

## Chapter 11

# Chitin Biodegradation in Marine Environments

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After cellulose and lignin, chitin is surely one of the most abundant biopolymers in the biosphere. However the amounts of chitin actually produced and accumulated in different ecosystems are difficult to evaluate accurately: according to Gooday (1), "*the annual production of chitin is enormous, but just how enormous is difficult to say*". A reasonable estimation for both annual production and the steady state amount of chitin in the biosphere is of the order of  $10^{12}$  to  $10^{14}$  kilograms (1-3). The estimated high biomass and production of chitin in marine ecosystems, together with its relatively recent commercial applications (for reviews see 4-10), led to extensive studies of this biopolymer for the last twenty years.

This polysaccharide is known to be one of the most decay resistant of all biomacromolecules. It is generally firmly associated with proteins *via* different kinds of cross-linkages (e.g. catechol and quinonic sclerotization, histidyl links, disulfur bridges; 11, 12) and the chitin-protein complexes are often mineralized (calcium carbonate with different mineralogies, silicate, etc.). These associations determine the rates and pathways of degradation in natural environments.

From an ecological point of view, the chitin biopolymer plays a key role in many biogeochemical studies as it contains both N and C, and so forms a link between the two most important cycles: rates of chitin production and biodegradation will affect C and N stocks and availability. Moreover, the high chelating capacities of this polysaccharide (in its native and deacetylated form, chitosan) affect the distribution and transfer of heavy metals and other elements through the whole water column and in surficial sediments (6, 13, 14).

### Chitin in Marine Environment: Distribution and Abundance

Chitin biosynthesis appears to be a primitive property of the eukaryotic cell (15-17). Different types of unicellular organisms produce chitin: diatoms (18-20), Rhizopoda, Foraminifera, Cnidosporidia, ciliates, and others (21-25). Yeasts, molds and fungi are

also well-known chitin producers (26, 27). This biosynthetic capacity is completely lacking in prokaryotic Archaea, bacteria and Cyanobacteria.

Chitin is widely distributed in the animal kingdom as a supporting molecule in skeletal structures (e.g. tubes, cuticles, shells, eggs; 15-18), mainly of epidermal origin. Chitin is used by many diploblastic organisms like cnidarians (mainly Hydrozoa but not by corals) and, in the protostomian lineage, triploblastic classes as bryozoans, brachiopods, nematodes, mollusks, pogonophorans, vestimentiferans, polychaete worms and, of course, arthropods. The main exceptions are platyhelminths and nemerteans. On the other hand, in the deuterostomian lineage (echinoderms, Enteropneusta, Pterobranchia, urochordates, ... that supposedly leads to vertebrates) chitin is absent, except in tunicates (peritrophic membrane, 28), and in the cuticular layer of a fish fin (29, 30).

Three different crystallographic structures were described in chitinous material differing in the number of chains in the unit cell and their organization. In  $\beta$ -chitin, each crystallite contains only one chitin chain, all chains running parallel. In the  $\alpha$ -chitin, there are two chains per unit cell, running in antiparallel directions. In  $\gamma$ -chitin (the existence of which is subject to debate), the crystallite should be formed by three chains, the central one running antiparallel between the two adjacent ones (16, 31). The distribution of the three crystallographic forms does not appear to be taxonomically related and the three forms can even coexist within the same organism as in the squid *Loligo* (beak in  $\alpha$ -chitin, pen with  $\beta$ -chitin and stomach cuticle in  $\gamma$ -chitin) or the brachiopod *Lingula* (16). According to Rudall (31),  $\beta$ -chitin and  $\gamma$ -chitin are associated with collagen-like structures or with neighboring collagen-secreting tissues, while  $\alpha$ -chitin replaces collagen-like structures.

The various chitinous structural tissues show striking differences in macromorphology, fine structure, chemical composition and physical characteristics. In living systems, chitin occurs in most cases in the form of microfibrils 2.5 to 3.0 nm in diameter, clearly demonstrated recently by electron microscopists. 18 to 25 individual molecular chains are arranged in two or three rows to form a single crystalline rod, with proteins bound at the periphery (32-35). In arthropod cuticles, the chitin-protein supramolecular organization first highlighted the presence of twisted liquid crystalline orders in biological structure (35). This model was subsequently extended to other biological polymers. The chitinprotein fibers appear as fiber reinforced composites. The matrix is an ordered set of crystalline chitin fibrils surrounded by sheaths of ordered proteins (35, 36). In arthropod cuticles, for example, the chitinprotein fibrils are disposed horizontally and parallel in successive planes. However, the direction of the main fibril axis rotates continuously from one plane to the following one. This constitutes the "twisted plywood model" of cuticular structure described by Bouligand (37, 38). The pseudohelicoidal structures of the organic matrix of cuticles are organized as cholesteric liquid crystals. Since this description, many publications confirmed this analogy between the three-dimensional organization of extracellular fibrillar material in several plant (cell walls) and animal phyla (vertebrate bone, fish scales, egg membranes, annelid, Pogonophora and Vestimentifera tubes, and others) and that of molecules in liquid crystals (35, 39). In mollusks, the rather less organized chitinprotein fibrils act as a structural skeleton on

which other structural and calcifying proteins are arranged (40). In the newly discovered worms Vestimentifera from the deep-sea hydrothermal vents, tubes are made of  $\beta$ -chitin with high crystallinity and proteins without mineralization (like in Pogonophora), but the crystallite has unusually large microfibrils, 50 nm in section and 3400 nm length, and atypical fibrillar structure (41). The latter form a liquid crystal-like structure, differing from the cholesteric arrangement, merely a nematic texture with random twist inversion (42). Large microfibrils are also observed in fungi, 9 to 27 nm in diameter and up to 1  $\mu\text{m}$  length.

The chitinoprotein fibrils of the organic matrix leave space for minerals but there is no apparent relationship between chitin content and degree of mineralization (except in gastropod operculi, 43), hardness or flexibility of the structure. These last properties mainly depend on the protein composition and abundance, but principally on the nature of stabilization forces involved (hydrogen bonded or Van der Waals interchain regular interactions, covalent bridging, quinonic sclerotization, disulfur bridges, etc).

### Distribution and Production of Chitin in Benthic Biocoenoses and Sediments

**Rocky Benthic Substrates.** Jeuniaux and collaborators studied the biomass and production of chitin by benthic communities on rocky substrates in the Bay of Calvi (Corsica, Mediterranean Sea; 2). Chitin biomass in infralittoral communities of photophilous algae (characterized by several species of *Cystoseira* between 5 and 30 m depth), sampled by diving, was principally due to sessile colonies of Bryozoa and Hydrozoa and to agile species of crustaceans on the other hand. The whole benthic biological cover contributed to a mean chitin biomass close to 1 g  $\text{m}^{-2}$ . In sciaphilous communities, in semi-dark caves or below overhanging rocks, the chitin biomass due to encrusting colonies of Hydrozoa and Bryozoa was significantly lower, but the contribution of large crustaceans, while highly variable, was sometimes very important. The mean chitin biomass value was tentatively estimated at 1.4 g  $\text{m}^{-2}$  (44-46).

The rate of chitin production by infralittoral communities living on rocky shores was estimated by measuring the amount of chitin accumulated after pioneering communities allowed to settle and to grow on naked substrates immersed at different depths (6 to 37 m) in natural conditions. One must assume that, in this case, predation and mortality are negligible. The species composition of the biological cover on the plates was, after a few months, roughly similar to that of neighboring benthic communities. The results (2) showed that during the first year, the pioneering communities developed and chitin production was low (ca 0.3 g  $\text{m}^{-2}$  year $^{-1}$ ); it was quicker during the second year (ca 1 g  $\text{m}^{-2}$  year $^{-1}$ ). Chitin production appeared to be lower at greater depths (750 mg  $\text{m}^{-2}$  around 19-30 m), compared to a shallower experimental sites (1200 to 1400 mg  $\text{m}^{-2}$  from 5 to 11 m,) (2, 45). In a *Posidonia* meadow, the main chitin producers are still Hydrozoa and Bryozoa: chitin production by the epiphytic biological cover increased from 0.5 mg  $\text{m}^{-2}$  of the leaf surface during spring and summer, to 1.3 mg  $\text{m}^{-2}$  during autumn and winter. Thus annual chitin production in the meadow of Calvi Bay was estimated at 75 mg  $\text{m}^{-2}$  year $^{-1}$  (44).

We have already noted that crustaceans are the main chitin producers in infralittoral benthic communities growing on rocky substrate (2). However, large decapod species inhabiting crevices (e.g. lobsters, crabs) were not really taken into account in these estimations. The importance of these crustaceans as chitin producers is obvious, owing to the part occupied by crabs and lobsters in fisheries and canning industries. Some authors (47) estimated that approximately 39000 tons of chitin is the amount available yearly as wastes of fisheries. Although, the specific productivity studies of natural populations are scarce, it was possible to calculate the annual production of chitin in a population of lobsters ( $1.5 \text{ g m}^{-2} \text{ yr}^{-1}$ ) based on a natural population of the spiny lobster *Panulirus homarus* inhabiting a small isolated reef of the Natal Coast (South Africa) (2, 48). Obtained value concerning a single species is on the same order as the estimations of chitin production obtained so far for the whole pioneering benthic communities growing on naked substrates.

**Sediment.** In order to evaluate chitin biomass, Poulichek (49) screened one hundred marine sediments of various origins. The chitin biomass of marine sediments is very variable, from 2 up to  $2800 \mu\text{g g}^{-1}$  of decalcified sediment. Most sediments have a low or very low chitin biomass content (67 % below  $100 \mu\text{g g}^{-1}$  of decalcified sediment). No significant difference related to depth nor climatic influence was observed, except that all sediments richer in chitin (above  $500 \mu\text{g g}^{-1}$  of decalcified sediment) are on the continental shelf (less than 200 m depth). Moreover, the chitin content is higher in coarse, much calcified sediments of organoclastic origin with Bryozoa, and shelly sands and gravels being the richest.

To our knowledge, no data on production of chitin in sediment are available. Nevertheless, considering potential "rain" of chitinous material arising from benthic communities or from plankton and settling suspended matter, the very low chitin content of most sedimentary environments provides an evidence for rapid degradation of this biopolymer in an open water. The latter process occurs via the microbial loop, and/or at the sedimentary interface, or in the surficial layers of sediments.

### Distribution and Production of Chitin in Open Water

**Planktonic Biocenoses.** The biomass and production of chitin by typical oceanic zooplankton was estimated in the bay of Calvi (Corsica) in the Mediterranean Sea (50, 51). Plankton was sampled and analyzed during two annual cycles (50) and the results expressed with respect to a square meter of surface water for a water column of 100 m depth. The zooplankton biomass was dominated by five species of copepods (belonging to the genus *Acartia*, *Calanus*, *Clausocalanus*, *Centropages* and *Oithona*: more than 90 % of total biomass) and by cladocera. The daily production of every species was estimated by the "cumulative growth method" of Winberge (52) adapted for species with several larval instars during the life span. Chitin content (expressed in % of dry weight) was found to be relatively constant during larval development and in adults of the same species. However, variations were noted between species, from 3.1 % in *Clausocalanus* spp. and 8.6 % in *Acartia clausi* to as much as 12.2 % in the dominant cladoceran *Evdadne* spp. (2, 3, 51). The mean daily

chitin production by planktonic crustaceans (copepods, cladocerans and decapod larvae) was calculated. Seasonal variations are important, with a maximum production of  $20 \text{ mg m}^{-2} \text{ day}^{-1}$  during the spring bloom in May, corresponding to a chitin biomass around  $400 \text{ mg m}^{-2}$ . Mean annual chitin production was estimated at approximately  $1.0 \text{ g m}^{-2} \text{ yr}^{-1}$ , for a mean chitin biomass of  $26 \text{ mg m}^{-2}$  (2, 3, 51). The values calculated for chitin production must be considered as minimum ones, as exuviae and peritrophic membranes were not taken into account (although it has been shown that part of the chitin of the cuticle is hydrolyzed and recovered by the molting animal before ecdysis).

Other data on chitin production by plankton are very scarce and merely concern fresh water environments, however, the some estimates (2), appear to suggest the biomass and production values of the same order of magnitude as those obtained for marine zooplankton.

