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Comparison of anaerobic and aerobic biodegradation of mineralized skeletal structures in marine and estuarine conditions

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Abstract. The knowledge of the biodegradation rates is essential to studies of the biogeochemistry and ecology of aquatic systems. It helps us to quantify the production and uptake rates of chemical components and their recycling, and to understand the mechanisms and rates of organic matter accumulation in sediments. Experimental studies of biodegradation processes in six types of mineralized skeletons were performed in shallow-marine waters of Calvi Bay, Corsica and in estuarine waters of Roscoff, Brittany. Three types of mollusk shells, sea urchin skeletal plates, crab cuticle and fish vertebrae were exposed to oxic and anoxic conditions over periods of 15 days to 30 months. After recovery of the substrates, protein assays, bacterial counts and organic carbon analyses were performed.

Quantitative protein assays and bacterial counts indicate that biodegradation of mineralized skeletal structures occurs at a slower rate in anoxic conditions than in oxic conditions. Bacterial analysis showed that in anoxic environment, less than 0.5% of the consumed organic matter is converted into bacterial biomass. The aerobic biodegradation rate was positively correlated with the organic content of the skeletons.

Anoxic biodegradation of skeletons occurred at much slower rates in estuarine sediments than in shallow marine sediments. Preservation of skeletal structures in estuarine conditions appears to be correlated with the abundance of dissolved organic matter rather than with high sedimentation rates.

Introduction

The elucidation of biodegradation processes is essential in biogeochemistry and ecology. Weathering rate is one of the key factors necessary to understand the recycling of the chemical components of organic matter or accumulation of organic detritus. Skeletal structures of all organisms contain organic matter (Poulicek 1982) that is more or less masked by mineral matrices of different compositions. While this organic matter is not accessible for most macroconsumers, it represents an essential energy source for heterotrophic microorganisms (Poulicek & Jaspar-Versali 1984). Furthermore, in the biogeochemical

recycling of organic matter, it is essential to know the biodegradation rates of all the important sources of organic matter, like that in the organoclastic fraction of shallow-water marine sediments.

Aerobic degradation processes of skeletal structures have been thoroughly studied (e.g. Edwards & Perkins 1974; Golubic et al. 1975; May & Perkins 1979; Ohwada et al. 1983; Poulicek & Jaspar-Versali 1984; Hook & Golubic 1990; Kidwell 1989). However, surprisingly little information concerning anaerobic biodegradation of skeletal structures can be found in the literature (May & Perkins 1979; Wuttke 1983; Kidwell & Baumiller 1990; Kristensen et al. 1991). Despite this obvious lack of information, a widely held assumption concerning anaerobic biodegradation is that anaerobic conditions are favorable for organic matter preservation in skeletal structures (e.g. Roger 1952; Didyk et al. 1978; Raup & Stanley 1978; Demaison & Moore 1980; Vigneaux et al. 1980; Glenn & Arthur 1985; Stein et al. 1986; Calvert et al. 1991). To test and verify this assumption, we exposed discrete skeletal structures over time periods of 15 days to 30 months to anoxic waters in two aquatic environments and compared biodegradation rates in these conditions to those in oxic conditions.

Quantitative microbial degradation rates were estimated from data obtained from protein assays on individual skeletons, both in oxic and anoxic conditions. In the rate studies in anoxic conditions, enzymatic activity evaluations and bacterial counts were also performed. These assays allow us to define whether or not the degraded organic matter is used for anaerobic bacterial growth. Bacterial counts and estimates of size are used to calculate biomass, using the Meyer-Reil (1987) conversion ratio. With those data, we can establish a mass balance between skeletal organic matter depletion and bacterial biomass.

Besides investigating biodegradation processes in shallow-water marine sediments, we also wanted to compare anoxic biodegradation processes in shallow marine waters with those in an estuarine environment. Estuaries have high sedimentation rates which may promote preservation of skeletal organisms (Raup & Stanley 1978; Towe 1987). However, in estuaries sedimentary organic matter is particularly abundant (e.g. Aston 1978; Berner 1982; Skyring 1987), favoring the growth and development of attached and free-living decomposer communities. It is already known that non-mineralized skeletons are decomposed rapidly in estuarine conditions (Hillman et al. 1989; Gooday et al. 1991). One of the goals of this investigation was to determine if mineralized skeletons will also undergo rapid biodegradation in estuarine conditions.

Materials and methods

Nylon (mesh 180 μm, size 12 × 13 cm) bags were used to expose skeletons and organic matter to microbial decomposers (Poulicek 1982; Hillman et al.

1989; Rieper-Kirchner 1989; Peduzzi & Herndl 1991). We positioned the nylon bags at the water-sediment interface in the aerobic biodegradation experiments and in 2 L closed glass jars (containing two sets of samples each) that were completely filled with bottom sediment in the anaerobic biodegradation experiments. In the closed jars, nylon bags were placed in the center of the jar, so that they were not in contact with the glass.

The skeletal structures (Table 1) were collected from freshly gathered organisms:

1. Mollusk shell material was isolated mechanically from other layers (e.g. periostracum), by using a milling machine. Crossed-lamellar structural material was obtained from *Tridacna gigas* (in oxic experiments) collected in the Philippines. *Mercenaria mercenaria* (in anoxic experiments) was collected along the French coast of the Channel. The *Pinna nobilis* specimens were collected from Calvi Bay, Corsica and the *Nautilus pompilius* from the Philippines.
2. The sea urchin, *Sphaerechinus granularis* (in oxic experiments) and *Arbacia lixula* (in anoxic experiments) were collected from Calvi Bay. The skeletal plates were brushed manually to remove spines and epidermis. Stereom was not separated from stroma.
3. The crab, *Carcinus maenas*, was collected during its intermolt period (C4 stage) along the Netherlands coast of the North Sea. The cuticle was taken from the cephalonotal shield and brushed manually to remove epidermis.

Table 1. Type and composition of skeletal structures used for this expermental study.

Skeletal Structure	Mineral phase	Total Organic Matter	
		Content (%)	Nature
Crossed-lamellar structure of *Tridacna gigas* (in oxic experiments) *Mercenaria mercenaria* (in anoxic experiments) (Bivalve, Mollusk)	Aragonite	0.283 ± 0.190 (n=8)	Chitinoproteic
Prisms of *Pinna nobilis* (Bivalve, Mollusk)	Calcite	1.338 ± 0.410 (n=11)	Chitinoproteic
Nacreous layer of *Nautilus pompilius* (Cephalopod, Mollusk)	Aragonite	3.557 ± 0.621 (n=11)	Chitinoproteic
Test of *Sphaerechinus granularis* (in oxic experiments) *Arbacia lixula* (in anoxic experiments) (Echinoïd, Echinoderm)	Magnesium-rich calcite	2.528 ± 0.584 (n=6)	Glycoproteic
Cuticle of *Carcinus maenas* (Decapod, Crustacea)	Magnesium-rich calcite	16.087 ± 0.836 (n=7)	Chitinoproteic
Vertebra of *Scorpaena porcus* (Teleost, Fish)	Hydroxy-apatite	17.175 ± 3.280 (n=4)	Glycoproteic

4. The fish, *Scorpaena porcus*, was collected from Calvi Bay, and the vertebrae were isolated using a scalpel.

Without chemical treatment, the skeletal structures were then crushed and sieved. The size fraction from 2 to 4 mm was further cleaned ultrasonically. Weighted dry aliquots of the skeletal materials were placed in the 180 μm mesh nylon bags.

One experimental site (A) was located in Calvi Bay, Corsica on a sand channel within a seagrass (*Posidonia oceanica*) bed at a depth of 37 m, at the outer edge of the grass meadow (Fig. 1). The temperature at this depth is between 13.2 and 16.4 °C; the salinity and the oxygen content of the overlying water are, respectively, 37.4–37.7 psu and 9.3–10.2 mg/l. The ambient sediment is coarse organoclastic sand, with a mean grain size of 0.489 mm. The bags containing skeletal structures were placed either directly on the sediment interface (oxic experiment) or in closed jars (anoxic experiment)

