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BACTERIA AS TRANSFORMERS OF THE ARENA OF PLAY OF OTHER ORGANISMS

FERRAN VALLESPINÓS & SÍLVIA MALLO

Institut de Ciències del Mar (CSIC). Passeig Nacional, s/n. 08039 Barcelona. Spain.

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SUMMARY

The following parameters were determined from a series of 70 samples taken from different estuary systems in the Iberian peninsula: oxygen uptake of the superficial sediment, number of viable heterotrophic bacteria, Eh, concentration of organic matter and percentage of fine-grained materials. The existence of positive and significant correlations between organic matter content, aerobic respiration and bacterial biomass is shown. Between 35% and 45% of the accumulated matter is mineralized annually by aerobic metabolism, and bacteria are the principal agents of this transformation. The greater organic load entails, however, conditions which are increasingly negative in redox potential, due to the demand of oxidants and the accumulation of reduced chemical species, which means that the circulation of the system's matter and energy through the bacterial compartment is gradually more important, with the installation of conditions that prevent the continuity of the eukaryote metabolism (with the exception of certain yeasts).

KEY WORDS: Bacteria, estuaries, respiration

INTRODUCTION

Coastal zones display a relatively higher concentration of organic matter (resulting from *in situ* primary production plus continental contributions), which increases the possibilities of heterotrophic metabolism. The functioning of estuary systems is clearly influenced by the metabolism of the bacterial populations which affects the cycles of matter and fluxes of energy. So there is an association between bacterial and particulate matter, which is seen most clearly in the processes of mineralization and the production of nutrients.

The most quantitatively significant part of these processes takes place at a sedimentary level, and the importance of the joining of the benthic and pelagic communities in coastal waters is recognised (NIXON, 1981). The consumption of organic matter is therefore associated with an important flow of nutrient towards the water column, in such a way that the rates of respiration in the sediment are indicators of the trophic levels of the system (EDMONDSON, 1966). At the same time, the activity of bacteria in the sediment is the principal factor responsible for the establishment of reducing conditions that call for the use, in the respiratory chains, of end acceptors of electrons different from molecular oxygen (nitrates, sulphates and carbon dioxide), in a sequence which depends on its relative energy (WATSON *et al.*, 1985), and which is itself accompanied by a diminishing of the Eh of the system, and consequently of the entire chemistry of both the sediments and the water column.

The participation of the various bacterial metabolisms (both aerobic and anaerobic) in the global balances of C and N features both a seasonal and spatial variability (MALLO & *et al.*, in press) associated with the availability of organic material, the thermic conditions and the value of the redox potential. In any case, the metabolism of the bacterial populations gradually establishes certain environmental conditions which make impossible the activity of the majority of the decomposing eukaryotic organisms (due to its chemical inadequacy), with the exception of the yeasts, whose importance is practically unknown. The final result is that virtually the entire flux of energy and matter ends up circulating through the bacterial compartment of the system, in a relation directly proportional to the existing quantity of organic material.

This study attempts to discuss some aspects of this model of the progressive "dominance" of the medium by the bacteria through the analysis of the information obtained in a series of coastal media of distinctive characteristics, in relation both to the quantity and quality of the inputs received and to the type of dynamics to which they are subjected.

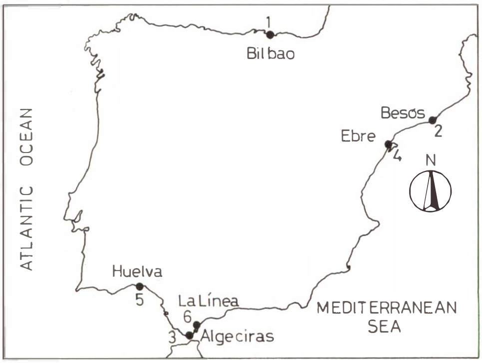

FIGURE 1. Distribution along the Spanish littoral of the sampling sites.