**Krill.** The role played by euphausiids in the production of chitin must be considered carefully in the present review, as krill is regarded as one of the most important components of the marine pelagic food web. Recent estimates of annual production of Antarctic krill varied from 16 to  $1350 \text{ } 10^6 \text{ tons per year}$  (53) or, more likely, from 100 to  $500 \text{ } 10^6 \text{ tons (wet weight) per year}$  (54). Jeuniaux and collaborators estimated chitin production by krill using results of exhaustive studies of populations in the North Atlantic and South Pacific oceans. Following Lindley (55), the chitin biomass of krill would be highest in the Norwegian Sea ( $0.6 \text{ mg m}^{-3}$ , principally *Meganyctiphanes norvegicus*), whereas in other parts of the North Sea and North Atlantic Ocean, chitin biomass of krill varies from 0.2 to  $0.4 \text{ mg m}^{-3}$ . Thus, the chitin production values (without exuviae) range from 0.2 to  $0.3 \text{ mg m}^{-3} \text{ yr}^{-1}$  in most ocean areas, to more than  $0.9 \text{ mg m}^{-3} \text{ yr}^{-1}$  in the North Sea and near the Atlantic. If the scattered vertical distribution of krill "swarms" is taken into account, chitin biomass and production in a water column of 100 m depth would vary respectively from 10 to  $30 \text{ mg m}^{-2}$  (biomass) and from 10 to  $40 \text{ mg m}^{-2} \text{ yr}^{-1}$  (production). The latter values are lower than those obtained for zooplankton in the Mediterranean Sea, but the calculated values for krill are probably underestimated (i.e. without exuviae being taken into account). The data of Ritz and Hosie (56) on a krill community in the South Pacific dominated by *Nyctiphanes australis*, are more reliable as they include the production of exuviae. For a mean annual chitin biomass of  $0.4 \text{ mg m}^{-3}$ , the total chitin production by exuviae was estimated to be  $16 \text{ mg m}^{-3} \text{ yr}^{-1}$ . After extrapolation for a water column of 100 m depth, a chitin biomass of  $20 \text{ mg m}^{-2}$  and a chitin production of about  $800 \text{ mg m}^{-2} \text{ yr}^{-1}$  was estimated (2, 3).

The total chitin production (calculated for adults + exuviae) would be  $1.1 \text{ g m}^{-2} \text{ yr}^{-1}$  (values overestimated according to 57). Other calculated values for *Euphausia superba* are in the range of 100 to  $500 \text{ mg m}^{-2} \text{ year}^{-1}$  (3). These values more nearly approach the  $1.0 \text{ g m}^{-2} \text{ yr}^{-1}$  obtained for the Mediterranean zooplankton.

**Particulate Organic Matter (POM).** Although the general and elementary composition of particulates has been widely reported, their chemistry has not been thoroughly analyzed. The difficulty in collecting sufficient amount of material for

such studies is limiting factor. Suspended material in open water is composed of both organic (from less than 1 % to more than 95 %) and inorganic components, with wide variability in composition (58, 59, 60). The input of detrital chitin to the open waters is certainly very high with the presence of fragments of planktonic crustacean cuticles, exuviae, and fecal pellets with chitinous peritrophic membranes, diatoms, and other contributors (58, 59) Figures 8-11.

Nevertheless, a thorough study of detrital chitin distribution in the North Sea reveals a very low chitin content in suspended matter measured during several cruises (Table I) and, thus, indirectly indicates that chitin degrades at a high rate (61).

**Table I Distribution of detrital particulate chitin in the suspended matter of the North Sea (61, 62).**

Season	Chitin ( $\mu\text{g/l}$ ) Mean [extr. values] (% org. matter)	Total POM ( $\text{mg/l}$ ) Mean [extr. values]	Organic compounds ( $\mu\text{g/l}$ ) mean [extr. values] (% total susp. matter)
<b>Winter</b>	1.7 [0.3 - 4.8]; 1.9	5.4 [0.5 - 30.0]	90.1 [19.0 - 305.0]; 1.7
<b>Spring</b>	4.9 [0.9 - 26.1]; 3.0	6.2 [1.3 - 24.8]	164.9 [27.5 - 669.3]; 2.7
<b>Summer</b>	3.1 [0.8 - 11.7]; 2.2	5.1 [0.7 - 19.9]	141.5 [20.6 - 387.1]; 2.8
<b>Autumn</b>	1.6 [0.2 - 6.6]; 1.7	2.5 [0.3 - 21.0]	90.8 [18.8 - 226.4]; 3.6

The concentration of detrital particulate chitin in January was the highest ( $\mu\text{g l}^{-1}$ ) in southern and eastern parts of the North Sea, and lower in the northern part, and in the Skagerrak (61, 62). The proportion of chitin in suspended matter ( $\mu\text{g mg}^{-1}$ ) showed the reverse trend (same tendency in the distribution of the content of the whole organic matter in the suspended material determined by ashing and with the distribution of particulate organic carbon (POC)). In March, the chitin content of suspended particles was much higher than in January, with the highest concentrations in the Skagerrak and along the eastern side of the North Sea (61, 62). This indirectly reflects the distribution of primary production, which begins earlier in the southern and the eastern North Sea than in the northern part. Distribution patterns in October were similar to those in January, but not as clear; July displayed intermediate values.

Chitin concentrations are generally very low compared to those of lipids and proteins. Proportions of proteins in the North Sea suspensions (70-85 % of the total organic matter) are always higher than those of other compounds, such as lipids (13-25 %) and chitin (2-3 %) (61, 62).

Other data on chitin distribution in suspended particles are scarce. Chitin concentration estimated near the mouth of the Delaware Bay (estuarine conditions) ranged from 4 to  $21 \mu\text{g l}^{-1}$  (63), very similar to the data obtained for the North Sea samples close to the mouth of Schelde river (61). The decrease in chitin concentrations from 21 to  $4 \mu\text{g l}^{-1}$  reflects the seasonal decline in primary production in Delaware Bay. Concentration of chitin in both, the Delaware Bay and the North Sea are also similar to those of the subarctic Pacific (Gulf of Alaska, 50°N, 150°W; 4 to  $10 \mu\text{g l}^{-1}$ ; 63), and to north occidental Mediterranean Sea ( $< 5 \mu\text{g l}^{-1}$ ; 58, 59).

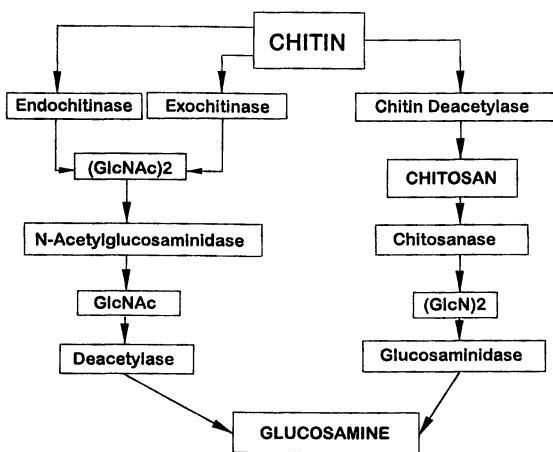


Figure 1. Alternative pathways of chitin biodegradation in marine environments.  
Note: GlcNAc: acetyl glucosamine;  $(\text{GlcNAc})_2$ : chitobiose;  $(\text{GlcN})_2$ : dimer of glucosamine.

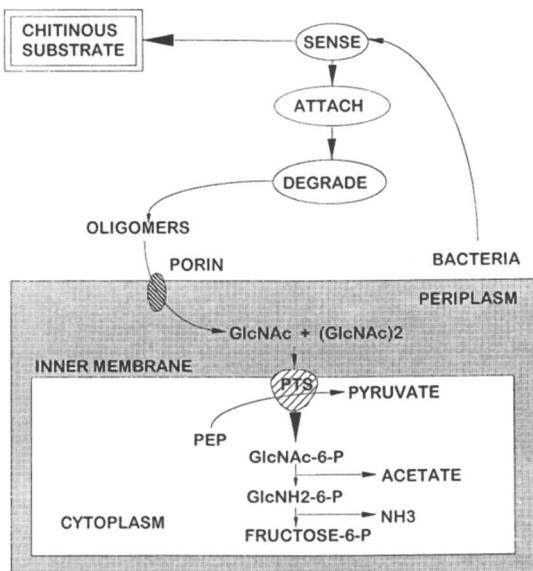


Figure 2. Chitin biodegradation processes by *Vibrio* (adapted and transformed from 127). Note: GlcNAc: acetyl glucosamine;  $(\text{GlcNAc})_2$ : chitobiose; GlcNAc-6-P: acetyl glucosamine-6-phosphate; GlcNH<sub>2</sub>-6-P: glucosamine-6-phosphate; PEP: phosphoenol pyruvate; PTS: phosphoenolpyruvate phosphotransferase system.

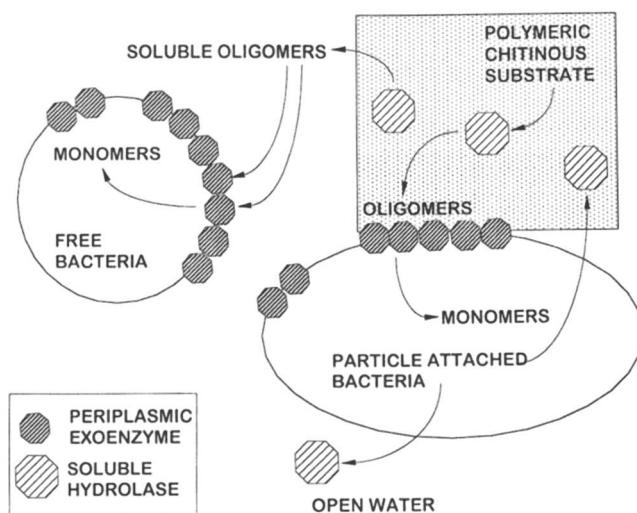


Figure 3. Conceptual model of cooperation between free and attached bacteria for organic polymer biodegradation (adapted from 60).

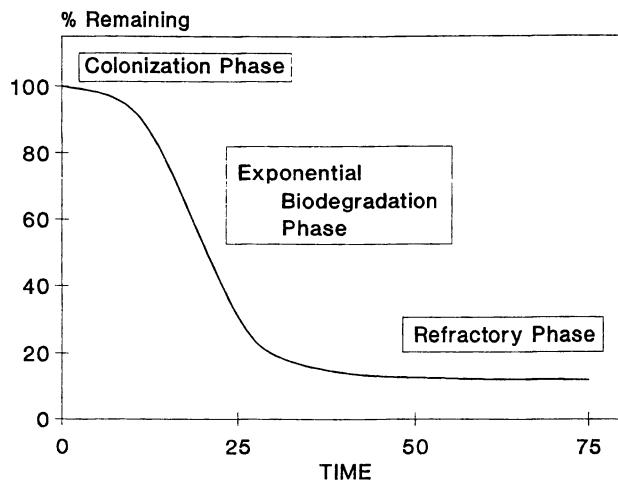


Figure 4. Chitin biodegradation kinetic curve according to our conceptual model.

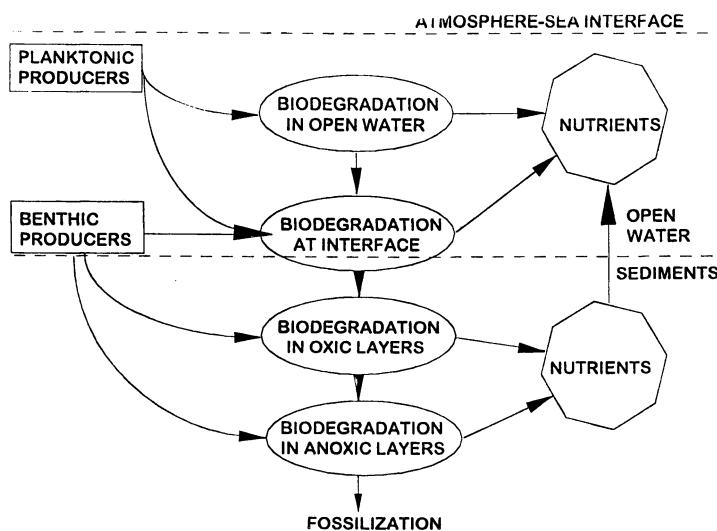


Figure 5. Box model displaying main interrelations between different compartments in marine environment

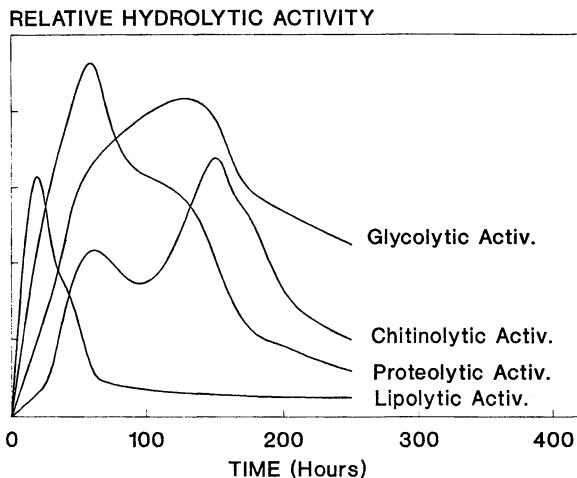


Figure 6. Succession of hydrolytic activities on experimental detritic material suspended in open water.

