Thompson, R Sarojini, R Nagabushanam (eds), *Marine Biodeterioration; Advanced Technique Applicable to The Indian Ocean*, Oxford & IBH Publishing Company Ltd, New Delhi, Bombay, Calcutta, pp 249–260
- Christensen J P, Packard T T (1977) Sediment metabolism from the northwest African upwelling system. *Deep Sea Res* **24**: 331–343
- Christensen J P, Owens T G, Devol A H, Packard T T (1980) Respiration and physiological state in marine bacteria. *Mar Biol* **55**: 267–276
- Estrada M, Martinez R, Mathot S (1992) Respiratory electron transport activity in plankton of the Weddell and Scotia Seas during late spring early summer: relationships with other biological parameters. *Polar Biol* **12**: 35–42

- Fuhrman J A, Ammerman J W, Azam F (1980) Bacterioplankton in coastal euphotic zone: distribution, activity and possible relationships with phytoplankton. *Mar Biol* **60**: 201-207
- Garfield P C, Packard T T, Codispoti L A (1979) Particulate protein in the Peru upwelling system. *Deep Sea Res* **26**: 623-639
- Iriarte A, Daneri G, Garciaabmt V M T, Purdie D A, Crawford D W (1991) Plankton community respiration and its relationship to chlorophyll a concentration in marine coastal waters. *Oceanol Act* **14**: 379-388
- Jeffrey W H, Paul J H (1986) Activity measurements of planktonic microbial and microfouling communities in a eutrophic estuary. *Appl Environ Microbiol* **51**: 157-162
- Kenner R A, Ahmed S I (1975) Measurements of electron transport activities in marine plankton. *Mar Biol* **33**: 119-127
- King F D, Packard T T (1975) Respiration and respiratory electron transport system in marine zooplankton. *Limnol Oceanogr* **20**: 849-854
- Linely E A S, Newel R C, Lucas M I (1983) Quantitative relationships between phytoplankton, bacteria and heterotrophic microflagellates in shelf waters. *Mar Ecol Prog Ser* **12**: 77-89
- Loeb G I, Neihof R A (1977) Adsorption of an organic film at platinum seawater interface. *J Mar Res* **35**: 283-291
- Lowry O H, Rosebrough N J, Fair A L, Randal R J (1951) Protein measurements with Folin-Phenol reagent. *Biol Chem* **193**: 265-275
- Maki J S, Little B J, Wagner P, Mitchell R (1990) Biofilm formation on metal surfaces in Antarctic waters. *Biofouling* **2**: 27-38
- Martinez D, Bastida R, Brankevich G (1984) Ecological aspects of marine fouling at the port of Ingeniero white (Argentina). In: *Sixth Int Cong Mar Corr Foul*, Athens 5-8 Sept 1984, Greece, pp 521-537
- Martinez R, Arnone R A, Velasquez Z (1990) Chlorophyll a and respiratory electron transport system activity in microplankton from surface waters of the western Mediterranean. *J Geophys Res* **95**: 1615-1622
- Mitchell R, Kirchner D, (1984) The microbial ecology of marine surfaces. In: Costlow J D, Tipper R C (eds) *Marine Biodeterioration: An Interdisciplinary Study*, US Naval Institute, Annapolis MD, pp 49-56
- Morikawa K (1988). Differences in plating efficiency of bacteria from river epilithon sampled from upper and lower surfaces of artificial substrata. *Microb Ecol* **15**: 217-228
- Packard T T (1971) The measurement of respiratory electron transport activity in marine phytoplankton. *J Mar Res* **29**: 235-244
- Packard T T (1979) Respiration and respiratory electron transport activity in phytoplankton from the Northwest African upwelling area. *J Mar Res* **37**: 711-742
- Packard T T (1985). Measurements of electron transport activity of microplankton. *Adv Aquat Microbiol* **3**: 207-281
- Packard T T, Williams P J LeB (1981) Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest atlantic. *Oceanol Acta* **4**: 351-358
- Parsons T R, Maitai y, Lalli C M (eds) (1984) *A Manual of Chemical and Biological Methods of Seawater Analysis*. Pergamon Press, New York, pp 63
- Setchell F W, Packard T T (1979) Phytoplankton respiration in the Peru upwelling. *J Plank Res* **1**: 343-354
- Sharma O M, Wagh A B (1990) The nature of particulate matter settled on solid substrata. *Oceanol Acta* **13**: 471-474
- Sharma O M, Bhosle N B, Wagh A B (1990) Methods for the removal and estimation of microfouling biomass. *Indian J Mar Sci* **19**: 174-176
- Sokal R R, Rohlf J F (1981) *Biometry, 2nd edition*, Freeman & Company, San Francisco
- Strickland J D H, Parsons T R (1972) *A Practical Handbook of Seawater Analysis*. *Bull Fish Res Board Can* **167**: 1-311
- Trevors J T (1984) The measurement of electron transport system (ETS) activity in freshwater sediment. *Water Res* **18**: 581-584
- Vosjan J H (1982) Respiratory electron transport system activities in marine environments. *Hydrobiol Bull* **16**: 61-68
- White D C, Benson P H (1984) Determination of biomass, physiological status, community structures and extracellular plaque of the microfouling film. In: Costlow J D, Tipper R C (eds) *Marine Biodeterioration: An Interdisciplinary study*, US Naval Institute, Annapolis MD, pp 68-74
- Williams P J LeB (1984) A review of measurements of respiration rates of marine plankton populations. In Hobbie J E, Williams P J LeB (eds) *Heterotrophic Activity in the Sea*, Plenum Press, New York, pp 357-390
- Zutic V, Tomaic J (1988) On the formation of organic coatings on marine particles: interaction of organic matter at hydrous alumina seawater interfaces. *Mar Chem* **23**: 51-68