MATERIAL AND METHODS

Seventy samples were taken from six sites distributed throughout the Iberian peninsula (Fig. 1), with environmental characteristics embracing a wide variety of conditions (in relation to organic matter content, percentage of fine-grained materials and redox potential). In every case the stations are coastal zones affected by continental contributions. The samples were taken in summer or pre-summer conditions. In the Besòs river and the Ebro river delta, a seasonal study was carried out, and winter samples were also available.

The samples were obtained by hand (on occasion with the help of scuba divers) using polystyrene cylinders 4.5 cm in diameter. In the Besòs area a box-corer was used. In each case the temperature was determined *in situ* with a portable probe (ORION 917001), along with the redox potential, using a platinum electrode (ORION RESEARCH s500 C-ORP) connected to a portable millivoltmeter (ORION 231).

The sediment was transferred to the laboratory under refrigeration, with its structure maintained unaltered. The analysis was always begun within four hours of the sample being taken. A dilution bank was prepared from a suspension of sediment (approximately 10% w:v) in sterile sea water obtained from the same station, for the counting of the total number of viable heterotrophic bacteria (aerobic and facultative), on Marine agar plates (DIFCO 0791-01). The samples were incubated at 30 °C for a maximum period of a week.

The levels of aerobic respiration were determined in 25 ml vials (with rubber septum and hermetically sealed) which contained suspensions of sediment and seawater. The potential oxygen uptake was measured by gas chromatography in relation to the changes in the composition of the gases in the head space of the vials, which were also incubated at 30 °C for 12 hours. In the conditions of the analysis, the

TABLE I. Principal characteristics of the sampled zones. A variable number of samples was obtained from each zone (70 in total), with which 16 groups of stations of equivalent significance were defined.

LOCATION	ORG. INPUT (Tm C d-^{1})	STATION GROUP	DEPTH (m)	WATER . TEMP (^{o}C)	ORGANIC MATTER (%)	FINE-GRAINED MATERIALS (%)	Eh (mV)
RÍA DE BILBAO	20	1.1	6-10	20.0	12.1-18.4	30.0-80.0	-150/-420
BESÒS DELTA	120	2.1	11-52	13.6-21.1	3.1-7.7	25.4-84.4	-180/-401
		2.2	52	14.0	4.7	77.4	-220
		2.3	62-63	13.0-14.0	1.8-2.5	24.5-79.5	-158/-218
		2.4	11-52	13.0-13.2	3.6-5.3	25.4-84.4	-126/-387
		2.5	52	13.1	3.6	77.4	161
		2.6	62-63	13.5-13.9	1.6-2.5	24.5-79.5	-210/-304
ALGECIRAS BAY	-	3.1	15	16.0	0.7-2.9	8.0-84.1	-
EBRO DELTA	30	4.1	1	3.5	0.4	3.0	179
		4.2	1	3.0	5.6	33.0	-106
		4.3	1-2	26.3-29.3	0.5-1.2	0.01-4.0	16/-280
		4.4	1-4	23.0-29.1	1.9-7.8	24.5-97.9	-73/-347
		4.5	4-6	24.0-29.0	2.4-6.2	22.5-72.5	-128/-290
RÍA DE HUELVA	5	5.1	10	15.2	0.3-0.8	3.1-3.9	180/210
		5.2	10	15.1	4.3-4.7	0.1-1.7	53/80
LA LÍNEA	-	6.1	10	15.0	0.3-1.4	0.4-5.7	-

greatest part of this uptake (always above 85%) corresponds to bacterial metabolism, as the results obtained with the addition of cyclohexymide (inhibitor of eukaryotic respiration) show.

A detailed decription of this technique and those used to determine the other accompanying parameters (granulometry, organic matter, etc.) can be found in MALLO (1989).

Information about all the aforementioned parameters is available for the majority of samples, although in some cases there are no data for counts of heterotrophic bacteria or of redox potential.