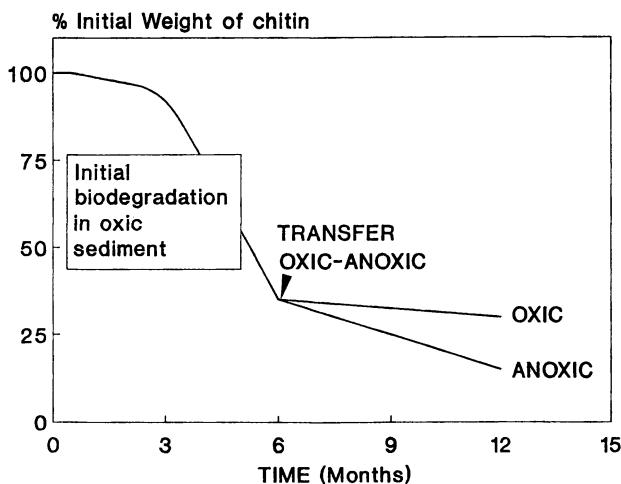


Figure 7. Transfer experiment between oxic and anoxic layers during chitin biodegradation

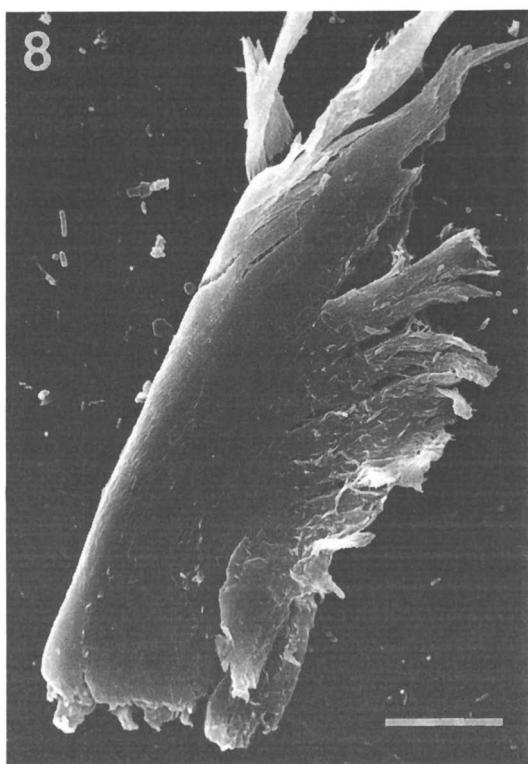


Figure 8. "Fresh" cuticular piece of zooplanktonic organism in the particulate organic matter (POM) collected at 75 m depth in Mediterranean Sea. Scanning Electron Microscopy (SEM) scale is 5  $\mu\text{m}$ .

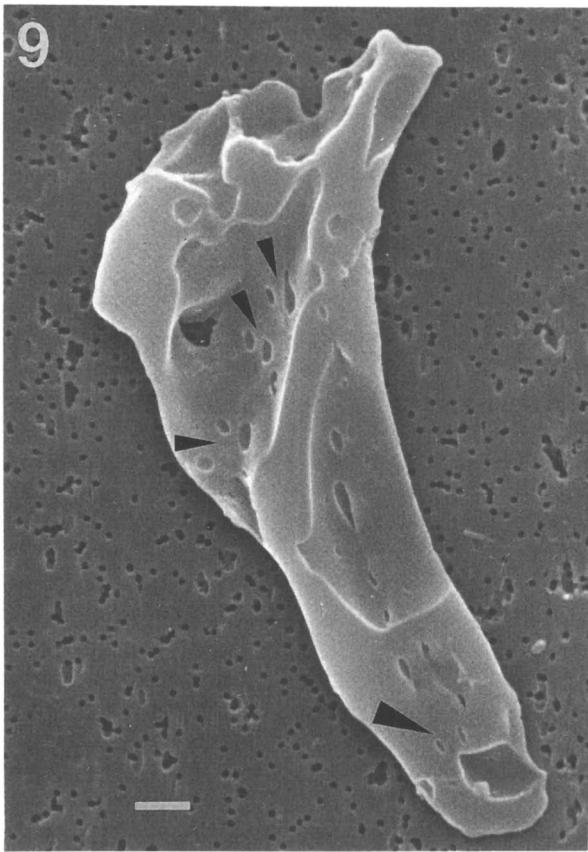


Figure 9. Weathered cuticular piece of zooplanktonic organism in the particulate organic matter (POM) collected at 675 m depth in Mediterranean Sea. SEM scale is 1  $\mu\text{m}$ . Observe "smooth" shape and bacterial pitting of the surface (arrows).

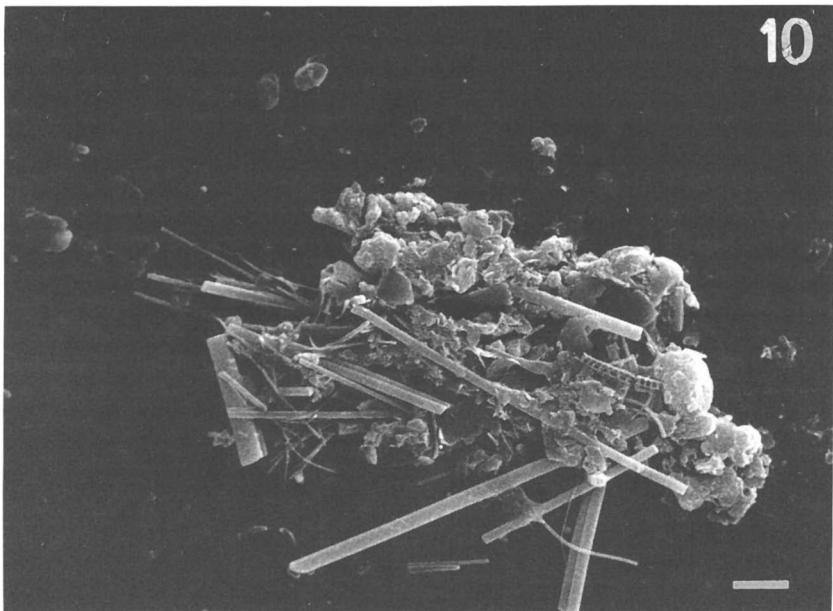


Figure 10. Partly disaggregated copepod fecal pellet without peritrophic membrane collected at 250 m depth in Mediterranean Sea. SEM scale is 10  $\mu\text{m}$ .

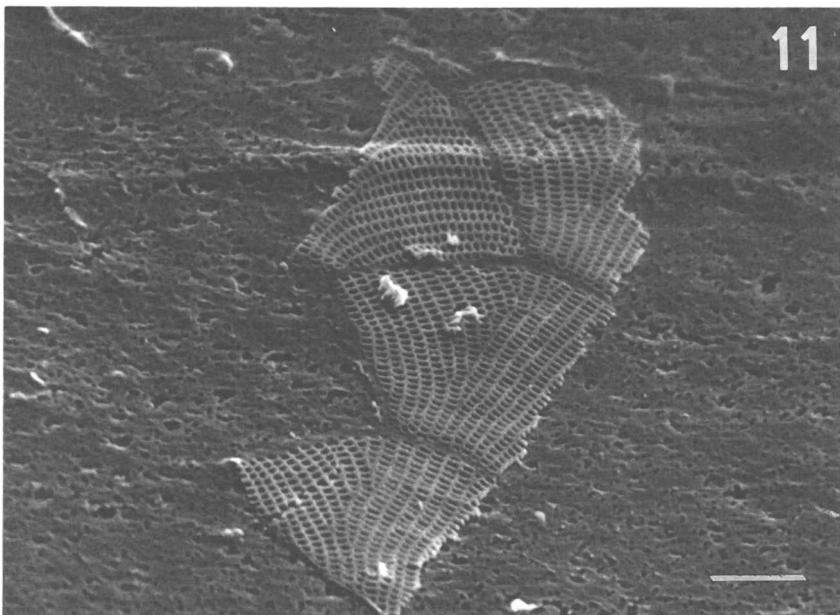


Figure 11. Isolated peritrophic membrane collected at 675 m depth in Mediterranean Sea. SEM scale is 1  $\mu\text{m}$ .

Assuming refractory character of chitin, its concentration would be expected to be highest in the Delaware Bay estuary and the eutrophic North Sea and lower in subarctic Pacific or mesopelagic samples of the Mediterranean Sea due to the higher production level in the former. However, heterotrophic bacteria (60, 61), readily use chitin like other compounds such as dissolved free amino acids or hexoses. The concentration of these compounds in the highly productive Delaware Bay and North Sea are similar to that found in the less productive subarctic Pacific (60, 61, 64-66).

Vertical fluxes of chitin associated with settling suspended matter were also measured in sediment trap experiments (63). The large decrease in chitin flux in the subarctic Pacific, from  $11.2 \text{ mg m}^{-2} \text{ day}^{-1}$  at 10 m to  $1.5 \text{ mg m}^{-2} \text{ day}^{-1}$  at 70 m supports rapid degradation of chitin. Chitin flux remained almost constant at about 1.3 to  $1.8 \text{ mg m}^{-2} \text{ day}^{-1}$  between 70 m and 500 m depth. The C flux decreased similarly with depth. The vertical flux of chitin in the Gulf of Alaska was 0.5 to 1.1 % of the total C and N flux (63). Muller et al. (67) observed that the percent of total C flux contributed by glucosamine was greater in deep (3.6 %) than in the surface waters (1.9 %).

In Table II, we use data of Honjo and coworkers (68) to calculate chitin fluxes in the deep sea (results from PARFLUX experiment with five sediment trap arrays deployed at four deep ocean stations: Söhm abyssal plain (Sargasso Sea), Demerara abyssal plain (tropical Atlantic), East Hawaii abyssal plain, and north central Panama Basin). We used mean proportions of chitin in particulate organic matter estimated in different settings (58-61, 63) to estimate the flux ranges. The total mass flux in these experiments was relatively constant below a depth of 1000 m, except in the Panama Basin, where it increases linearly (Table II). Biogenic materials are very abundant and accounted for 60 to 90 % of the material collected in sediment traps (68). There is a general trend of increase proportion of lithogenic material with depth: the main biogenic particles are fecal pellets, crustacean cuticle exuviae and fragments, amorphous aggregates including free cells, fragments, pigmented granules ("olive cells" of authors) and "waxy" particles. This composition is very similar to that found in deep Mediterranean samples (58, 59).

Calculated chitin fluxes are much higher ( $6\text{-}280 \mu\text{g m}^{-2} \text{ day}^{-1}$ ) than in other settings (except Hawaii) and appear frequently to stay at high levels, even in very deep stations. Nevertheless, all investigations (58-63, 67, 68) indicate that the chitin flux associated with settling particulate matter is relatively small ( $< 0.3 \text{ mg m}^{-2} \text{ day}^{-1}$ ). The material associated with the chitin (e.g. protein and carbohydrates in fecal pellets) may still be a large fraction of the total C and N flux. Chitinolytic marine bacteria associated with settling POM, in superficial down to mesopelagic layers (58, 59), could degrade and utilize the portions of the chitin fibrils that are readily susceptible to hydrolysis (13, 60, 69, 70). The relatively undegraded chitin that sinks lower to the ocean depths (below the most productive, mixed area, 58-59) may be heavily cross-linked with sclerotized proteins which limits further degradation by bacterial exohydrolytic activity. This may explain the large turnover times ( $> 2000$  days for the detritic chitin pool below the mixed layer, 63).

**Table II Flux of suspended material in four deep stations as recorded in sediment traps experiments (PARFLUX data recalculated according to 68)**

Localization	Depth m	Mass flux mg/m <sup>2</sup> /day	Biogenic material % total flux	Organic material % tot. flux	Chitin (*) µg/m <sup>2</sup> /day
<i>Sargasso Sea (Söhm abyss. plain)</i>	976	19.6	90.1	27.2	27-51
	3694	18.4	84.0	10.8	10-19
	5206	13.0	-	29.6	19-36
<i>Demerara abyssal plain</i>	389	69.4	91.9	19.7	68-128
	988	49.2	83.0	17.4	43-81
	3755	46.4	76.2	10.3	24-45
	5068	47.0	69.3	10.5	25-47
<i>East Hawaii abyssal plain</i>	378	11.4	-	59.5	34-64
	978	7.5	-	16.2	6-11
	2778	17.1	92.6	14.0	12-23
	4280	16.8	91.7	10.7	9-17
	5582	11.1	84.8	13.5	7-13
<i>North Panama Basin</i>	667	114.1	81.1	22.5	128-242
	1268	104.5	80.1	19.2	100-189
	2265	125.1	73.3	17.0	106-200
	2869	158.0	68.7	17.0	134-253
	3769	179.3	58.2	13.6	122-231
	3791	179.6	58.9	16.5	148-280

\* calculated from data in (68)

The sinking rates of individual chitinous particles are too low to contribute significant amounts of organic matter to the deep-sea sediments (Figures 8, 9). Smayda (71) reported sinking rates ranging from < 0.1 m day<sup>-1</sup> up to > 500 m day<sup>-1</sup> for very large and heavy particles, Lännergren (72) recorded a range of 0-9 m day<sup>-1</sup>, and Bienfang (73) found from 0.3-1.7 m day<sup>-1</sup>. A more important route is the sinking of fecal pellets (74-83). Turner (84) determined sinking rates for fecal pellets of the copepod *Pontella* to be 15-150 m day<sup>-1</sup>, and Fowler & Small (85) estimated rate of 240 m day<sup>-1</sup> for euphausiid fecal pellets. The sinking rate of individual fecal pellets was not constant due to their small volume and density, the factors very susceptible to variations in water column micro-structure. Even assuming laboratory conditions and the fact that the plankton in experimental enclosures were fed on unialgal diets rather than a natural mix of phytoplankton cells, values obtained give an indication of the likely rates in the sea. Knauer *et al.* (74) estimated the flux of fecal pellets in the northeastern Pacific to be about 1000 fecal pellets m<sup>-2</sup> day<sup>-1</sup>, whereas Wiebe *et al.* (75) measured a flux of 160 fecal pellets m<sup>-2</sup> day<sup>-1</sup> off the Bahamas. Furthermore, the confirmatory laboratory studies found sinking rates of 50-940 m day<sup>-1</sup> with a mean of 159 m day<sup>-1</sup>. Some fecal pellets therefore sink quickly enough to supply chitin to deep-sea sediments. But even whilst sinking, organic matter is likely to be broken

down by bacterial activity (81-83, 86, 87) (Figure 12). Thus, the amount of organic matter reaching the sediments will be reduced.