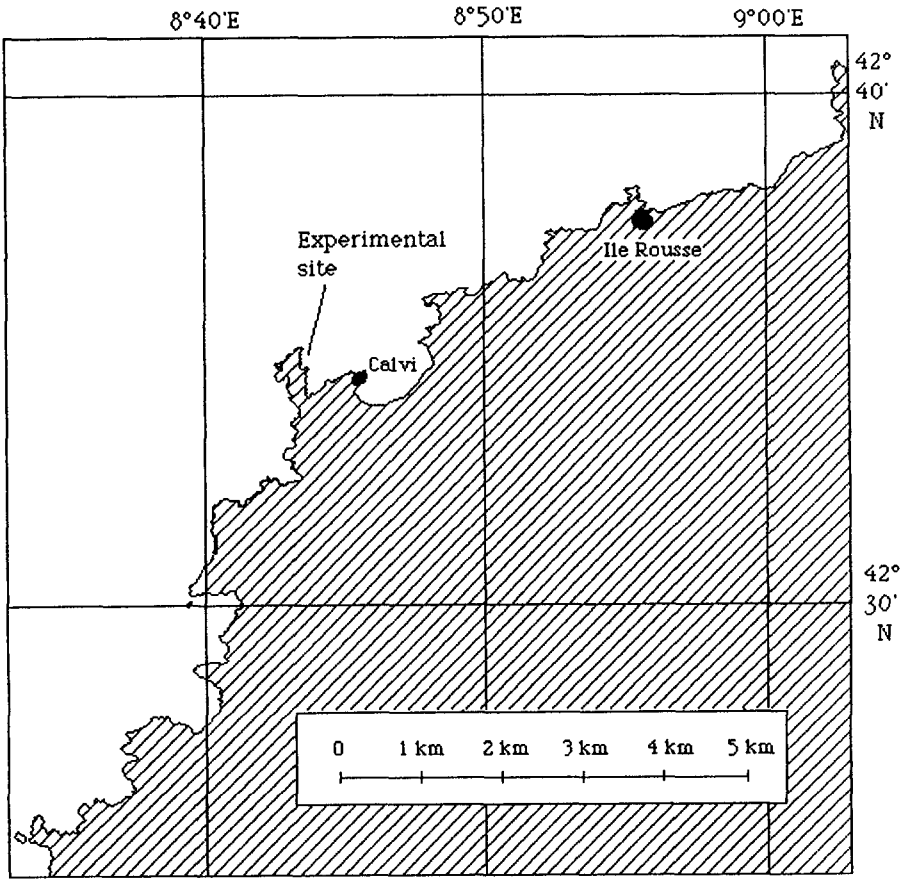

Fig. 1. Shallow-water marine experimental site (A) in Calvi Bay, Corsica.

set in place above the sediment (Table 2). The careful placement of the experimental equipment was done by scuba-divers thus minimizing disturbances to the environment. Eight control bags were inserted directly into the anoxic sediments at site A (by inserting a 15 cm diameter × 50 cm long plastic cylinder into the sediment, extracting the sand, placing the bag in the hole, removing the cylinder and replacing the sand) to permit comparisons with closed-jar experiments.

Table 2. Characteristics of the two expermental sites.

Sites	Environmental conditions		Sediment
Calvi Bay	aerobic	sediment-water interface	Organoclastic sand
(Corsica)	anaerobic	anoxic jar*	Organoclastic sand
Site A	anaerobic	anoxic sediment *in situ* **	Organoclastic sand
Guillec estuary (Brittany) Site B	anaerobic	anoxic jar *	Mud

* Anoxic experiments were perfomed using 2 L glass jars. Bags containing skeletal structures were placed in these jars. which were filled up with sediment from experimental sites and set in place for periods of 15 days to 30 months.

** Control samples in nylon bags were placed 40 cm below the sediment interface.

Our second site (B) was located in the Estuary of the Guillec (French Brittany, Roscoff, France, Fig. 2). The sediment is estuarine mud, with a mean grain size of 0.052 mm. At this location, jars containing skeletal structures could not be placed directly in the estuary because of the high sedimentation rate, physical disturbances and 'human predation'. The materials were placed in an aquarium at Liège University, Belgium, under controlled environmental conditions. The temperature was varied between 14.0 and 19.0 °C, recreating the Channel seasonal temperature fluctuations. The salinity of the water was 34.2–34.7 psu, and the oxygen concentration was 9.0–9.5 mg/l. The environmental conditions of the experiments are summarized in Table 2.

The dry weight and protein content of the total organic matter and the mineral matrix of pre-weighed samples were determined gravimetrically by leaching of the mineral phases in 1N HCl at room temperature for 8 hr, and reweighing the dried organic residue. After a subsequent 6 hr treatment with 0.5 N NaOH at 100 °C and centrifugation, the protein content was determined in neutralized supernatant using the B.C.A. (Bicinchoninic Acid; Pierce chemicals) method, which is based on the biurtet reaction with the peptidic link (Creighton 1984). This method is more sensitive and less subject to error (Smith et al. 1985) than the other assay methods available.

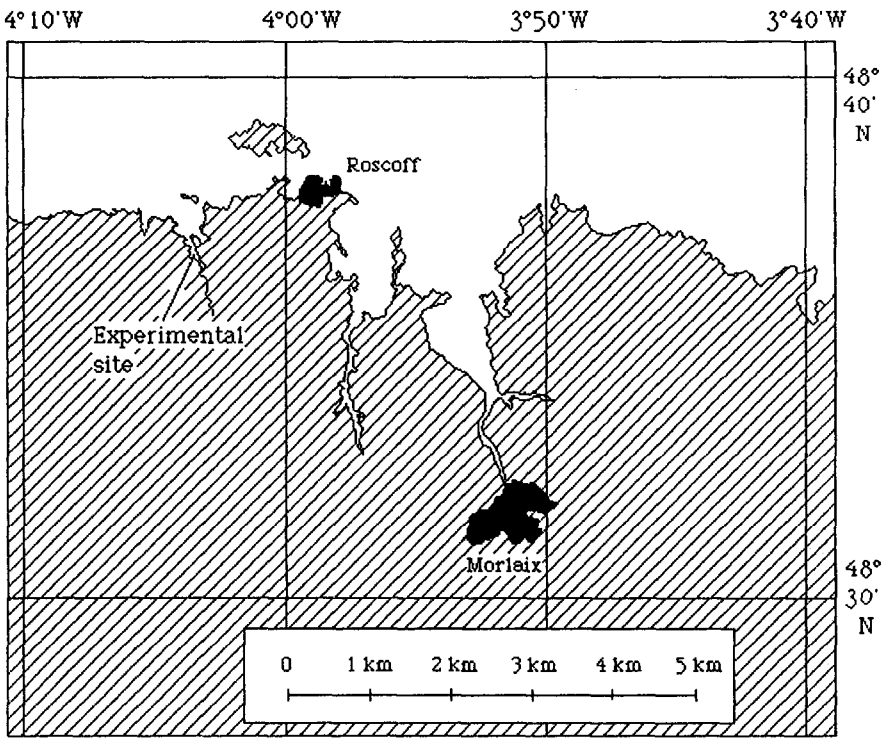

Fig. 2. Estuarian experimental site (B) in French Brittany.

Scanning electron microscopic (S.E.M.) observations were performed on fixed (12 hr in 4% glutaraldehyde in 0.2 µm filtered seawater) and post-fixed (6 hr in 1% $Os0_4$ in 0.2 µm filtered seawater) samples using a Jeol JSM 840 microscope.

The evaluation of enzymatic activity was performed using Api Zym kits (Sherwood Medical), which is a faster, but less precise method than the classical assay, on 100 µl of interstitial water or skeletal structures that were manually ground. Solutions were frozen to destroy cells and to free enzymes. Incubation time was 4 hr at 37 °C. Nineteen enzymatic activity determinations (in nM of hydrolyzed substrate $\times$ g^{-1} $\times$ h^{-1}) of each sample were combined to yield the total enzymatic activity.

Direct counts of bacteria were performed by the acridine orange technique (Hobbie et al. 1977), with a Leitz Diaplan microscope equipped with a Leitz Orthomat E Photosystem (excitation wavelength of 450 to 490 nm, cutoff filter of 515 nm). Cells were detached from skeletons or sediment by ultrasonic cleaning for 40 seconds (power 75 W; Branson Sonifier II Ultraschall Desintegrator Modell 450). All cells were counted using a minimum of 35 fields of vision per sample. A total of 350 bacteria per field were sized using an eyepiece micrometer, and classified into rods, cocci and vibrio. Biovolume

estimations were approximated based on the assumption that rods are cylinders with 2 hemispherical caps and cocci are spheres. Biovolumes were converted into biomass using the Meyer-Reil (1987) ratio; 0.11 g of organic carbon per cm^3. All analyses were performed on two samples from each experiment. The error bars on the graphs represent the standard deviations of the analyses.

Results

Protein assay

To ensure that the processes observed in anoxic jars are identical to those in ambient anoxic sediment, control samples in nylon bags were placed directly into anoxic sediment at site A. Figure 3 shows that after 6 and 12 months of exposure, the protein contents were similar for samples enclosed in the jars and for those inserted directly in the anoxic sediment.

The following results concerns skeletons placed either at the sediment-water interface or in closed jars and used for quantifying the extent of biodegradation of the skeletal structures. Figs. 4 to 6 show that the mollusk mineral phase exhibits barely any weight loss, whereas its decrease in protein content is significant, especially in oxic conditions. Variations of the protein content of this structured material are insignificant in the estuarine environment (site B).