RESULTS

Taking the sediment studied as a whole, the concentration of organic matter oscillates between 0.25 and 18.4%; the proportion of fine-grained materials, between 0.10 and 97.9%, and in general there is a relation between the two parameters. The number of viable heterotrophic bacteria varies between 2 x 10^{5} and 4 x 10^{7} cell g^{-1}. The most negative redox potential value determined was -420 mV. This information is summarised in Table I, in which a series of groups is established for each zone that include stations of equivalent characteristics. The grouping was obtained in each case by the application of a multivariate analysis (principal components) to the group of chemical and biological parameters considered (up to 15), which include those used in this study (MALLO, 1989; VALLESPINÓS *et al.*, 1989).

Although the samples include a variety of types of coastal marine systems, a strong lineal and positive relation (r= 0.94) is found between the amount of organic matter accumulated in the surface sediment (and which includes both the matter produced *in situ* and the imported matter) and the mineralised organic matter, measured by the oxygen uptake according to the technique described (Fig. 2).

In those cases in which the quantity of organic matter apported to the system is known, it has been related to the influenced area and divided by an average value of oxygen uptake. The results are shown in Table II, and it can be observed that between a third and a half of the contributions are mineralized by aerobic means by the bacterial populations of the superficial sediment. Moreover, the results seem to point to an inverse relation between this proportion and the total inputs, as will be discussed below.

In relating the concentration of organic matter of the sediment to the bacterial populations (Fig. 3) it is observed that an increase in the total number of heterotrophic bacteria (aerobic and facultative) is produced as a result of the enrichment in the organic matter content (r= 0.62). The greater availability of carbon and nitrogen therefore enhances the presence of richer bacterial flora which participates in the degradation of the organic matter accumulated in the superficial sediment. This implies the consumption of an equivalent quantity of oxydants (oxygen, free or incorporated in mineral molecules) and the corresponding accumulation of reduced chemical species. These processes taken as a whole necessarily involve a diminishing of the redox potential of the sediments, a diminishing that is the result of the combination of bacterial metabolisms (flux of electrons generated by the organotrophic processes).

TABLE II. Percentage of benthic remineralization in relation to the total of organic matter deposited in each system.

LOCATION	ORGANIC INPUT (g C m^{-2} y^{-1})	BENTHIC REMINERAL. (g C m^{-2} y^{-1})	%
RÍA DE BILBAO	3,600	1,280	35
BESOS DELTA	1,700	580	34
EBRO DELTA	250	100	40
RÍA DE HUELVA	110	50	45

The relation between the consumption of organic matter and the redox potential of the sediments summarizes a set of complex thermodynamic balances, but it is evident that the highest rates of mineralization of the excess of accumulated organic matter is translated into a diminishing of the Eh value, as shown in figure 4, with a value of r= 0.49.

The quotient between the concentration of organic matter and the uptake of oxygen (OM/OU) measures, to an extent, the turnover of material incorporated in the superficial sediment. If this value is referred to the bacterial biomass (Fig. 5), a significant (r= 0.73) and positive lineal relation is found, which implies that, contrary to expectations, the sediments with a population more abundant in heterotrophic bacteria (aerobic and

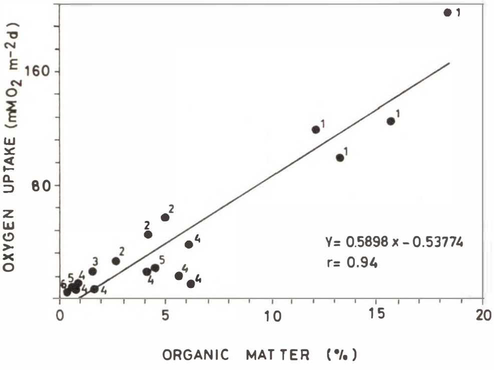

FIGURE 2. Relation between the rate of mineralization (measured as oxygen uptake) and the content of organic matter in the sediment. It corresponds to average values for distinct groups of stations (1: Ría de Bilbao; 2: Besòs Delta; 3: Algeciras Bay; 4: Ebro Delta; 5: Ría de Huelva; 6: La Línea).