Honjo & Roman (83) studied the rate of breakdown of copepod fecal pellets and found that degradation of the peritrophic membrane of the pellet took 3h at 20°C, however, at temperature close to that of the deep ocean (i.e. 5°C), peritrophic membranes remained intact for up to 20 days. After degradation of the membrane, the pellet lost its integrity and broke up into small amorphous aggregates (Figures 10, 11). Therefore, if fecal pellets "survive" bacterial degradation whilst sinking through the warm surface waters, there is a good chance that they could survive to sink further down. Iturriaga (88), studying the bacterial alterations occur in sedimenting organic matter, found that at 20°C dead phytoplankton decomposed at a rate of 35 % day<sup>-1</sup> whereas zooplanktonic remains decomposed at 18 % day<sup>-1</sup>, and at 5°C the rates were lower (about 3 % day<sup>-1</sup> for phytoplankton and 8 % day<sup>-1</sup> for zooplankton). Comparable decomposition rates were recorded by Poulichek and coworkers (69, 70, 90, 91) and Harding (89) who found that killed copepods decomposed within 3 days at 20°C and within 11 days at 4°C. Harding (89) commented that copepods, which die in the surface waters, are unlikely to be recognizable as copepods if caught by a plankton net within one or two days of death.

**Conclusion.** The results considered above allow us to propose a mass balance of chitin production and accumulation in the oceans. Table III summarizes the biomasses and fluxes of chitin.

Assuming chitin production in a pelagic systems to be  $2.3 \times 10^9$  tons per year, and chitin mean biomass in POM as 5 µg l<sup>-1</sup>, then on a global basis, calculated residence time of chitin in the open ocean is 103 days. This value is very close to the 119 days estimated for turnover in cold surface waters (approx. 12°C, surface to depth of 50 m), based on the chitin flux and concentrations of suspended chitin (63). It is also similar to the turnover time of 140 days at 15°C calculated by Seki (92, estimation based on chitin degradation and probable number of chitinolytic bacteria in the water column). Taking into consideration the mean ocean depth (3729 to 3824 meters according to 93), and the time needed for fine particles to settle through the water column, this can explain the low chitin fluxes at intermediate and abyssal depths (6 to 280 µg m<sup>-2</sup> day<sup>-1</sup>), even if shallower fluxes are much more greater (1 to 11 mg m<sup>-2</sup> day<sup>-1</sup>). The very effective biodegradation pathways of chitin and chitinoproteic complexes in the open water environment can only explain the latter phenomena. It also explains the generally low chitin content in the sediments, even where fast sinking fecal pellets, sheltering relatively undegraded chitinous particles, and skeletal pieces of macrofauna (molluscan shells, crustacean cuticles, etc.) are embedded in the sedimentary layers.

### Biochemical Aspects of Chitin Biodegradation

**Chitin Biodegradation Enzymes.** Chitin is degraded by at least two different enzymes (15, 16), i) the endoenzyme chitinase (more precisely defined as poly-β-1,4-(2-acetamido-2-deoxy)-D-glucoside glycanohydrolases, E.N. 3.2.1.14), which splits

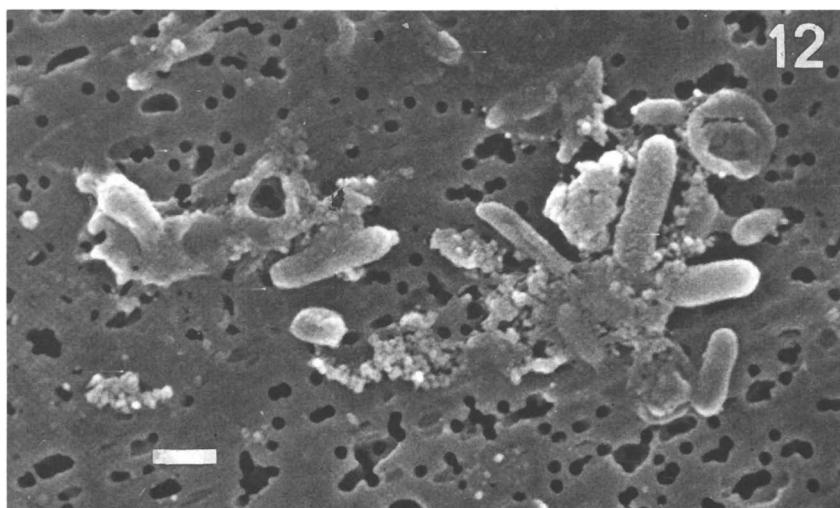


Figure 12. Bacterial clusters associated with small organic particles in the POM collected at 675 m depth in Mediterranean Sea. SEM scale is 1  $\mu\text{m}$ .

**Table III Chitin production and accumulation in a global perspective  
(compiled from 2, 3, 17, 44, 45, 49, 51, 61-63)**

<b>PRODUCTION</b>	<b>Extent</b> $10^6 \text{ km}^2$	<b>Chitin production</b>	
		$\text{g m}^{-2} \text{ year}^{-1}$	$\text{tons } 10^3 \text{ year}^{-1}$
<b>Pelagic systems</b>			
Zooplankton	360	1.0 *	360 000
Krill		5.3 **	1 938 000
Other shrimps		?	?
<b>Benthic systems</b>			
Rocky substrates	1.4		
Epifauna		1.0	1 500
Large Decapods		1.5	2 100
Reefs	1.5	?	?
Continental slope	30	?	?
Sediments	320	?	?
<b>Total</b>	360		<b>&gt;&gt;2 301 600</b>
<b>ACCUMULATION</b>		<b>Chitin biomass</b>	
	$10^6 \text{ km}^2$	$\mu\text{g g}^{-1}$	$\mu\text{g l}^{-1}$
<b>Pelagic system</b>			
MOP	360		1 - 26
<b>Benthic systems</b>			
Sediments	320	2 - 2 800	

\*for a water column, 100 meters depth; \*\*for a water column 500 meters depth

chitin into oligomers, and ii) *N*-acetyl- $\beta$ -D-glucosaminidase (2-acetamido-2-deoxy- $\beta$ -D-glucoside acetamido-deoxygluco hydrolase E.N.3.2.1.30), which degrades these products to monomers (4, 15, 94). Both enzymes are in most cases soluble and generally secreted. The exochitinase activity, splitting the glycosidic bond two residues distant from the non reducing end of each chitin chain, was described by Ohtakara (95, 96), and separated from endochitinase activity by DEAE-cellulose column chromatography (97-99). Endochitinase cleaves bonds randomly along the chitin strand to form loose ends that can be further hydrolyzed by exochitinase (100).

Chitinases comprise families 18 and 19 of glycosyl hydrolases (based on their amino acid sequences, 99). The family 19 is homogeneous and contains only plant enzymes whereas family 18 is more diverse, containing chitinases from plants, fungi, bacteria and viruses. Family 18 includes eukaryotic chitobiases (i.e. *N*-acetyl- $\beta$ -D-glucosaminidase), but prokaryotic enzymes constitute family 20 together with hexosaminidase (98, 99). The isoelectric point and optimum pH of the reactions is usually in the range of about 5.0-5.5 for both enzymes, except in the digestive system of reptiles (15, 16). Their apparent molecular masses are between 40 and 120 kDa for endochitinases, 60 kDa for exochitinases, and around 100 kDa for *N*-acetyl- $\beta$ -D-glucosaminidase. Numerous chitinases have been purified, the corresponding gene

isolated, cloned and the primary and tertiary structures of the enzymes determined (111, 112).

Chitinases are widely distributed hydrolases synthesized by bacteria, fungi, higher plants and digestive glands of animals whose diet includes chitin (15). They allow many plants and animals to perform vital functions such as digestion, growth and defense against parasites and chemical attack. The widespread distribution of endo- and exochitinases and *N*-acetyl- $\beta$ -D-glucosaminidase in bacteria, molds and Protozoa suggests a primitive nature of their function. In animals, diploblastic Metazoa are characterized by both ecto- and endodermic secretion of these enzymes. The ectodermic secretion of *N*-acetyl- $\beta$ -D-glucosaminidase is maintained whereas most Protostomia (except nematodes and, of course, arthropods, generally loses that of endochitinase where it functions in molting (15, 101). The endodermic secretion of endochitinase is preserved in most invertebrates (except for groups with very specialized food containing no chitin). In Deuterostomia, and especially in vertebrates, a marked tendency towards a loss of this function is clearly observed (regressive evolution by so called "enzymapheresis") (102). In fungi, there are three roles attributed to chitinases (103) involving [1] the gross autolysis associated with the release of spores, [2] a nutritional role as in the case of saprophytes (enabling fungi to use insect or other fungal debris as a food source or, for pathogen species, to penetrate their arthropod or fungal host), [3] a morphogenetic role in the growth and differentiation of hyphae. In plants, chitinase is ubiquitous, found in seeds, leaves, stems and roots (104-108), but present in various quantities. *In vitro* studies have shown the ability of plant chitinase to attack and to degrade fungal cell (104). Moreover, the enzyme products found may act as elicitors of phytoalexin formation and thereby contribute to plant defense (109, 110).

Endo- $\beta$ -*N*-acetylmuramidases, more commonly known as lysozymes (mucopeptide *N*-acetyl muramoylhydrolase, E.N. 3.2.1.17), have been isolated from a great variety of sources, animals, plants and microorganisms (4). Endo- $\beta$ -*N*-acetylmuramidase hydrolyses the glycan of the bacterial cell walls to oligosaccharides of *N*-acetylglucosaminyl-*N*-acetylmuramic acid type, while endo- $\beta$ -*N*-acetyl glucosaminidases give saccharides with *N*-acetylglucosamine at the reducing end. However, the ability of lysozyme to degrade chitin has been reported in several studies (4, 113).

**Chitosan Biodegradation Enzymes.** Chitosan is a polymer composed of  $\beta$ -1,4-linked glucosamine residues. It is usually obtained through artificial deacetylation of chitin with concentrated NaOH solution. The occurrence of "natural" chitosan was evidenced by the discovery that it is a major component of cell wall of mucoraceous fungi, formed by concerted action of chitin synthetase and chitin deacetylase (114-116).

A new class of enzymes, chitosanases (E.N. 3.2.1.99) active in hydrolyzing chitosan, was proposed by Monaghan (117). This kind of enzyme is probably widely dispersed (activity found in 25 fungal and 15 bacterial strains out of 200, 118). The molecular weight of chitosanase was estimated to be about 31 kDa, and its isoelectric point at pH 8.3. Optimum pH is 5.6, but the enzyme is stable from pH 4.5 to 7.5. It

is inactivated more quickly at alkaline than at acidic pH. In *Streptomyces*, extracellular chitosanase synthesis is induced by glucosamine. The purified enzyme hydrolyzes chitosan, but not chitin or carboxymethylcellulose (119). Chitin deacetylase (*i.e.* chitin amidohydrolase) has been purified and characterized (120, 121), and cDNA isolated, characterized and sequenced (122). The enzyme (EN.3.5.1.41) is an acidic glycoprotein (30 to 67 % carbohydrate content) of 75 to 150 kDa with very narrow specificity for  $\beta$ -1,4-linked *N*-acetyl-D-glucosamine homopolymers (at least three to four residues) in *Mucor*, but less stringent in *Colletotrichum* (123). The effectiveness of the latter enzyme in deacetylating various chitin substrates is ranked as follows: carboxymethyl chitin>glycol chitin>amorphous chitin>crystalline chitin. Optimum pH for the reaction varies, from around 4.5 to 5.5 in *Mucor* to 8.5 in *Colletotrichum* (123). Three factors can affect the deacetylation process of chitin and chitin derivatives, i) the properties and mode of action of specific strains of deacetylase, ii) the structural properties of chitin (depending on the nature of stabilization forces, and thus the origin of the chitinous material), and iii) the mode of interaction between the enzyme and the chitin molecule, partly under environmental control (123). Chitin alteration is a result of weathering processes, under biological mediation or not, which can modify the conformation of the polymer and, as a result, influence the interaction between chitin and the enzyme and the subsequent deacetylation reaction.

### **Chitin Biodegradation by Microorganisms**

Chitin mineralization is primarily a microbial process, and chitin can act as the sole source of carbon and nitrogen for many microbes (bacteria and fungi). Its degradation is usually assumed to be via the "traditional" pathway, *i.e.* via chitinolysis to *N*-acetylglucosamine. However, the alternative pathways can be defined (124, 125), and, namely the "chitosan pathway" is of ecological importance (Figure 1).

According to the traditional models, chitin is depolymerized by either endo- or exochitinases, generally secreted as soluble, diffusive hydrolases. The action of "endo" hydrolases results in the formation of a variety of oligosaccharides of varying chain length. The endochitinases can rarely act on chains with less than three acetyl glucosamine residues, and the rate of hydrolysis is proportional to the degree of polymerization. Consequently, the result of endocleavage is a mixture of short chain oligosaccharides of which the disaccharides (chitobiose) are dominant. The exochitinase commonly acts after endochitinase has exposed a significant number of chain ends. Its action may result in the direct formation of disaccharides. The oligosaccharides formed are hydrolyzed by an acetylglucosaminidase, freeing acetyl glucosamine monomers that can be directly assimilated by the cells.