In the case of sea urchin skeletal plates incubated in anoxic conditions (Fig. 7), changes in mineral phase weights never exceed 0.5%. The loss of protein

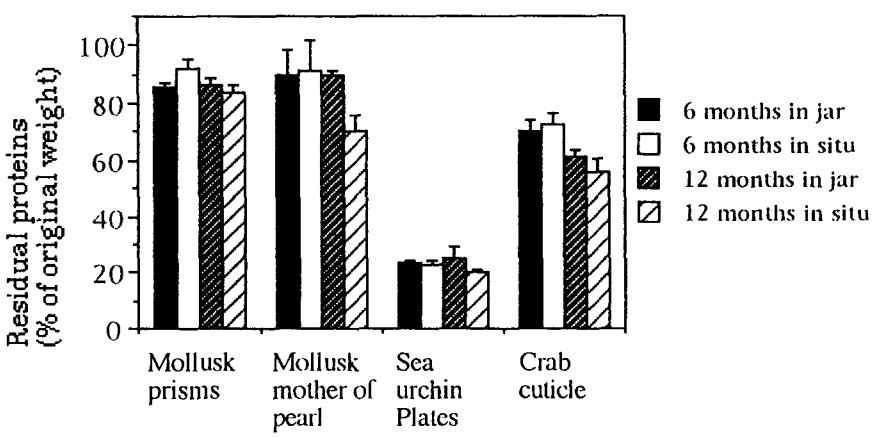

Fig. 3. Protein content of skeletal structures incubated in anoxic conditions at site A (shallow-water marine environment). Skeletons were incubated 6 or 12 months either in closed jars or 40 cm below the sediment interface.

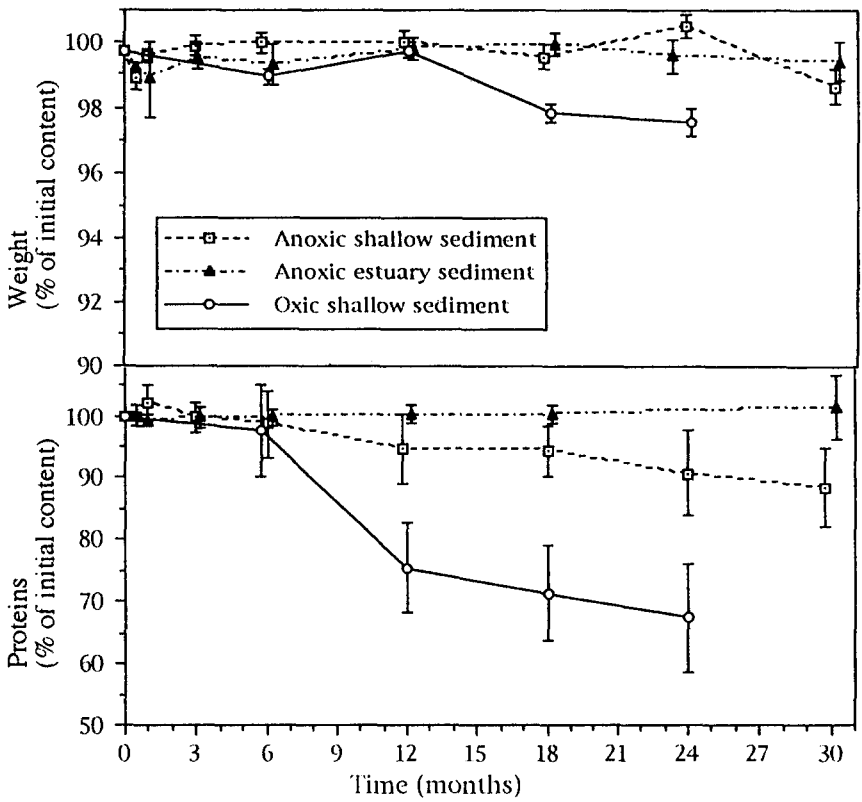

Fig. 4. Residual mineral phase and protein content (both in % of initial weights) in mollusk crossed-lamellar structure versus time of exposure to decomposers.

occurs at a rapid rate during the first month of the anoxic incubation period and is then stabilized.

The mineral phase weight of the crab cuticle (Fig. 8) declines during the first 3 months of the experiment and is followed by a period of nearly constant weight.

The fish vertebrae (Fig. 9) incubated in the oxic environment are totally demineralized after 12 months of degradation. In all the environments studied, the protein loss is important during the first month of the experiments.

For all the skeletal materials studied (Figs. 4–9), the decrease in protein content occurs faster in oxic conditions than in anoxic conditions. In anoxic conditions, protein decay is more pronounced at the shallow-water marine site A than at the estuarine site B.

A comparison was made among biodegradation rates for different skeletal structures in anoxic marine conditions (Fig. 10). The protein content of fish vertebrae is lower than that found in any other skeletal structure after incubation.

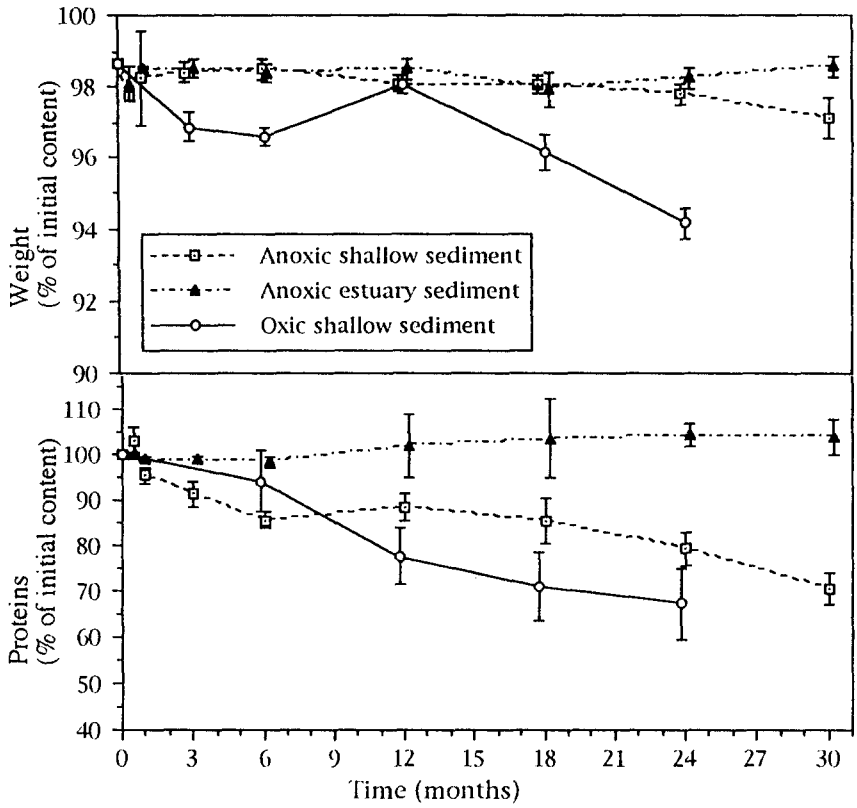

Fig. 5. Residual mineral phase and protein content (both in % of initial weights) in mollusk prisms versus time of exposure to decomposers.

Enzymatic activity evaluation

The evaluation of enzymatic activity was only performed in the experiments in anoxic conditions (Fig. 11). The enzymatic activity measured in undisturbed marine or estuarine sediments is negligible ($\leq$ 10 nM g^{-1} h^{-1}). At the shallow-marine site A (Fig. 11, top), activities are highest for skeletal substrates than in the interstitial waters of the jars. The reverse situation can be observed at the estuarine site B (Fig. 11, below).

Direct counts of bacteria

Direct counts of bacteria were only performed in the anoxic conditions at site A (Fig. 12). The number of rods and cocci are similar in sediments and mother of pearl. Cocci were found to be very abundant in sea urchin skeletal plates. All types of bacteria are most numerous in crab cuticle. The ratios of

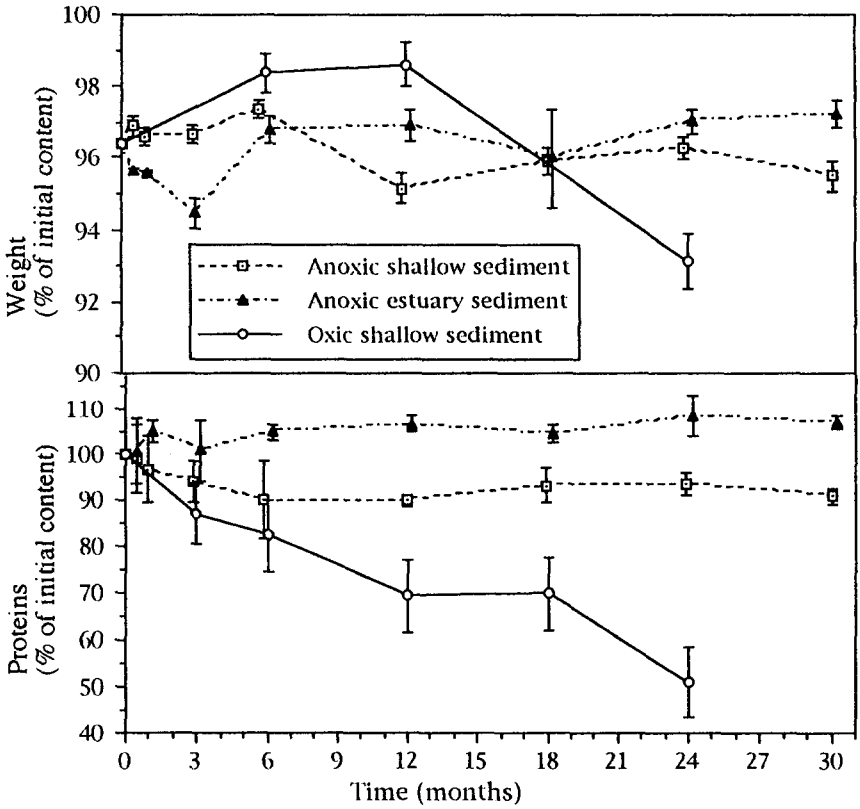

Fig. 6. Residual mineral phase and protein content (both in % of initial weights) in mollusk mother of pearl versus time of exposure to decomposers.

the mean number of rods to cocci were calculated using the sum of the two incubation times in Fig. 12. Results are: 2.6 for sediment *in situ*; 3.2 for jar sediments; 13.3 for mother of pearl; 1.5 for sea urchin skeletal plates and 1.5 for crab cuticle.