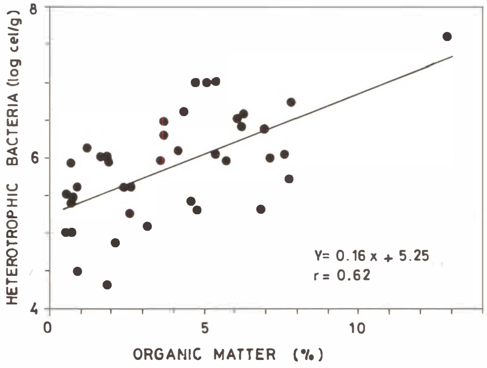

FIGURE 3. Relation between the organic matter content of the sediment and the logarithm of the number of viable heterotrophic bacteria (aerobic and facultative).

facultative) remineralize the organic matter in a relatively slower fashion.

DISCUSSION

In the conditions of the analysis, the uptake of oxygen is a measure integrated in all the oxidative processes that take place in the sediment. The comparison between the data presented in this study (which range from 0.16 to 200 mM m^{-2} d^{-1}) and those published by other authors who have studied the communities of the sediments of shallow water is difficult, given the wide disparity both between methods of measurement and presentation of results. However, all the uptakes found are similar to those described for other estuarine environments (NOWICKI & NIXON, 1985), even in the case of the highest values of oxygen demand, which correspond to the Ría de Bilbao (BOYNTON *et al.*, 1980; HOPKINSON & WETZEL, 1982; VAN ES, 1982).

Where it was possible to follow the temporal variation (Ebro Delta), it was seen that a high variability exists and that the oxygen is consumed more slowly in winter (9.5 mM m^{-2} d^{-1}) than in summer (36.8 mM m^{-2} d^{-1}). This seasonal difference in oxygen flow is similar to that described in other coastal sediments of equivalent characteristics (BALZER, 1984; HALL *et al.*, 1989), and corresponds to a Q_{10} of 1.9. The proximity of this value to that assigned to marine bacteria (JOHNSON, 1936) in the interval of sediment temperatures discovered during the entire cycle (8.2-29.8°C) and the results of the experiments in which cyclohexymide was added, suggest that the greater part of this demand is exercised by the populations of heterotrophic bacteria involved in the degradation of the organic matter, which use the oxygen as a final acceptor of electrons in their respiratory chains. In fact, a relation is found in the body of available data (Fig. 3) between the organic content of the sediment and an index of bacterial biomass (in this study the count of viable heterotrophic bacteria on plates).

In comparing the results obtained in this study from distinct types of sediment, with a variable quantity of detritic matter (HARGRAVE, 1972) and which also feature an unknown refractory fraction to bacterial attack (RILEY & CHESTER, 1971), everything seems to indicate that the determinations of oxygen uptake rates are useful as a measure of total biological activity (on the whole bacterial) in the sediments (PAMATMAT, 1971). This relation is illustrated in figure 1. On the other hand, in comparing the benthic metabolism of oxygen uptake, transformed in terms of carbon (for which the quotient

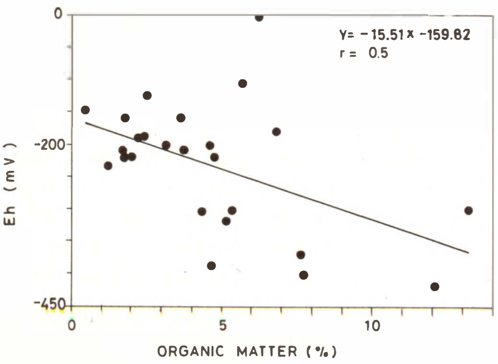

FIGURE 4. Relation between Eh (redox potential) value and the organic matter content of the sediment.

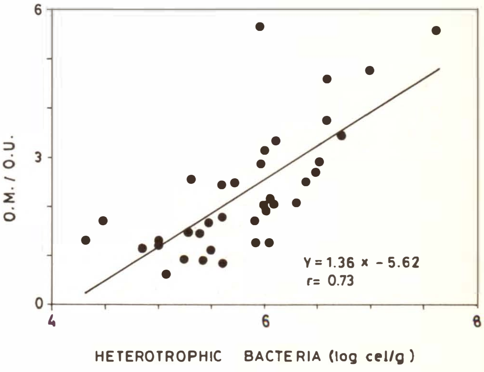

FIGURE 5. Relation between the organic matter content of the sediment and rate of turnover.