The "chitosan pathway" involves a partial or total deacetylation step, where chitin is transformed into chitosan. Chitosan is further hydrolyzed by chitosanase to oligomers of glucosamine. The hydrolysis of these oligomers by glucosaminidase frees glucosamine residues as direct substrates for cells.

**Bacterial Processes.** Chitin turnover is essential for recycling carbon and nitrogen in marine ecosystems. By 1937, ZoBell & Rittenberg (126) had shown that marine chitinovorous bacteria are ubiquitous, very abundant, diverse, widely distributed and able to live in extreme environments such as deep water.

However, how can we understand the process of chitin mineralization by such bacteria? This multi-step process can be subdivided into stages, each of them complex and highly regulated. The bacteria must be able to sense the presence of an adequate substrate, *i.e.* chitin, and/or come into contact with it by active directional swimming or by random collision. Then, they attach to the polymer and degrade it to oligosaccharides, and further into N-acetylglucosamine (or glucosamine via the chitosan pathway), either extracellularly (in the periplasm) or intracellularly. These steps were detailed in the case of *Vibrio furnissii* (127-129). Vibrionaceae are among the commonest marine bacteria, rod shaped Gram negative, flagellated and mobile, facultative anaerobes.

A key step in the chitin biodegradation process is the adhesion of marine bacteria to chitin-containing particulates. *Vibrio* species were therefore surveyed for their ability to bind to immobilized carbohydrates. One strain of *V. furnissii* adhered to glycosides of three sugars, N-acetylglucosamine (the preferred ligand), D-mannose, and D-glucose (127). A single lectin disposed at the tips of fimbriae is responsible for binding to the three sugars. Sugar specific lectins are widely distributed among bacteria and the process of lectin-mediated adhesion to insoluble carbohydrates is considered to play a very important role in marine fouling and biodegradation processes. Schrempf (130) described a lectin (CHB1) secreted by several streptomycete species and specific for  $\alpha$ -chitin, but not binding to  $\beta$ - or  $\gamma$ -chitin nor chitosan. *Vibrio* cells adhering to the chitin analogue divided at the same rate as cells in liquid culture, but the population gradually shifted to a large fraction of free swimming cells, a process that may be necessary for colonization. Metabolic energy is required for cell adhesion to the glycosides. Both the initiation and maintenance of lectin-mediated adhesion requires continuous protein synthesis, and so lectin activity is a major priority of these cells (127). The adhesion/de-adhesion apparatus is apparently used as a nutrient sensorium (to continuously monitor the nutrient status of the environment, 131). In an incomplete medium (or presumably when the environment is unfavorable), cells de-adhere, presumably to migrate to a more favorable environment. This adhesion/de-adhesion behavior of *V. furnissii* (127) probably catalyzes the first step in colonizing chitin.

A second step was shown to be chemotaxis to chitin hydrolysis product (128). *V. furnissii* swarms toward chitin oligomers  $[GlcNAc]_n$ ,  $n=1-6$ , at initial concentrations as low as 10  $\mu\text{mol}$ . Two (or more) independently induced receptors (perhaps the most potent reported for bacteria) with overlapping specificities, recognize  $[GlcNAc]_n$ , with  $n = 2-4$  (128). Expression of the receptor(s) for  $[GlcNAc]_5$  and  $[GlcNAc]_6$  apparently requires special induction conditions (128). The chemotactic response was greatly affected by growth and conditions and the presence of nutrients in the environment. Chemotaxis to the sugars increased 2- to 3-fold when the cells were starved. Nutrients, especially compounds that feed into or are part of the Krebs cycle, were potent inhibitors of taxis to the sugars (128). Since most

of the catabolites are inhibitory, chemotactic behavior is displayed mainly when the Krebs cycle is operating at the low rate, and is inhibited when the cycle functions at high rate. When the environment fulfills the metabolic needs of the bacteria, then the chemotaxis system is either inhibited or not induced. Thus, the adhesion/de-adhesion system and chemotactic behavior both optimize chitin resource utilization by the bacteria (Figure 2).

It is not clear whether and when microbial cells release extracellular (i.e. soluble) enzymes during growth, but it is clear that polymers like chitin, potential substrates used for metabolism, must be hydrolyzed to yield oligosaccharides before they can act as substrates for periplasmic or cytosolic disaccharases (132). The importance of extracellular hydrolysis is evident in the observation that often 80 to 100 % of seawater isolated strains can utilize *N*-acetylglucosamine whereas less than 20 % can hydrolyze chitin. This cooperative chitin degradation is often attributed to different pools of bacteria, free (i.e. open water bacteria) and particles bound (60) (Figure 3).

Some steps in the catabolism of the oligosaccharides are known in *V. furnissii* (129). Acetyl glucosamine (GlcNAc) and its oligomers ( $[GlcNAc]_2$  and  $[GlcNAc]_3$ ) are very rapidly consumed by intact cells. Tetramer ( $[GlcNAc]_4$ ) is utilized somewhat more slowly. During these processes, there is virtually no release of hydrolysis products by the cells (129). The oligosaccharides resulting from random hydrolysis of chitin by extracellular (secreted) exo- or endochitinases enter the periplasmic space (via specific porins ?) and are hydrolyzed by a unique membrane-bound endoenzyme (new enzyme called by the authors chitodextrinase, 129) and an exoenzyme (*N*-acetyl- $\beta$ -glucosaminidase). Chitodextrinase cleaves soluble oligomers (but not chitin) to di- and trisaccharides, while the periplasmic *N*-acetyl- $\beta$ -glucosaminidase hydrolyzes the terminal acetyl glucosamine from the oligomers. The end products in the periplasm, GlcNAc and  $[GlcNAc]_2$  (possibly  $[GlcNAc]_3$ ), are catabolized as following several pathways (129) (Figure 2):

(a) Disaccharide pathway, where a  $[GlcNAc]_2$  permease is apparently expressed by *Vibrio furnissii*. Translocated  $[GlcNAc]_2$  is rapidly hydrolyzed by a soluble cytosolic *N*-acetyl- $\beta$ -glucosaminidase, and the GlcNAc is phosphorylated by an ATP-dependent, constitutive kinase to GlcNAc-6-P.

(b) Monosaccharide pathway, where a periplasmic GlcNAc is taken up by the phosphoenolpyruvate phosphotransferase system, yielding GlcNAc-6-P, the common intermediate for both pathways.

Finally, GlcNAc-6-P generated by both pathways is deacetylated and deaminated, producing fructose-6-P, acetate and NH<sub>3</sub> (Figure 2).

Chitobiose,  $[GlcNAc]_2$  is probably the "true" inducer of the chitin degradative enzymes and, depending on its concentration in the environment, differentially induces the periplasmic and cytosolic *N*-acetyl- $\beta$ -glucosaminidase (129). The disaccharide pathway appears to be the most important when the cells are confronted with low concentrations of the oligomers, but when there is a greater supply of  $[GlcNAc]_2$ , both monosaccharide and disaccharide pathways are used.

**Chitin Susceptibility to Biodegradation Processes.** Highly crystalline  $\beta$ -chitin occurs in the absence of other polymer only in diatoms (132) and this kind of chitin can be hydrolyzed directly. In most cases, however, chitin is associated with other macromolecules that control its reactivity. Chitin and chitosan are associated with other polysaccharides in the fungal cell wall, while chitin is associated with proteins in animals. Therefore, where chitin occurs naturally in association with proteins or other polysaccharides, it might be expected to have properties slightly different from those of isolated chitin, particularly its susceptibility to biodegradation.

The composition and the architecture of the fungal wall are known in several groups of fungi. Chitin is probably a universal component of fungal cell walls (27). The basic pattern of organization of the hyphal walls of most fungi includes an inner layer composed of chitin microfibrils in a  $\beta$ -glucan matrix. The latter matrix also contains proteins and an outer layer of  $\alpha$ -glucan (glucan in the chytrids; chitosan and polyglucuronic acid in the zygomycetes and mannoproteins and glucans in the ascomycetes and basidiomycetes) (133,134). A layer of a  $\beta$ -glucan mucilage may also be present on the outer surface, and treatment with  $\beta$ -glucanase is essential before chitinase can effectively attack the walls. Thus,  $\beta$ -glucan and protein may form the matrix of a fibrillar chitinous layer, which forms the inner compact surface of the wall, but is looser and uneven on the outer side (27). What is quite specific to fungi is that chitin is continuously subjected to various chemical modifications such as deacetylation or covalent linking to glucans and/or proteins (133). The involvement of protein in the cell walls has not been clarified, but protein molecules may link chitin to carbohydrates. The glucan-chitin complex is highly resistant because the glucan chains are linked to chitin through their reducing ends via amino acids, particularly lysine (135, 136). Amino acid analysis shows significant amounts of threonine, serine, aspartic acid and glutamic acid, which may contribute, to the linkages between carbohydrates and protein moieties in the cell walls. Also noteworthy is the high content of alanine and glycine, since both amino acids are involved in the cross-linking of peptidoglycans in bacterial cell walls. Both chitin and  $\beta$ -glucan chains are connected by hydrogen bonds (27, 133, 135). This results in a cross-linked network of  $\beta$ -glucan-chitin complex, which confers rigidity and chemical stability to the cell wall.

There is an experimental evidence of covalent bonding between chitin and proteins in animals, especially in arthropods (4, 137, 138). The covalent proteoglycan is, however, accompanied by other proteins, specifically or non-specifically associated with the former by weaker forces and forming a chitinoproteic complex. *N*-acetylglucosamine and chitin can react with  $\alpha$ -amino acids, peptides and cuticular proteins to give a glycoprotein complex (139). Chitin, whether in its  $\alpha$ - or  $\beta$ -form, is covalently linked to arthropodins or sclerotins in cuticles or other types of proteins in mollusk shells, to form more or less stable glycoproteins. The latter process occurs probably through aspartyl, glutamyl, seryl, glycyl and histidyl residues (usually carboxyl to the amino group of glucosamine but other types of bond may also occur) (140-143). In the internal shell of cephalopods, the greater part of the protein moiety can be removed, while the remaining chitin is bound to a protein rich in aspartic acid (4). Enzymatic studies and acidic hydrolysis of cuticles have also led to the

conclusion that there is a stable linkage between proteins and chitin through a non-aromatic amino acid (144, 145).

Structural proteins from animal exoskeletons are generally more or less stabilized through different kinds of bonding, such as hydrogen, hydrophobic, and ionic bonds, Van der Waals interchain regular interactions, disulfur bridges and sclerotization. The last two stabilization forces have an important consequences on the biodegradation processes.

According to Peter *et al.* (138), very little is known about the molecular mechanism of sclerotization and, despite many efforts to analyze the chemical details of that process, and a number of excellent reviews (145-146), this important process is still a matter of controversy. The sclerotization process in arthropods and mollusks begins in the cuticle or the periostracum by an enzymatic oxidation of a diphenolic substrate (for example hydroquinones, catechol, *N*-acetyl dopamine, DOPA, 146, 147). This oxidation yields the corresponding o-quinone and/or p-quinonemethide (reactive intermediate). According to the results of a model reactions and analyses of cuticular extracts, crosslinking of cuticle proteins then results from Michael type conjugate addition and Schiff's base formation with free peptidic amino groups. Chitin may also be involved in the o-quinone or p-quinonemethide mediated formation of crosslinks, either via unacetylated amino groups or hydroxy oxygen (138). Moreover, non-covalent interactions between oxidation products of the sclerotization agents and polypeptides and/or chitin may contribute to the stability of sclerotized chitinoproteic complexes (136, 138). The resulting chitin-tanned protein complex gains considerable stability as well as hardness, rigidity and resistance to enzymatic hydrolysis.

Disulfur bridges (as in keratin) are generally of a little importance in the stabilization of invertebrate skeletal proteins except in the case of *Halocynthia* tunical cuticle, and in *Vestimentifera*. These deep-water "worms" build dwelling tubes that can reach lengths of one meter in *Riftia*, and some tens of centimeter in *Tevnia*. The tubes are made of a chitinoproteic complex (148-150) of a very high chemical stability, interpreted by authors as the result of strong self-interaction of proteins via numerous sulfur bridges.

The chitin-protein complexes may be complemented by the deposition of other substances, such as waxes and lipoproteins, giving the complex some impermeability. Animals for a number of different functions have extensively exploited such chitinous structures. The chitin-protein framework also provides a structural organic framework around which mineral may be deposited (calcification and silicification) (40, 151).

In our opinion, one of the most important controls on the rate of environmental chitin degradation is the level of chitin accessibility by hydrolytic activities. Chitinous structures almost always consist of a covalently-linked complex of chitin and proteins or glucans. In addition, the proteins themselves can form stable structural bonds (disulfide bonds, sclerotization of chitinoproteic complexes) rendering chitin even less accessible. This explains why some chitinous structures are much more resistant to degradation than others in the same experimental setting.

## Morphological, Ecological and Biogeochemical Aspects of Chitin Biodegradation

**Pathways of Biodegradation.** The process of chitin biodegradation can be conceptually divided into three successive steps (Figure 4).