The size distribution of the bacterial rods (Table 3) and cocci (Table 4) showed that the more abundant cocci class shows a volume of 0.065 μm^3, 4 times lower than that of the most represented class of rods (0.262 μm^3, Table 3).

The results of the rods and cocci biomass calculations (Table 5) show that cocci biomasses are clearly less than those of rods.

Data from bacterial biomass were used, together with organic matter loss (calculated from Figs. 4–9) to establish an approximate mass balance between the consumed organic matter and bacterial biomass in anoxic conditions, using 0.5 (Bjornsen 1986) as the conversion factor between organic matter content and biomass. Those data (Table 6) show that less than 0.5% of the organic matter consumed in anoxic conditions is converted into bacterial biomass.

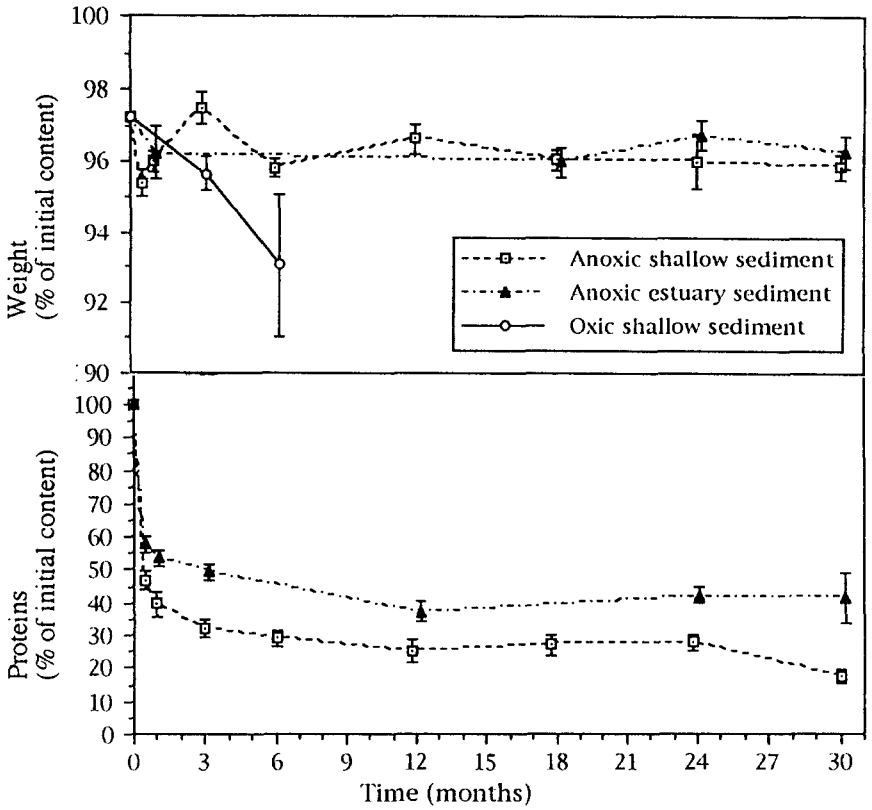

Fig. 7. Residual mineral phase and protein content (both in % of initial weights) in sea urchin skeletal plates versus time of exposure to decomposers.

Scanning electron microscopy

Scanning electron microscopic studies were done of the surfaces of the non-incubated and incubated skeletal substrates. After 3 months of degradation in the oxic, shallow-water, marine environment of site A, a dense fungal network and diatoms had developed on the substrate (Plate 1B). In anoxic conditions, fungal networks were not observed, but burrows were commonly present. For example, after 6 months degradation in the anoxic marine environment at site A, about 2 μm diameter and 80 μm to 1 mm long tunnels can be seen in the mollusk substrate (Plate 1C). Those tunnels are situated on the periphery of the prism, in close contact with the periprismatic organic matrix. The morphology of these tunnels and their correspondence to the morphology of the organisms observed *in situ* (Plate 1D) suggest that the tunnels are a result of fungal activity. Interestingly, some diatoms appear to be alive after 2 years of anoxic incubation (Plate 1E).

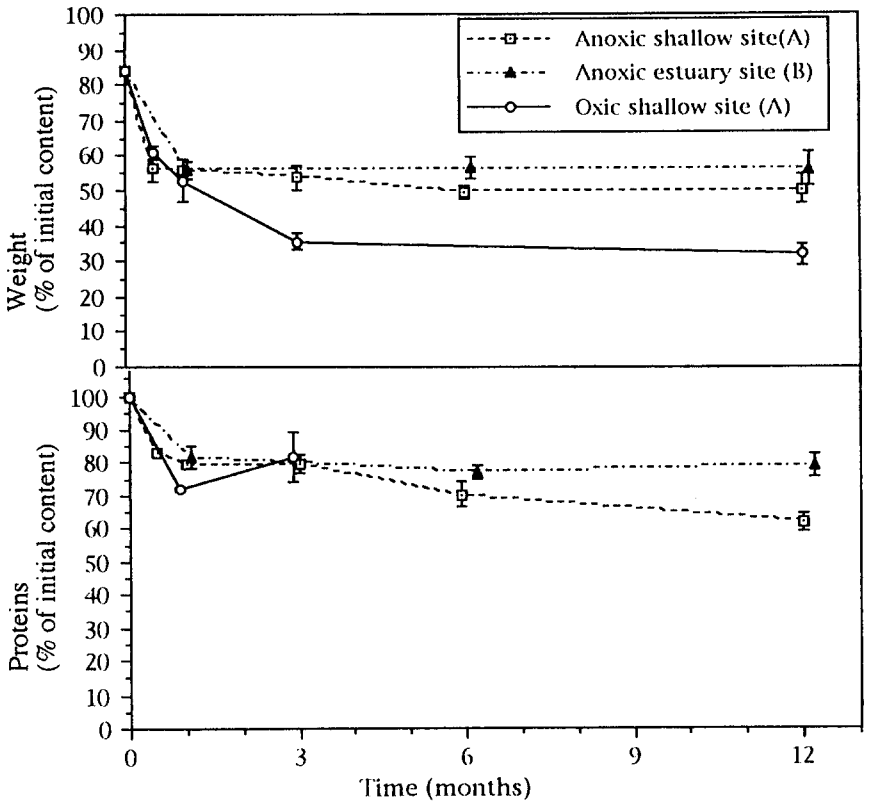

Fig. 8. Residual mineral phase and protein content (both in % of initial weights) in crab cuticle versus time of exposure to decomposers.

On sea urchin skeletal plates, both in the oxic and anoxic marine environments, the substrates are colonized mainly by bacteria (Plate 1F), inducing pitting of the mineral phase.

Plate 2G shows a control fish vertebrae. In anoxic conditions (both at site A and B), the decomposing microorganisms do not cause major alterations of the vertebrae surface; only dissolution and/or bacterial pitting is observed after 1 year of incubation at site A (Plate 2H). On the contrary, the decomposers in the oxic environment destroy quickly and thoroughly the vertebrae structure (Plate 2I to 2L).

The various forms of organisms observed through S.E.M. during this study were subdivided on basis of their morphology. The number of forms and size of all the organisms observed are given in Table 7. In the oxic zone, both the number of forms and the size of the organisms are higher than in the anoxic environment.

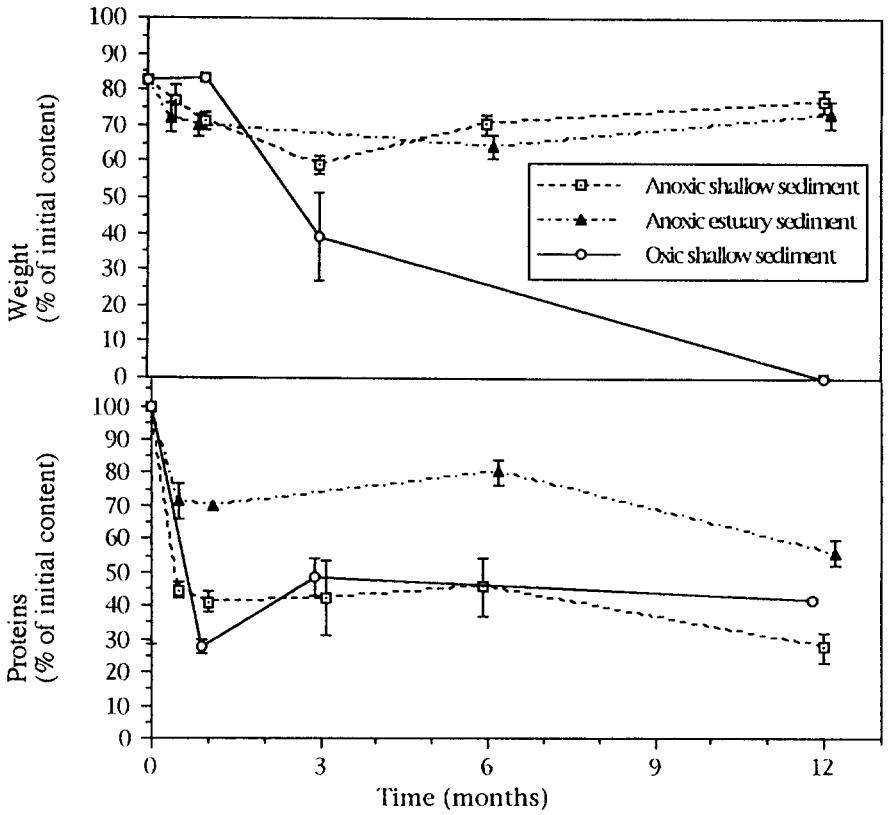

Fig. 9. Residual mineral phase and protein content (both in % of initial weights) in fish vertebrae versus time of exposure to decomposers.