RQ = 1 has been used; NIXON 1980) with the primary production total (P, measured or estimated) in each one of the stations considered, it is evident that in estuarine systems an external contribution of organic matter (I) is required to allow the adjustment of budgets (in terms of carbon and nitrogen).

In the sampled stations as a whole, the situation is usually I >> P. In spite of the organic matter input having in some cases a "natural" origin (drainage from cultivated fields in the Ebro Delta), and in others stemming from processes of pollution by waste waters (Ría de Bilbao or Besòs Delta), the final result is not very different, in that the bacterial activity in the sediment is always carried out in a fairly equivalent manner, so that by aerobic means 35-45% of the organic matter accumulated in the sediment is remineralized annually (Table II). Finally, although the data currently available does not allow an absolute statistical validity, the relative weight of the aerobic processes in the overall bacterial activity appears to be less in sediments with higher organic matter content (and with a lower redox potential).

This is also supported by the fact that the lower the turnover of matter accumulated in the sediment (estimated using the relation MO/OU), the higher its organic content. It must be borne in mind that these sediments correspond to the most polluted zones (Ría de Bilbao and Besòs Delta); the presence of high concentrations of micropollutants (principally heavy metals; Borràs, pers. comm.) partially inhibits the bacterial activity. Furthermore, they are sediments with the most negative redox potential (see Table I), and the use of molecular oxygen in the respiratory chains (WATSON *et al*., 1985) is not to be expected below a value of Eh of +200 mV. The relationship shown in figure 5 is indicative of the growing importance of the anaerobic processes as the oxygen demand by populations of heterotrophic bacteria reduces the value of the redox potential. The alternative use of other electron acceptors also affects the balance of the consumption of organic carbon accumulated in the sediment (SØRENSEN *et al*., 1979), for as the nitrates and sulphates oxidize 1.25 and 2 moles of organic carbon respectively, the oxygen acts in an equimolecular relation.

The degradation of organic matter is accompanied by a consumption of oxidants and an accumulation of reduced chemical species, the effect being a diminishing of the redox potential. The estuarine systems in which I >> P are especially suitable for studying the dynamics of this process, in terms of both time and space, amongst other reasons because they usually represent an unbalanced medium, in which the greater availability of material liable to be oxidized by the bacterial populations exercises a demand for oxidants that rapidly reduces the value of the redox potential, as shown in figure 4. Once the availability of oxygen is exhausted, the decomposition of organic matter continues through the use of NO^{-}_3, Fe^{+3}, SO^{2-}_4, CO_2 and anaerobic fermentations. The process occurs precisely in this sequence due to respiration involving reactions of phosphorylation tied to transport of electrons, and thus with oxide reduction reactions that depend on the value of the Eh of the medium.

In agreement with the data presented, which refers to a wide variety of estuaries, the bacterial populations of the sediment work as active transformers of the quality of the medium, with a high oxygen demand for the metabolisation of the available organic matter, which causes its reduction and rapid exhaustion. The intensity of this process is directly related to the quantity of accumulated organic matter, in such a way that in those systems with greater external contributions conditions are more rapidly established that make the metabolism of all the eukaryotic organisms impossible (with the exception of those yeasts capable of fermenting, whose contribution in terms of the balance of matter is unknown). However, some products of these alternative respiratory mechanisms (e.g. H_2S) are toxic for the majority of organisms.

It must thus be concluded that the overall tendency of these processes is a gradual control of the medium by the bacterial populations of the sediment, in the sense that the percentage of matter and energy that circulates through them is higher as more strictly reducing conditions are established. Moreover, these conditions are themselves the result of the activity of the same bacterial populations in the initial phases of aerobic oxidation of the organic matter. The final result is that the bacterial compartment of the sediment is only the agent in the mineralization and production of nutrients, which in turn affects the conditions of the water column. The comparison between samples taken from systems with different characteristics shows that the availability of organic matter constitutes the principal factor in explaining the temporal and spatial variability of the activity of the bacterial populations.

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