The first step is the colonization process during which microorganisms settle on the detrital material, multiply and begin degradation. The amount of material biodegraded is almost negligible. The duration of this phase varies from a few hours (in the case of zooplanktonic remains in open water) to some months (mineralized skeletons within sediments such as mollusk shells, echinoid plates, etc.).

The second step involves the most active biodegradation. Many microorganisms adapted to the substrate, weather and mineralize the organic components of the chitinous skeleton. The duration of this phase varies from a few days (zooplanktonic remains in open water or at the water-sediment interface) to several months (mineralized skeletons within the sediments). The rate of chitin (or other compounds) degradation during this phase is generally a negative exponential, of first or second order.

The last step involves the slowing down of the process during which all labile compounds have been mineralized and only refractory molecules or complexes remain. Only very specialized microorganisms can manage to degrade such compounds. This phase last until the material is totally broken down (from some weeks up to several years).

In addition to the above model of degradation, we propose a non linear parametric transition functions that allows direct comparison between several parameters such as different settings, seasons, etc. The logistic curve based upon parameters relating to actual biological phenomena (e.g. microbial growth) is preferred for such comparison where % of chitin remaining in the material (Y) is plotted against time (X) according to the following equation:

$$\text{Chitin remaining} = a + \frac{b}{1 + \exp [-(x-c)/d]}$$

where *a*: transition height=maximum amount of degradable material (before refractory phase begins); *b*: transition center=when maximum degradation rate is supposed to be observed; *c*: transition width calculated as 2.197 *c* (=duration of the exponential degradation phase, after colonization steps and before refractory phase); *d*: shape factor.

Chitin biodegradation processes are quite different in the main compartments of the marine environment (Figure 5).

**Open Water Conditions.** Planktonic communities are especially rich in chitin producers due to the abundance of crustaceans. Through mechanical and biodegradation processes (including ingestion, egestion, digestion processes,

coprorhexia, etc.) (69, 70, 77, 90), this chitinous material is partly incorporated into suspended matter in the oceans.

Poulichek and co-workers designed an *in situ* experimental device to study zooplankton biodegradation in the open water (70, 90, 91, 152-154). Dead organisms are first degraded from inside by autolytic processes (69, 70, 90, 91, 152-154). These processes induce the lysis of most muscles and organs during about 50 hr *post mortem* (at 13 to 22°C in an oligotrophic setting, Figures 13, 14). This autolysis results from the activity of the hydrolytic enzymes of the digestive tract and other organs and of the symbiotic microorganisms in the intestine (69, 70, 153-154). The latter stage occurs 6-20 h *post mortem* and leads to the opening of the cuticle generally along the articular membrane between two sclerites (where the cuticle is less or not hardened by quinone tanning, Figure 13) (70, 89, 154). The heterotrophic microorganisms can invade the corpse through that gap and further degrade it (Figure 14). At 50-100 h *post mortem*, the chitinoproteic layers of the cuticle are deeply altered (Figures 16, 17), so that pieces of appendages (legs, antenna, etc.) are lost (69, 155). These fragments can form macroscopic aggregates often known as "marine snow", falling relatively fast through the water column (69, 90) (Figure 15). The formed aggregate material is degraded during sedimentation by a rich heterotrophic microorganism community (Figures 14, 15) (70, 89, 153, 155-157).

A wide variety of hydrolases have been detected during biodegradation. The importance of each enzymatic activity varies with time (Figure 6) (69, 70, 154). Lipolytic activities, which are mainly autolytic, occur first, culminating after 12-40 h. Proteolytic and glycolytic activities develop next, and culminate 80-120 h *post mortem*. This prepares the cuticles for hydrolysis by chitinolytic activities, that reach a maximum between 60 and 70 h (first squad) and 120 and 180 h *post mortem* (second more functional squad). All mentioned activities are due mainly to the development of successive populations of heterotrophic microorganisms (Figures 14, 15, 17) regulated by ciliates and flagellates (Figure 14). Autolytic processes are negligible after 50 h. The number of active bacterial strains on the experimental material (measured as plate count or microbial ATP estimations) increases according to a logarithmic curve during the first 100-150 h *post mortem* then decreases slowly, reflecting the progressive hydrolysis of metabolizable substrates (70, 153, 154).

The hydrolytic activities described above lead to a rapid decrease of the organic content of the animal remains (69, 70, 154). The overall decrease of the organic content follows the negative exponential kinetic curve. The fate of chitin is essentially similar. After a short delay during which proteins and lipids gradually disappear, the chitin content of the material reduces quickly (maximum variation between 50 and 120 h *post mortem*). After about 150 h, approximately 90% of the initial chitin have disappeared but the chitin remaining at the beginning of the refractory phase is only very slowly mineralized and is likely to settle on the sediment-water interface.

**Biodegradation Processes at the Sediment-Water Interface.** Material of planktonic origin settling onto sediment has generally already undergone extensive biodegradation. However, since most of decomposing microorganisms remains

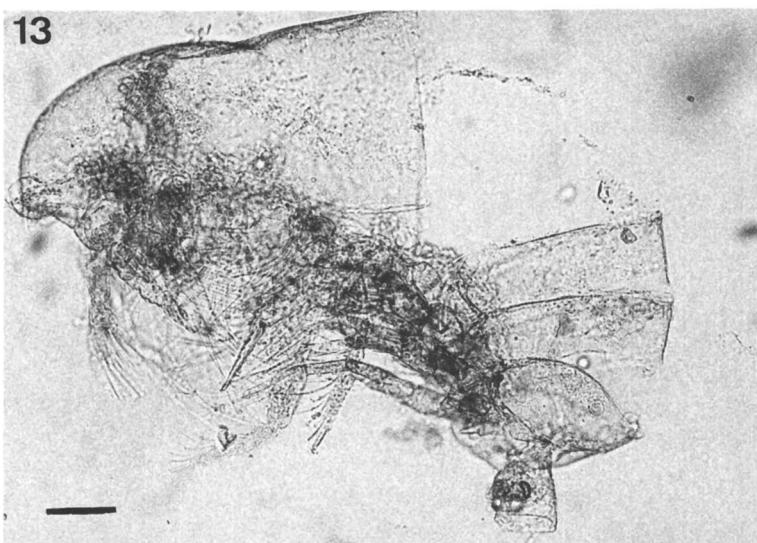


Figure 13. Empty and broken copepod "ghost" after 72 hours incubation *in situ* (Calvi Bay experimental setting at 37 m depth, temperature 16°C). Light Microscopy (LM) scale is 100 µm.



Figure 14. Detail of Figure 13 showing muscles and cuticular remains embedded in a rich sheath of bacteria. Note abundant Ciliates. LM scale is 10 µm.

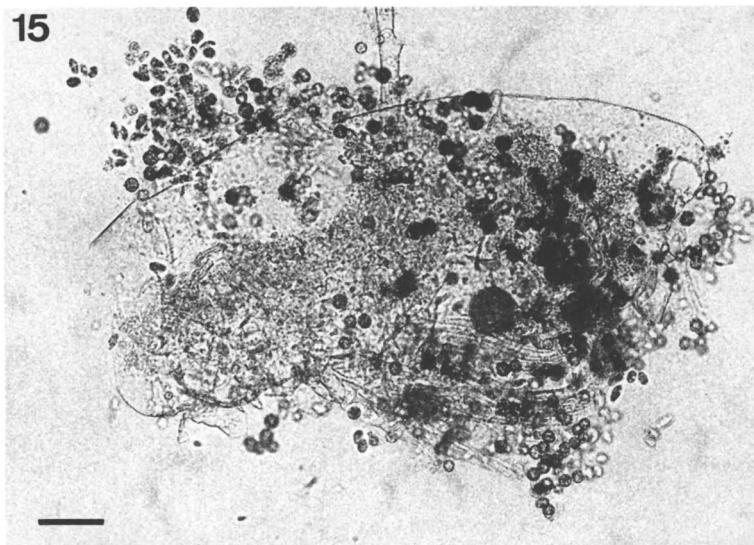


Figure 15. Cuticular remains aggregates with bacteria and ciliates constituting "marine snow" after 120 hours incubation *in situ* (Calvi Bay experimental setting at 37 m depth, temperature 16°C). LM scale is 100 µm.

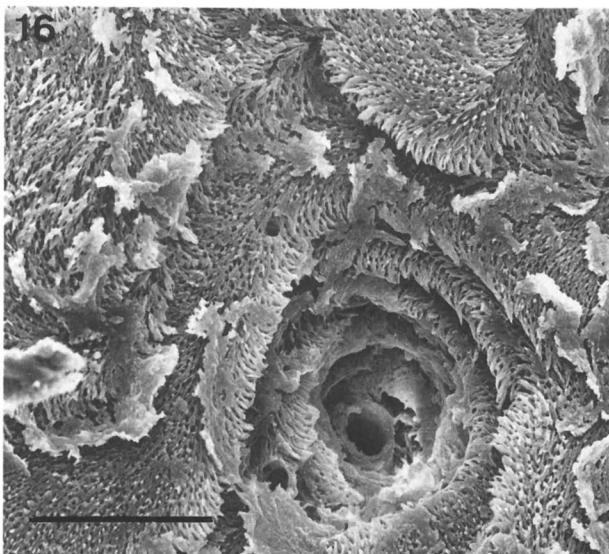


Figure 16. Bored and exfoliating crustacean cuticular surface after 72 hours incubation *in situ* (Calvi Bay experimental setting at 37 m depth, temperature 16°C). SEM scale is 10 µm.

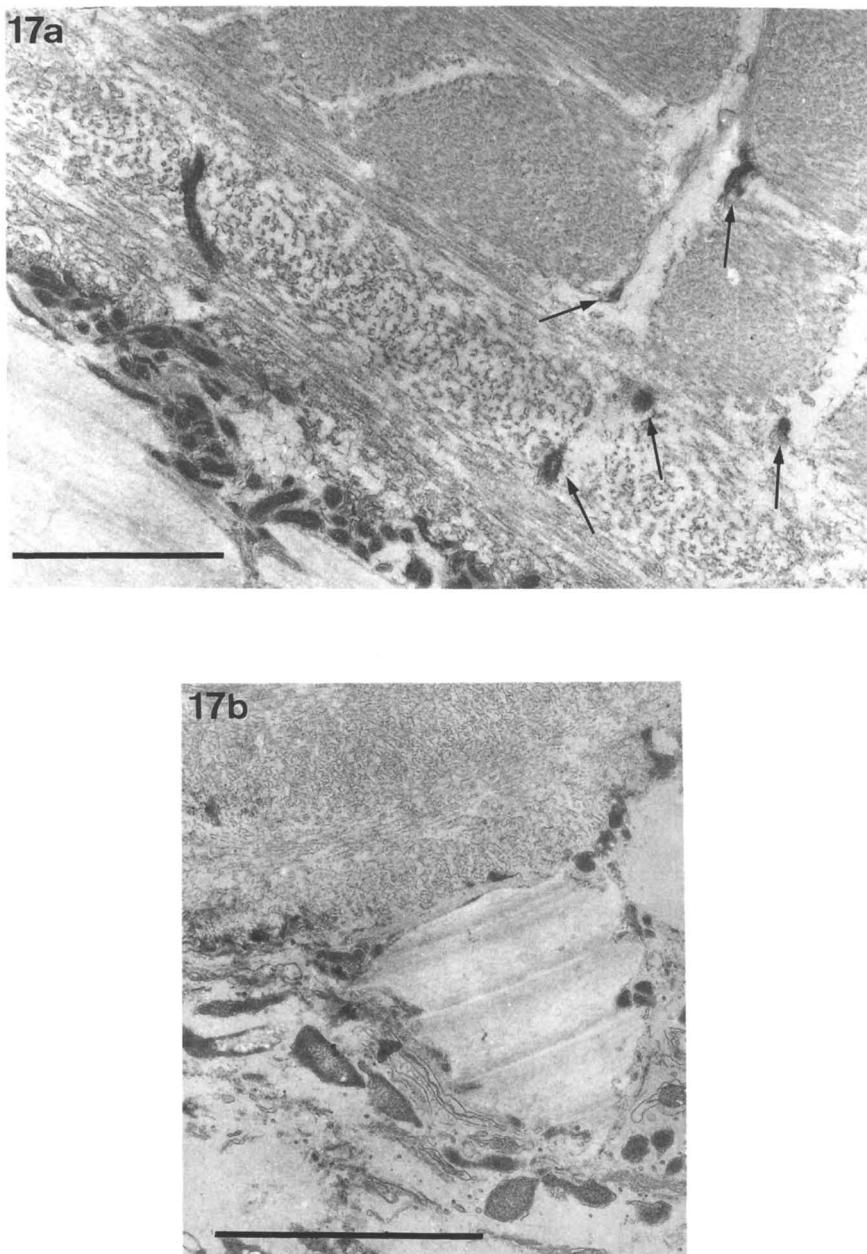


Figure 17. Bacterial weathering of the calcified layers of a crustacean cuticle (*Carcinus maenas*), Calvi Bay experimental setting at 37 m depth, 72 h immersion. Transmission Electron Microscopy (TEM) scale is 5  $\mu\text{m}$ . a) Bacterial mat biodegradation from the internal side (membranous layer); pore canals are used as preferential routes for deeper invasion (arrows); b) Detail of bacterial weathering of the membranous layer.

attached to the settling material (Figure 21), the matter deposited on the sediment surface continues to degrade (69, 70, 153). The apparent decrease of the process is mainly due to the prior loss of most labile compounds. The material now consists mainly of more or less refractory molecules, and chitin remains (generally less than 5 to 10 % of the initial content). In shallow water, mechanical disruption and abrasion occur, due to hydrodynamic forces resuspending material. Weakening of the remaining cuticle leads to complete fragmentation and pulverization of the planktonic material (153). Such mechanical disruption does not occur deeper in the oceans and chitin incorporation into the oxic sedimentary layers should be possible. Whatever the input, it has been shown that the number of chitinoclastic bacterial strains is a logarithmic function of the chitin content of the sediment at the interface (49). This means that a slight increase of the chitin content readily available should induce an immediate growth of chitinoclastic bacteria resulting in the rapid degradation of this input. In such conditions, the bacteria behave as typical opportunistic organisms.