Discussion

Evaluation of methods

The only treatment the skeletal structures were subjected to before incubation was sonication. Because the organic matter is masked within the mineral phase of the skeletons, this procedure probably does not significantly damage the organic matter matrix of the skeletons.

Unfortunately, it was not possible to place all of the skeletal structures studied directly into anoxic sediments *in situ*, because of technical reasons related to scuba-diving at 37 m depth. Thus, to simulate the environmental conditions of anoxia in this environment, we chose to use closed jars filled with water and sediment collected at the experimental site. Experiments were performed to verify that anoxic jars are an acceptable means of studying biodegradation of skeletal structures under anoxic conditions. Oxygen, pH and

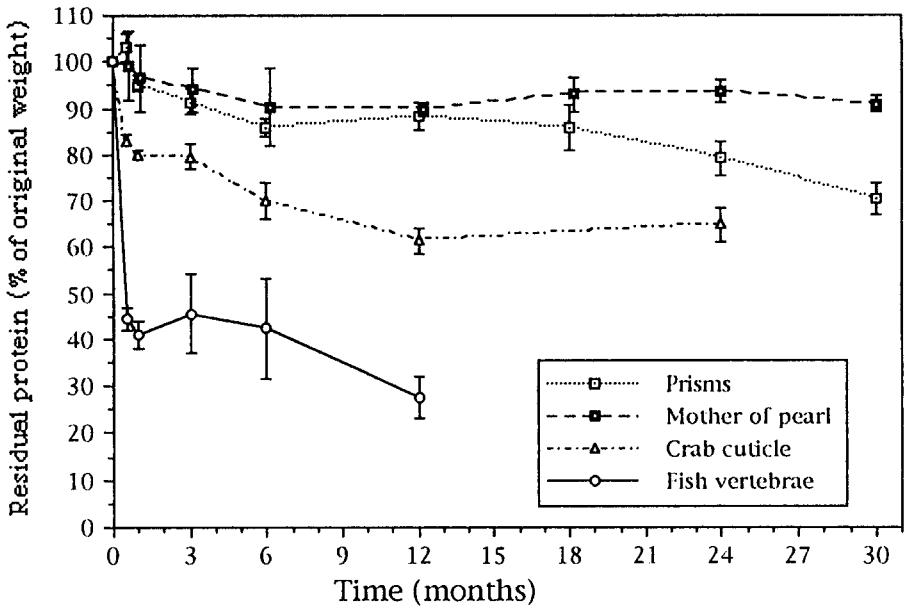

Fig. 10. Protein content of skeletal structures incubated in the anoxic jars at site A (shallow-water marine environment). Data shown in this figure are taken from Figs. 4 to 9.

Eh measurements were taken of jar waters and sediments. No chemical gradients in these parameters were observed within the waters and sediments of the jars, that is, in all cases, the jar systems were found to be completely anoxic. For example, less than 0.6 mg O_2 per liter was found in the closed jars after only two days of immersion, and this concentration decreased significantly with time of incubation (Simon 1992). Demaison and More (1980) mentioned in their study that an environment can be considered anoxic when the oxygen concentration is less than 0.725 mg O_2 per liter. Therefore, the experimental jars can be considered as anoxic systems after only two days of incubation. Furthermore, the bacterial counts (Fig. 12) were similar for sediment of anoxic jars and sediment taken *in situ* in the anoxic zone, about 2×10^8 rods and 10^8 cocci/g of sediment. These value are in agreement with Meyer-Reil's (1987) results for Baltic Sea sediments. Moreover, the protein content (Fig. 3) is similar in skeletons that were immersed for 6 and 12 months either in closed jars or placed directly in anoxic sediment. However, some differences do exist between the jar experiments and those done *in situ*. Fig. 3 shows that mother or pearl is degraded faster after 12 months of incubation *in situ* (72% of original weight) than in the closed jar experiment (92% of original weight). It is possible that small differences increasing with time of exposure do exist between the jar and *in situ* experiments, but we consider that the jar experiments reasonably well represent *in situ* conditions, at least for the time-scale of this study.

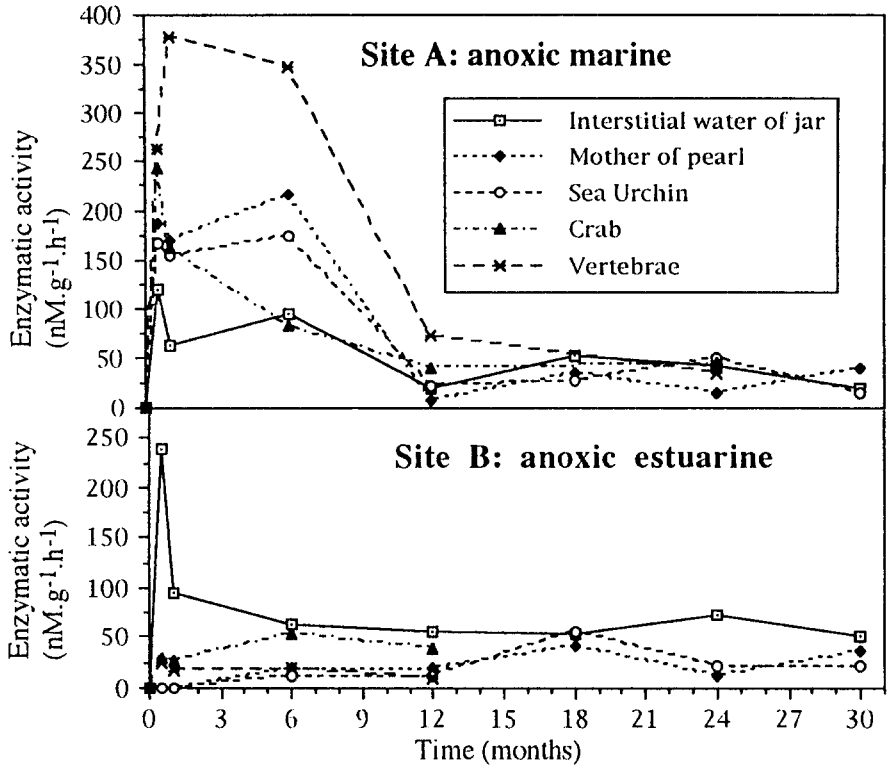

Fig. 11. Sum of enzymatic activities (using Api Zym kits) estimated on skeletons or on sediment from anoxic jars. Incubation at site A (anoxic marine), top, or at site B (anoxic estuarine), below.

Two to four samples were used for each incubation time. A two controlled factors anova analysis on protein results suggest there is statistically no significant difference between two samples in the same jar or in two different jars [F(3.2) = 0.07]. This appears to demonstrate that micro-environmental effects do not interfere severely in our protocol.

Biodegradation of skeletal structures in shallow water (Site A)

a) Indications of microbial colonization. The first indication of biodegradation activity is seen in the enzymatic activity data (Fig. 11). In the shallow water experiment at site A (Fig. 11, top), enzymatic activities are detectable (between 100 and 270 nM g^{-1} h^{-1}) after only 15 days of incubation (the shortest incubation time of our study). These activities decrease after 1 to 3 months, probably because of the depletion of labile organic matter substrate (Litchfield et al. 1974; Cahet 1980; Gagosian 1980; Peduzzi & Herndl 1991). It should be noticed that the activities are generally higher on skeletal surfaces (up to 370 nM g^{-1} h^{-1}) than in interstitial water (less than 125 nM g^{-1} h^{-1}), suggesting that microbial activity related to biodegradation is concentrated on available