**Biodegradation in Oxic Marine Sediments.** Since accumulation of particulate chitin in open water as a result of downward flux appears unlikely, the chitin content of sediments is related to their composition. Furthermore, the organoclastic sediments typically harbor much more chitin than terrigenous, volcanic or authigenic ones. Mollusk shells and bryozoans are the main contributors to sedimentary chitin (49, 159).

In the oxic layers of marine sediments, biodegradation of the chitinoproteic matrices of animal skeletons occurs mainly through the activity of microborers (for literature review see 160-168). A well-defined sequence of microorganisms settle onto detrital fragments and some of them are able to bore holes of 1-150  $\mu\text{m}$  in diameter (Figures 18-20). Although, the cyanobacteria are abundant, bacteria distributed within the organic matrices and fungi are the main biodegraders (69, 161, 167) (Figures 18-20). Marine borers, of calcified tissues, are involved in constructive and destructive processes. Some of these 'borers' pit and corrode carbonate surfaces while others bore into them, changing the pH of seawater in some micro-environments and possibly by secreting chelating metabolites (Figures 16-18, 20). The mechanism by which endoliths bore remains unknown, though it is thought to be by chemical rather than by mechanical means. These processes increase the porosity and weaken the surface layers of inhabited substrata, particularly when the chitinoproteic matrices inside the skeletons are deeply altered. Moreover, microphytes provide food for herbivores (e.g. gastropods, Polyplacophora) promoting this way, carbonate removal by abrasion (169).

Endolithic microphytes are also involved in producing "constructive" and "destructive" micrite envelopes around carbonate particles thus protecting from further biodegradation. These microphytes bore into substrates at 0.5 to 50  $\mu\text{m day}^{-1}$ . Filament densities within detrital substrata may range from 50,000  $\text{mm}^{-3}$  to 500,000  $\text{cm}^{-2}$ . In experiments, typical densities of boreholes are of the order of 150,000 to 400,000  $\text{cm}^{-2}$  in mollusk shells and echinoid plates after one to four years. There is no significant difference between experimental settings (Mediterranean Sea, English Channel, Papua New Guinea or the Caribbean Virgin Islands). Most genera of

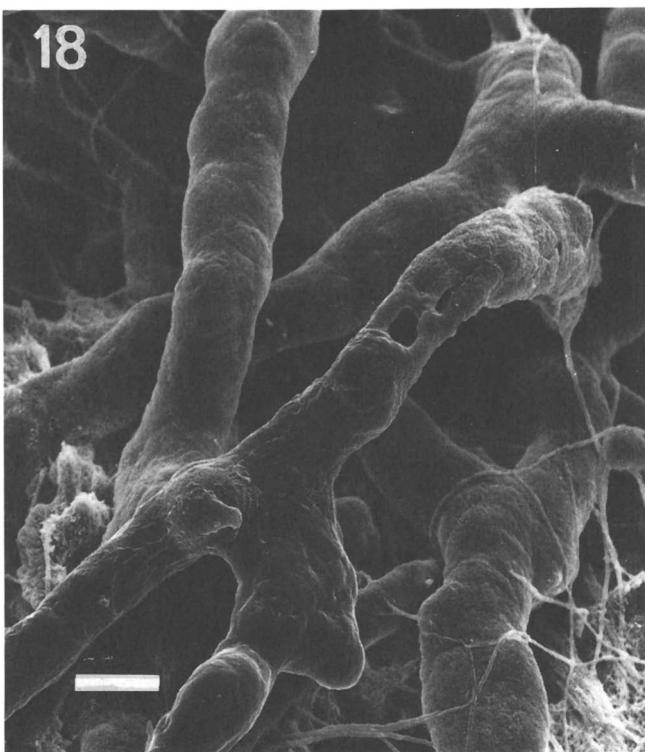


Figure 18. Different kinds of fungi boring into a mollusk shell after 6 months incubation in situ at water-sediment interface (Virgin Islands experimental setting at 15 m depth, temperature 26°C). SEM scale is 10  $\mu\text{m}$ .

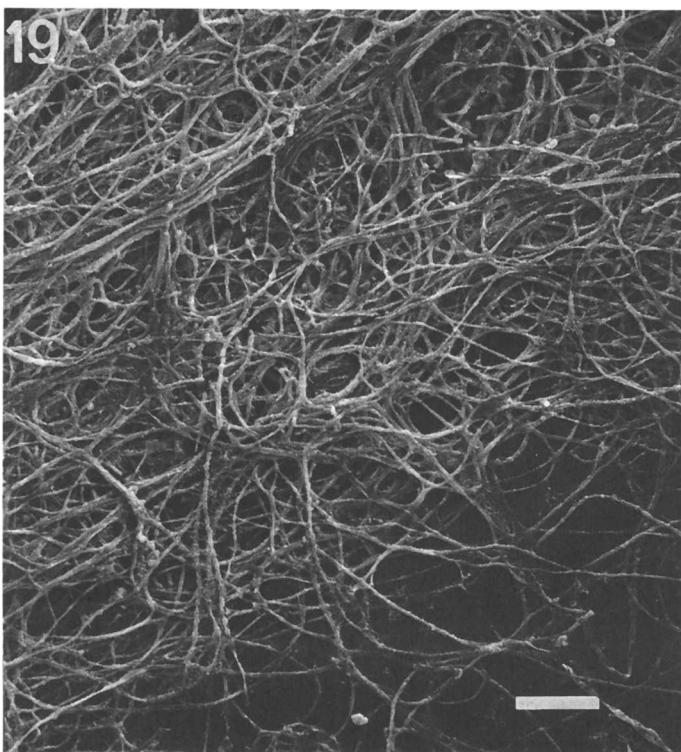


Figure 19. Filamentous bacteria associated with chitinoproteic organic matrices in a mollusk shell after 9 months incubation *in situ* at water-sediment interface (Calvi Bay experimental setting at 37 m depth). SEM scale is 10  $\mu\text{m}$ .

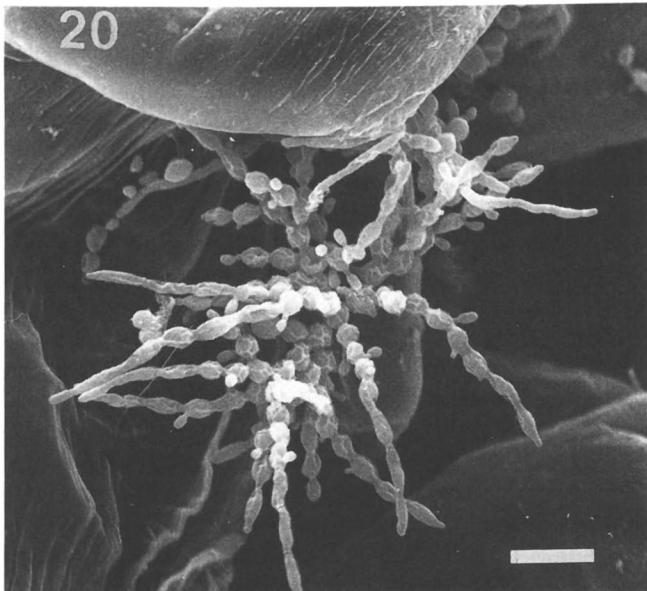


Figure 20. Undetermined organism of fungal affinity boring into a detritic mollusk shell at water-sediment interface (Indian Ocean, 4870 m depth). SEM scale is 10  $\mu\text{m}$ .

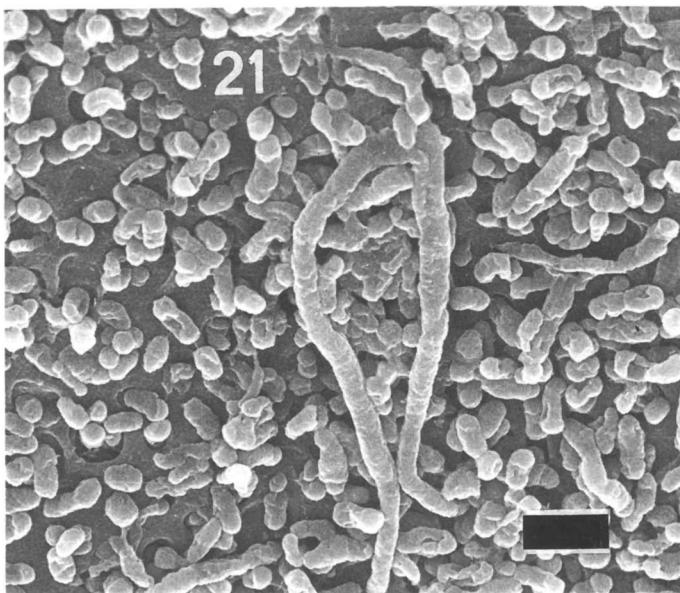


Figure 21. Different kinds of bacteria attached to a planktonic crustacean cuticular remain collected in a sediment trap disposed in nepheloid layer (Mediterranean Sea, 560 m depth). SEM scale is 5  $\mu\text{m}$ .

endolithic microborers have a worldwide distribution, although there is some endemism at the species level.

Endolithic algae, adapted to life with very little light, occurred at surprisingly great depths within oceans, with *Plectonema*, a cyanobacteria, being found down to 370 meters on the continental slope near Florida (169). Fungi, which are not light-limited, have been reported from 4780 meters (Figure 20) (160, 163, 166, 167).

Most of the vacated boreholes (Figure 16) (only 10-15% is occupied by the borer) are used by bacteria to reach the organic veils inside the skeleton (161, 163, 164, 167). With the exception of cyanobacteria, most microorganisms isolated from weathered skeletons are able to secrete extracellular hydrolases, like proteolytic and chitinolytic enzymes, when cultivated *in vitro* (161, 167). These hydrolytic activities also occurred in detrital skeletons (161, 167, 168). Estimations of enzymatic activity inside weathering skeletons show a great diversity of hydrolases and high levels of hydrolytic activity (163). Chitinolytic activity is important in mollusk shells and crab cuticles, and can explain a quick decrease of chitin content in experimental samples. More than 90 % of the initial chitin content in crustacean cuticles disappears in less than two months (Figure 17a, 17b, 23, 24), whereas the same percentage loss takes more than one year in mollusk shells (162, 163) (Figure 22a, b). The kinetics of chitin loss are the same in different experimental settings: once the microborers settle on the experimental material and invade the cortical layers (low hydrolytic activity), there is a fast decrease in chitin content, synchronous with the exponential growth of endolith populations. After this rapid biodegradation, the rate reduction corresponds to lowering of the concentration of the readily available growth substrates (refractory phase). The remaining organic compounds are mainly refractory or structurally bonded (tanned proteins of the chitinoproteic complex), thus being much less accessible.