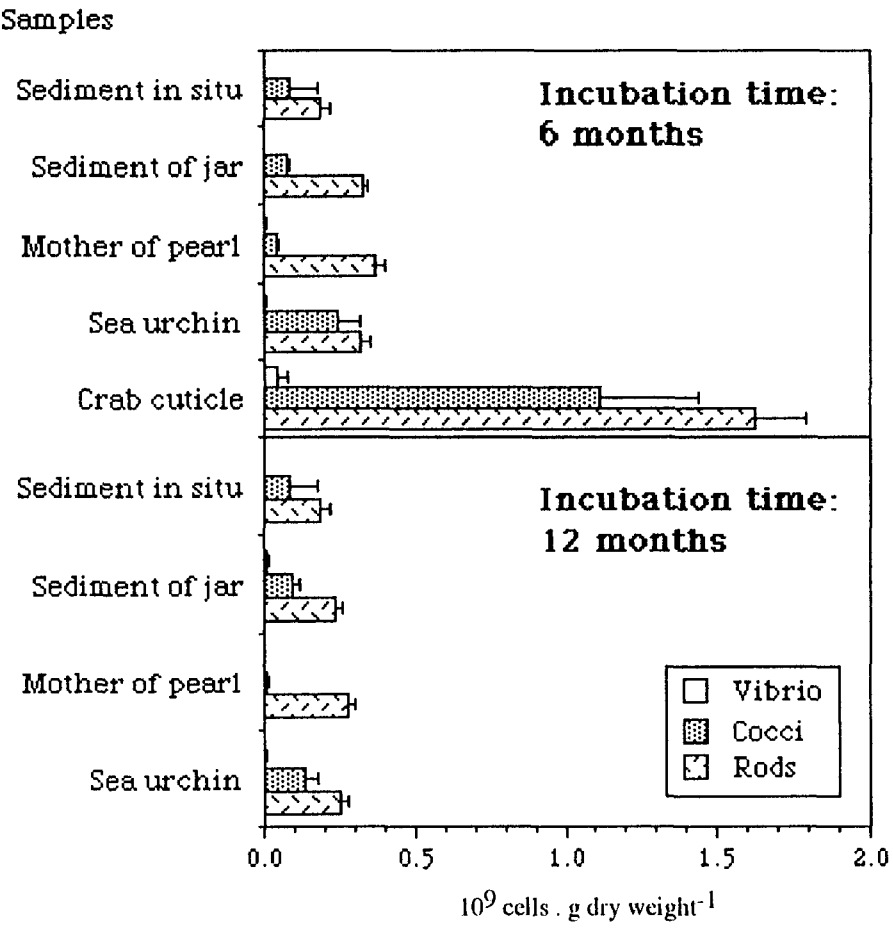

Fig. 12. Bacterial direct counts in samples from anoxic jars or sediment from site A after immersion for 6 or 12 months.

surfaces. The colonization of these biosurfaces is illustrated in the S.E.M. photomicrographs of Plate 1B to 1F and Plate 2J to 2L, showing bacteria, diatoms and fungi on the surface of the skeletons. The S.E.M. observations also show that the presence of fungi is directly correlated with the presence and distribution of the organic matrix of the skeletons. For example, Plate 1C shows that fungal burrows are situated on the periphery of the calcite prisms, in close contact with the periprismatic organic matrix. This observation indicates that fungal boring activity is probably a means of reaching the organic matrix of the mineralized skeletons.

The colonization of skeletons, which is probably a good indicator of the activity of bacteria that biodegrade skeletal material, can be estimated from bacterial counts. The colonization of skeletal structures (Fig. 12) is equal to

Table 3. Size of rods measured through epifluorescence microscope on skeletons or sediment incubated in anoxic conditions at site A.

Type of sample	% of each type	Length (μm) Class	Length (μm) Mean	Diameter (μm) Class	Diameter (μm) Mean	Volume (μm^3)
Bacteria in the sediments	80	1.25 - 1.75	1.50	0.45 - 0.55	0.50	0.262
	12	0.90 - 1.10	1.00	0.55 - 0.65	0.60	0.226
	5	3.50 - 6.50	5.00	0.35 - 0.45	0.40	0.612
	3	2.00 - 2.40	2.20	0.45 - 0.55	0.50	0.393
Bacteria in skeletons	95	1.25 - 1.75	1.50	0.45 - 0.55	0.50	0.262
	5	5.00 - 8.00	6.50	0.35 - 0.45	0.40	0.800

Table 4. Size of cocci measured through epifluorescence microscope on skeletons or sediment incubated in anoxic conditions at site A.

Type of sample	% of each type	Diameter (μm) Class	Diameter (μm) Mean	Volume (μm^3)
Bacteria in sediments and skeletons	90	0.40 - 0.60	0.50	0.065
	8	0.60 - 0.80	0.70	0.180
	2	0.90 - 1.10	1.00	0.524

Table 5. Bacterial biomass calculated for sediments and skeletons (in µg of C/g dry weight) incubated in anoxic conditions at site A.

	Rods biovolume (μm^3/g)	Cocci biovolume (μm^3/g)	Rods biomass (μg of C/g)	Cocci biomass (μg of C/g)
Sediment	4.72×10^7	8.34×10^6	5.2	0.917
Mother of pearl	7.31×10^7	2.08×10^6	8.03	0.229
Sea urchin	6.74×10^7	1.25×10^7	7.42	1.037
Crab	4.50×10^8	4.17×10^6	49.47	0.459

or higher than that in the sediment matrix, suggesting that bacterial activity concentrates on the skeletal particles, probably in relationship with weathering processes. It should be pointed out that the mean grain size of the sediments is less than that of the skeleton fragments. Therefore, sediments have a larger surface/volume ratio than that of skeletal structures. Thus, a given mass of sediment has a larger surface area than the same amount of skeletal material. Because the bacterial count results are expressed in number

Table 6. Percent of organic matter (expressed in % of dry weight) converted into bacterial biomass. O.M. is organic matter.

	Weight of consumed O.M. (μm dry weight)	% of O.M. converted into rods biomass	% of O.M. converted into cocci biomass	% of O.M. converted into rods + cocci biomass
Mother of pearl	35570	0.45	0.01	0.46
Sea urchin	17696	0.08	0.01	0.09
Crab cuticle	48261	0.20	0.002	0.20

of bacteria per gram of sample, this means that the skeletons ar more densely colonized by bacteria than the sediment per unit of area (Fig. 12). The higher bacterial concentration on skeletal particles reflects the interaction between the bacteria and the skeleton, i.e. biodegradation processes. Furthermore, both the abundance (Fig. 12) and size (Table 3) of rods are greater than that of cocci (Table 4 & Fig. 12). Thus, the biomass of rods is 6 to 10 times larger than cocci biomass (Table 5), especially on skeletal structures. Also, rods are more numerous (Fig. 12) and bigger (Table 3) on skeletal structures than on sediment particles. This is a reflection of higher metabolic activity when associated with skeletons, probably because of biodegradation processes, than when attached to the matrix sediment. We note that the size of rods observed in our samples (Table 5) is larger than that given by Velimirov and Walenta-Simon (1992) for the same site. The latter data were obtained for pelagic bacteria, whereas our studies dealt with sedimentary bacteria.

The ratio of the mean number of rods to cocci on skeletal structures shows distinct differences (Fig. 12). Once again, differences between this ratio in sediment and skeletons reflect the biodegradational interactions between bacteria and skeletons. Sea urchin skeletal plates appear to be more densely colonized by cocci than other types of skeletons. S.E.M. photomicrographs show that the gaps in structure of sea urchin skeletal plates allow the development of cocci in cavities (Plate 1F). On the contrary, the data also suggest that the original smooth shape of mother of pearl (e.g. Watabe 1965; Grégoire 1967) is probably not suitable for cocci development. The differences between the ratio of mean number of rods to cocci on skeletons can be attributed either to the composition of each skeletal structure, or to the ultramorphology (rough or smooth), making it more or less suitable for microorganisms to develop.

We can establish an approximate mass balance between the consumed organic matter (calculated from the protein loss, Figs. 4–9) and bacterial biomass in anoxic conditions. Because we used well-defined skeletal structures, we can calculate the dry weight of organic matter consumed by bacteria. Data given in Table 6 show that the consumed organic matter converted into bacterial biomass (vibrio are not taken into account, because their number is negligible; see Fig. 12) never exceeds 0.5%. This is a small value, but not

surprising, because anaerobic metabolism is energetically less efficient than aerobic (e.g. Froelich et al. 1979; Demaison & Moore 1980; Aller 1982; Reeburgh 1983; Hamilton 1984). However, it should be remembered that even if bacterial biomass is small, the turn-over rate of the bacterial community may be rapid and bacterial productivity high.

b) Quantification of weathering processes. Now that we have discussed the microbial biomasses present on mineral substrates, we are in a position to relate this activity to biodegradation processes. Protein assays allow us to quantify the biodegradation processes. First, we will compare biodegradation rates in anoxic conditions for the different skeletal substrates. Fig. 10 shows that the degradation of protein occurs very rapidly in crab cuticle and fish vertebra. After one month of incubation, 17 and 56% of the original protein of the crab cuticle and fish vertebrae, respectively, is lost by biodegradation. For the mollusk shells, the rate of protein loss is less; after 30 months of incubation, only 8% and 30%, respectively, of the original protein of the mother of pearl and calcite prisms were lost.

The Figs. 4–9 give us more precise data, both on the fate of the skeletons in different environments, and on the connection between organic and mineral matter weathering. Indeed, it can be seen in the Figs. 4–9 that protein loss is concomitant with the mineral loss. This observation is predictable. The organic matrix is within the skeletons, thus, the decomposing organisms have to remove the mineral phase to reach the organic matter.

S.E.M. Plates 1C and 1D show precisely that the mineral weathering is associated with organic matter weathering. The degree of mineral alteration coincides with the distribution of organic matrix. The mineral weathering mechanism is still unknown. It may be organic acid secretion, or chelation, or a combination of both mechanisms (Alexandersson 1975; Le Campion-Alsumard 1979). Another important factor in mineral phase alteration is the biodegradation of organic compounds (Reimers & Suess 1983). All biodegradation reactions generate organic acids and CO_2, which forms carbonic acid (e.g. Aller 1982; Reimers & Smith 1986). This process may be responsible for the vertebrae dissolution, as shown on Plate 2H.

Figures 4–9 show that the decay rate of protein and the rate at which the mineral phase loses weight are roughly positively correlated, showing a direct link between mineral dissolution rates and organic phase biodegradation. This link is most apparent for the crab cuticle, where the mineral phase loses 25 to 50% of its initial weight and in the fish vertebrae, where 10 to 100% of the mineral phase weight is lost. Variations in the mineral phase weight of the mollusk shells (Figs. 4–6) or sea urchin (Fig. 7) never exceed 2 to 3%, so it is difficult to demonstrate a link between biodegradation and mineral phase dissolution rates because of these small changes. Differences related to the relative solubilities of aragonite, calcite and magnesian calcite (e.g. Chave et al. 1962; Mackenzie et al. 1983) cannot be assessed using our data for the time-scale of this experiment.

Because only the organic phase provides energy to the decomposer, we will

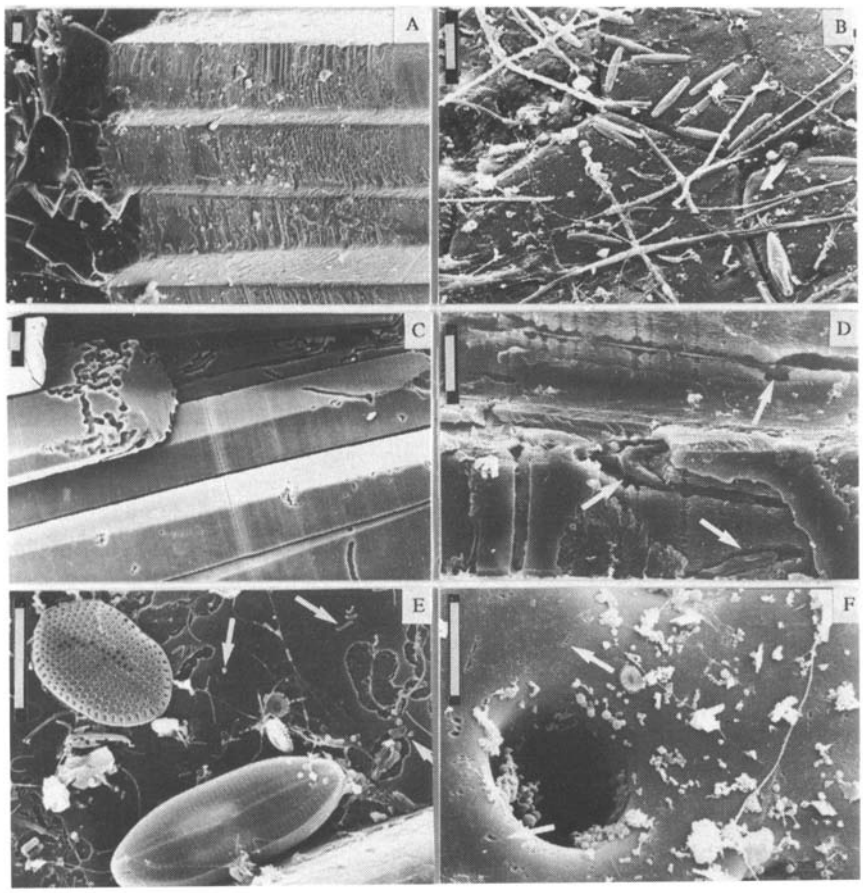

Plate 1
(Scale bar 10 μm)

A: Fresh, non-immersed mollusk prisms. The prisms are surrounded with a 1 μm thick organic matrix.

B: Prisms after 3 months oxic incubation in shallow-water, marine conditions, site (A). Colonization by fungi and diatoms.

C: Prisms after 6 months anoxic incubation in shallow-water, marine conditions, site (A). Fungal burrows are situated on the edge of the prisms, along the periprismatic organic matrix.

D: Prisms after 3 months anoxic incubation in shallow-water, marine conditions, site (A). Boring fungi (arrows) observed *in situ*.

E: Prisms after 2 years anoxic incubation at the shallow-water, marine site (A). Diatoms and bacteria (arrows). Alterations of the organic matrix are apparent.

F: Sea urchin skeletal plates after 2 years anoxic incubation at the shallow marine site (A). Bacteria and mineral phase pitting can be seen (arrows).

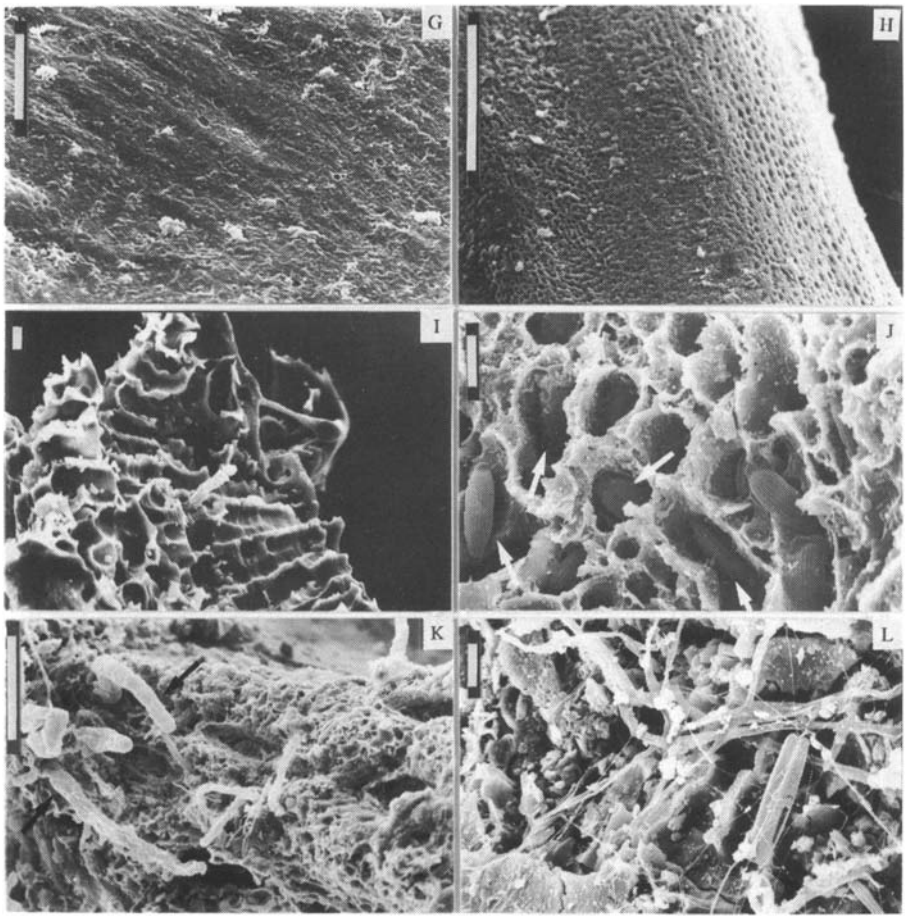

Plate 2

(G, I, J, L: scale bar 10 μm, H & K: scale bar 100 μm)

G: Fresh, non-immersed fish vertebra.

H: Vertebra after 12 months anoxic incubation at the shallow marine site (A). The dissolution of the mineral phase show the organic framework of the vertebra.

I: Vertebra after 1 month oxic incubation at the shallow marine site (A). An endolithic fungi can be sen in the center of the photomicrograph. The extremely altered shape of the vertebra is evident.

J: Vertebra after 3 months oxic incubation at the shallow marine site (A). Diatoms (arrows) are located in cavities within the vertebra.

K: Vertebra after 3 months oxic incubation at the shallow marine site (A). Several cyanobacteria (arrows) on the vertebra. Complete destruction of the structure is evident.

L: Vertebra after 1 year oxic incubation at the shallow marine site (A). Bacteria, diatoms and fungi can be seen on the totally desorganized vertebra.

Table 7. Size and number of organism groups observed for all skeletal structures using S.E.M.

Groups of observed organisms	Aerobic conditions		Anaerobic conditions	
	Number of forms	Diameter (in μm)	Number of forms	Diameter (in μm)
Bacteria	22	0.1-1	8	0.2-1.5
Diatoms	8	4-25	5	4-8
Cyanobacteria	42	1-50	-	-
Rhodophyta	1	3-8	-	-
Chlorophyta	7	5-50	-	-
Fungi	27	1-40	2	1.4-2.5
Sponges	4	50-1000	-	-

focus our attention on the fate of the organic matter. In anoxic conditions, the protein loss for the three mollusk shells studied (Figs. 4 to 6) is not large. Their protein content is about 90% of its initial content after 30 months of immersion. However, in the oxic environment, biodegradation is more pronounced, and 50 to 70% of the initial protein contents of the shells are lost within two years of incubation. The rate of degradation of protein occurring in the oxic environment at the same site (A) is higher than that in anoxic conditions.

The sea urchin skeletal proteins (Fig. 7) that can be measured are those from the stereom, stroma and collagen fibrils (Curney & Mc Nichol 1969; Dubois 1988). The rapid biodegradation of protein occurring during the first weeks of the experiments involving these substrates, 55% of their protein degraded after one month of immersion (Fig. 7), is probably due to biodegradation of the organic matter of the stroma. The stroma of the sea urchins is not shielded by mineral matter, whereas that of the stereom is.

In the case of sea urchin skeletal plates and mollusk shells, the period required for complete oxic degradation of the organic matter is about 10 to 20 years (Poulicek et al. 1988). These skeletons normally initially undergo oxic biodegradation, that might be followed by burial in anoxic sediment by processes of physical sedimentation or bioturbation, where anoxic biodegradation will continue to degrade the organic matter. In these skeletal materials, both oxic and anoxic processes contribute to the biodegradation of the skeletal structure. In contrast, crab cuticles and fish vertebrae, skeletons with high organic matter content, are degraded faster (Figs. 8 & 9) than mollusk shells or sea urchin skeletal plates. The intense protein loss that occurs during the first month of incubation of these latter substrates in the anoxic jars cannot be attributed solely to oxic decomposers. The closed jars rapidly become anoxic, after only 2 days of incubation, thus the anaerobic decomposers are at least partly responsible for this steep protein loss. The rapid degradation of protein is probably due to the intense bacterial colonization of the sub-

strates, as documented by the high bacterial counts. Figure 12 shows that crab cuticles are more densely colonized by both bacterial rods and cocci than any other skeletal structures studied. Despite the similarity of the initial organic matter contents of crab cuticle and fish vertebrae, biodegradation occurs at different rates in these materials. This could be attributed either to the high chitin content of the crab cuticle or to its mineralogical composition. Chitin and protein biodegradation appear to occur approximately at the same rates (Simon 1992), thus the impact of the mineralogy of the skeletons on weathering processes should be examined. Hydroxyapatite is a more soluble phase in the marine environment than all the carbonate substrates used in this study (e.g. Atlas and Pytkowicz 1977). The S.E.M. pictures show that for all carbonates skeletons studied, the weight loss is probably due to pitting of skeleton and burrowing by microorganisms (Plate 1C & 1D) rather than bulk dissolution. Again, in the case of the fish bones (Plate 2H), there is more evidence for dissolution than biodegradation in anoxic conditions, the regular organic framework of the vertebrae emerging due to skeleton dissolution. Skeletal mineralogy is probably responsible for the greater degree of weathering observed for fish vertebrae than for crab cuticle (although those two skeletons have the same organic matter content before incubation).

The high biodegradation rates of crab and fish skeletons, with high organic content, are particularly obvious in oxic conditions (Figs. 8–9). In the first month of oxic incubation, rapid protein decay occurs, followed by an increase in protein content, probably resulting from colonization of the skeletal substrates by decomposers, as shown in Plate 2I–K. While anoxic biodegradation only leads to dissolution and/or pitting of the surface of the vertebrae, in the oxic environment, fish vertebrae are colonized by numerous aerobic organisms (Plate 2K–L). As a result of colonization and biodegradation, fish vertebrae nearly totally disintegrate after only one year of aerobic biodegradation. Figure 9 shows that even the mineral matrix disappeared. The organic matter would probably be dispersed by abrasion (the skeletons were extremely crumbly after 3 to 6 months or aerobic biodegradation) or ingested by benthic macrofauna, if the skeletons in the experiments were not held in place by the nylon bags. This process happens so rapidly (within one year) that there is only a small probability that these skeletons would be buried into deeper anoxic sediment in most environments with typically small sedimentation rates. Nevertheless, if bioturbation (e.g. Aller 1982) did actively transport skeletons with high organic content into deeper anoxic sediments, anoxic biodegradation would take place, as shown in Figs. 8 and 9. This may explain why vertebrate and crustacean skeletons, in comparison to mollusk shells and sea urchins, are rarely preserved as fossils in sediments.

c) Why is anoxic organic matter biodegradation slower than oxic? Most previous work suggest that the slower rate under anoxic conditions results from the degradation of labile organic matter in the oxic zone. Indeed, a skeleton will usually undergo oxic weathering before being buried into the anoxic zone of the sediment. As a result of oxic weathering of labile organic

matter, only refractory compounds generally reach the anoxic zone of the sediment (Henrichs & Reenirgj 1987), leading to an apparently lower efficiency of the anoxic bacteria in degrading organic matter. In our experiments, however, we placed the same skeletons in oxic and anoxic environments. Another limitation is that anoxic reactions involving organic matter degradation are thermodynamically less efficient than oxic decomposition (e.g. Froelich et al. 1979; Demaison & Moore 1980; Aller 1982; Reeburgh 1983; Hamilton 1984). Interpretation of the S.E.M. observations provide some additional arguments. Plate 1C–E confirms the presence of fungi (Johnson 1976; May & Perkins 1979) and diatoms (May & Perkins 1979; Stal & Krumbein 1984) in anoxic conditions. The morphological results given in Table 7 show that both size and number of groups of organisms observed in oxic environments are larger than those seen under anoxic conditions. Also, macrofauna are usually absent from anoxic zones (Fenchel & Riedl 1979; Degens & Mopper 1976; Berner 1980; Demaison & Moore 1980), and meiofauna less abundant than in oxic zone (de Bovée 1975; Fox & Powell 1987; Meyers et al. 1988). The grazing of the microorganisms by predators, which tends to maintain the microorganisms in their growth phase of development, is thus restricted in the anoxic environment. As a result of the absence of macrofauna in the anoxic environment, the process of bioturbation is limited in this zone; only bioturbation processes due to microorganisms (Östlund et al. 1989) and meiofauna are important in the anoxic environment. The process of bioturbation is assumed to enhance organic matter biodegradation rates (Berner 1980).

Our S.E.M. analysis also showed that the larger oxic microorganisms (data summarized in Table 7) colonize and destroy the whole skeletons, while smaller anoxic microorganisms only disorganize the edge of the skeletons. It is possible that the slower biodegradation rates observed in the anoxic zone result also in the smaller size of the anaerobic microorganisms, even though the smaller organisms usually have a higher metabolism. The weathering activity of the anoxic microorganisms on the skeleton structure is in the micrometer range, while most oxic decomposers are at least 20 times larger, and their impact on skeletal disintegration rates is greater.

The calculation of the rate constants, the comparison between weathering in temperate and tropical waters and the efficiency of the organic carbon burial will be discussed in another publication.

Biodegradation of skeletal structures in an estuary (Site b)

Estuarine environments are known to have large primary productivity and abundance of decomposing organisms (Aston 1978; van Es & Laane 1982; Jørgensen & Sørensen 1985). Non-mineralized skeletal structures show rapid decay in estuarine environments (Hillman et al. 1989; Gooday et al. 1991). On the contrary, mineralized skeletal structures placed in anoxic estuarine conditions show a lesser degree of biodegradation than those in shallow-water

environments (Figs. 4–9). Moreover, our evaluation of enzymatic activity (Fig. 11, below) indicates very low values for skeleton materials and higher values for the interstitial waters of anoxic jars incubated under estuarine conditions (site B). The same analysis performed in the anoxic marine environment (Fig. 11, top) showed high hydrolytic activities on the skeletons and low activities in the interstitial water. This may indicate that bacterial biodegradation activity in the estuarine case is associated with another type of substrate. Estuaries are rich in dissolved organic matter (D.O.M.; e.g. Aston 1978; Berner 1982; Skyring 1987). Decomposing microorganisms appear to use readily accessible D.O.M. instead of the organic matter masked and difficult to get at in skeletal structures. The relative preservation of mineralized skeletal structures is also enhanced by the high sedimentation rates occurring in estuaries (Drake 1976), which lead to rapid burial of skeletal structures and removal from the zone of maximum bacterial activity.

Conclusions

In shallow-marine oxic waters, aerobic biodegradation seems to occur at a faster rate than biodegradation in anoxic regimes. This study showed that in oxic conditions the biodegradation rate is particularly high for skeletal structures with high organic content, in which most biodegradation appears to be complete within about one year. This period is shorter than the burial rate of particulate material in most sediments. Thus, the probability of burial of these skeletons with high organic content in the deeper anoxic zone of the sediment is small. For skeletons with lower organic content (mollusk shells and sea urchin skeletal plates), oxic biodegradation will normally be followed by biodegradation after burial in the anoxic zone. Among other reasons, it is likely that anoxic biodegradation processes are slower than oxic, because of the smaller size of anaerobic microorganisms. In estuaries, an abundance of readily accessible organic matter, particularly dissolved organic carbon, enables decomposers to use efficiently this non-mineralized organic matter as an energy source. Therefore, the organic matter of skeletons, which is masked by mineral matter and energetically less efficient for decomposers, is little decomposed, and preservation of the inorganic skeletal matter is enhanced in these sediments.

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