**Biodegradation in Anoxic Marine Sediments.** Although anoxic superficial sediments cover only 10% of the sea floor, they underlie most of the shallow highly productive areas of the world oceans. These sediments accumulate more than 90% of the total organic matter buried in sediments annually (69). Hence processes of anaerobic decomposition are very important in determining to what extent some chitinous compounds "survive" rapid mineralization in the surface biotic layers and are buried long term. Most aerobic microorganisms can oxidize a wide range of substrates to CO<sub>2</sub>, whereas individual anaerobic microorganisms metabolize (often incompletely) a rather restricted range of molecules. Although, the microorganisms inhabiting anaerobic microenvironments are much less diversified, the anaerobic communities can be very efficient in decomposing relatively refractory molecules (164-168, 170). Thus, aquatic anaerobic communities (164-168) can decompose most of the organic polymers. Anaerobic biodegradation of skeletal substrates is mainly due to bacteria, diatoms and fungi (165, 170). Despite the fact that few forms of decomposer are common to both aerobic and anaerobic conditions, the biodegradation patterns are very similar in both cases. The same colonization curve is observed, culminating in the same densities of borers (163, 167). The results of estimates of enzymatic activity show that, in the case of mollusk shells, there is no significant

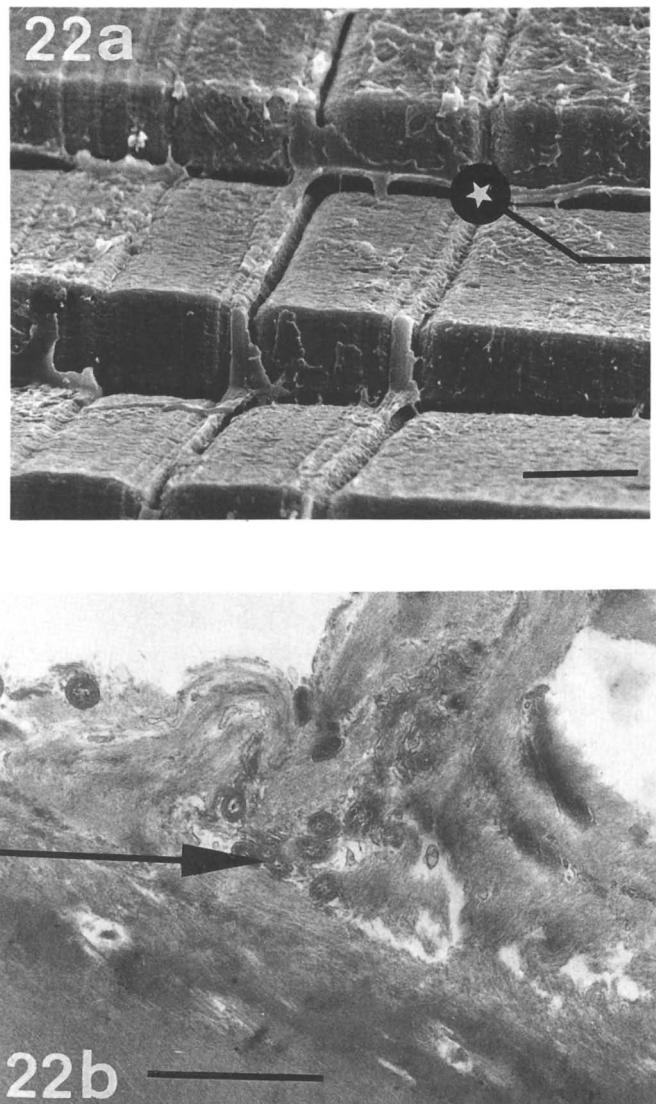


Figure 22. Prismatic layer of *Pinna nobilis* shell weathered for 12 months in Calvi Bay experimental setting in oxic sediment at 37 m depth. a) The organic matrices between crystallites almost disappeared. Area marked with a star enlarged in Figure 22b. SEM scale is 10  $\mu\text{m}$ . b) Bacteria within organic matrix remains. TEM scale is 1  $\mu\text{m}$ .

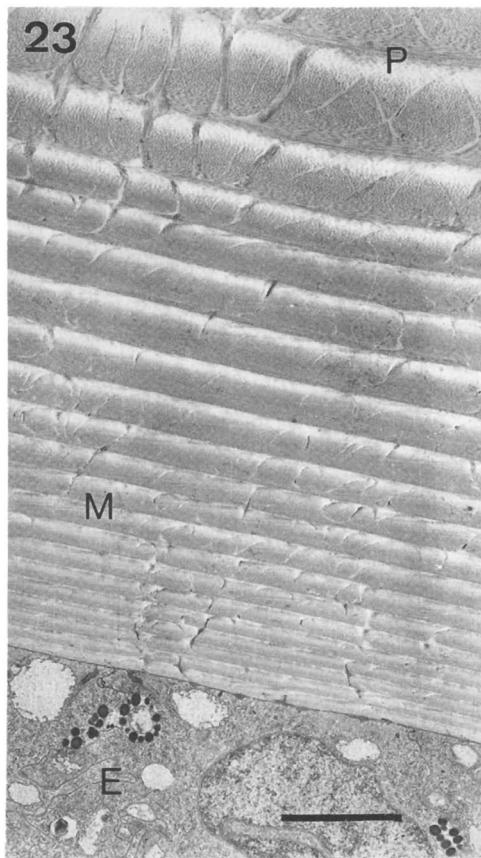


Figure 23. Fresh cuticle of the crab *Carcinus maenas*. TEM scale 1  $\mu\text{m}$ . Note: E, epithelium; M, membranous uncalcified layer; P, principal calcified layer.

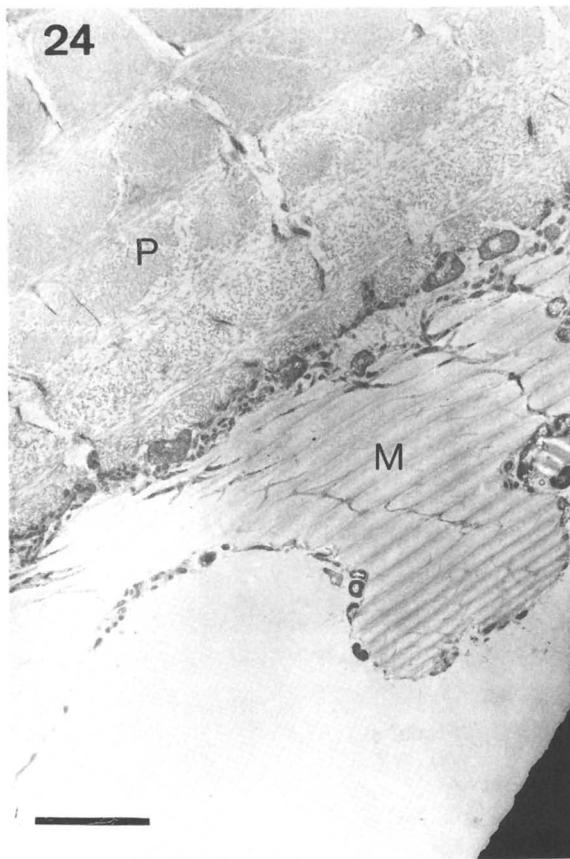


Figure 24. Bacteria weathering the cuticular layers of *Carcinus maenas* after 12 days in Calvi Bay experimental setting at water-sediment interface, 37 m depth: membranous layer (M) almost completely disappeared, begin of principal layer (P) alteration. TEM scale is 10  $\mu\text{m}$ .

difference between hydrolytic levels (166, 168). However, in other samples, where anaerobic hydrolytic processes appear lower, the kinetics of biodegradation appears essentially similar for chitin, with comparable rate constants (166-168). Slowing down of the process after initial rapid biodegradation is also observed, but the composition of the refractory compounds may be somewhat different in the two experimental conditions, as shown by the results of transfer experiments (69) (Figure 7). Skeletal material (mother of pearl) was laid down *in situ* in oxic sedimentary layers for six months, after which, part of the experimental material was transferred to anoxic sedimentary conditions (other samples remained in the same environment for a further six months period). After a rapid decrease of the organic content during the first six months (70% degradation), the extraction of the organic compounds slowed down, a normal phenomena at the beginning of the refractory phase (speed constant  $K_{aer}=0.91$ ). The material that was transferred to anaerobic conditions weathered three times faster than that remaining in aerobic conditions ( $K_{ana}=3.00$ ). The latter phenomena can be interpreted as a reflection of the adaptation of anaerobic microbiocenoses to the biodegradation of the less labile compounds that remained after aerobic degradation. The cumulative effect of both processes (aerobic and anaerobic weathering) results in optimal recycling of the chitinous compounds of skeletal substrates.

**Biodegradation in Deep-Sea Environments.** Enormous bulk of mineralized skeletal structures, sheltering chitinoproteic complexes, settles onto deep-sea sediments, sometimes in the form of deep calcareous oozes. Extraction of organic matter from calcareous skeletons, for example Pteropod or other Mollusk shells, occurs at any depth in marine sediments where microborers are ubiquitous (13, 163, 167, 169, 171). In the aphotic environment, fungi (Figure 20), colorless cyanobacteria and bacteria are supposed to function mainly heterotrophically, thus using the organic matrix of skeletal remains as a nutrient source (69, 163, 171). Most endolithic organisms live in close contact with the organic sheaths around crystallites (164, 165). Thus, the extracellularly secreted enzymes are able to hydrolyze the organic compounds of those sheaths, as experimentally shown in shallow depth (13). Despite the importance of the deep-sea ecosystem, data on enzymatic degradation under high hydrostatic pressure are scarce (172, 173): chitinase is a highly barotolerant enzyme, active and very stable under deep-sea conditions (1000 atm and 2°C), and psychrophilic and barophilic bacteria can secrete this hydrolase under *in situ* conditions.

Preliminary observations *in situ* around deep-hydrothermal vents indicate that the Vestimentifera tube is a very stable structure (174). An experimental *in situ* approach showed that after a 6 months of exposure, and in contrast with exoskeleton fragments of the crab *Bythogreya*, the tube samples appeared relatively unaltered, and the relative amount of chitin was comparable to that of the control samples (Figures 25, 26). This was confirmed with an experiment at shallow depths in Calvi Bay (174).

The results of experiments with the vent crab exoskeleton in both environments are comparable: a rapid and significant decrease (30 % after 12 days) of

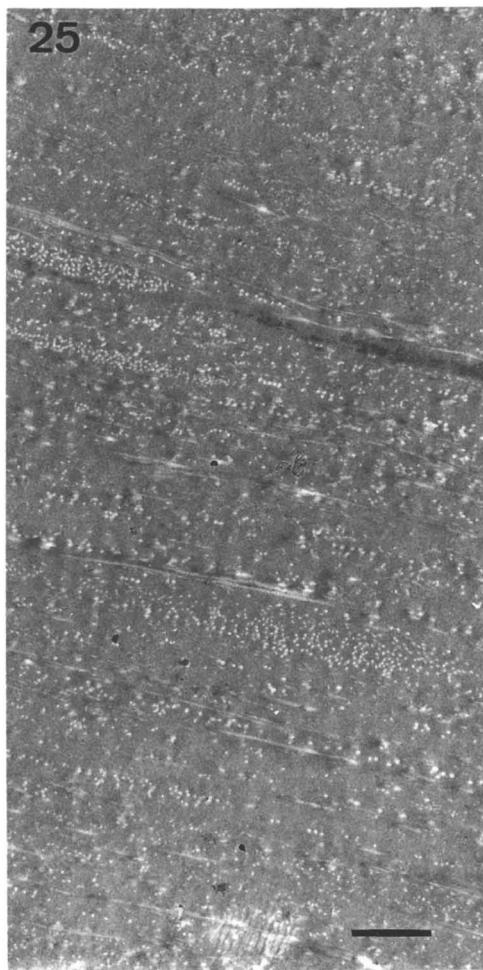


Figure 25. Structure of the fresh *Riftia pachyptila* (Vestimentifera) tube. White dots are chitin microfibrils. TEM scale is 1  $\mu\text{m}$ .

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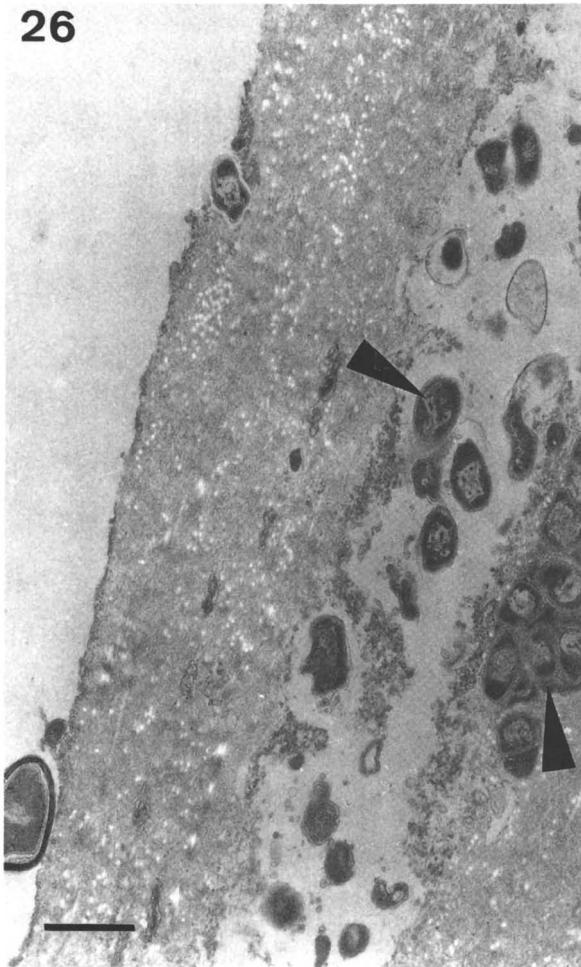


Figure 26. Structure of the most cortical layer of an *in situ* weathered *Riftia pachyptila* tube. Relatively unaltered structure after 12 days *in situ* incubation around hydrothermal vents (depth 2600 m). Clusters of bacteria embedded in the tube walls (arrows) are also frequent in fresh material (not illustrated). TEM scale is 1  $\mu\text{m}$ .

the total organic content in the vent site, is paralleled in the Calvi site where quite 80 % of this fraction is dissolved after 20 days (174). The latter observation agrees with experiments on sclerites of the shore crab *Carcinus maenas* in Calvi Bay (162, 175) (Figures 23, 24). While the loss of organic material in the vent crab results mainly from a decrease of the chitin content (32 % and 80 % after 12 and 20 days respectively), the alkalo-soluble protein content degrades more slowly. In contrast to the vent crab, the *Riftia* tube exhibits a radically different "biodegradation" profile. The whole organic content of the samples is stable over a period of about 3 weeks, either in the vent site or in shallow environment (174). After 6 months, there is a loss of about 20 % of material mainly the protein fraction. There was almost no variation in the chitin content of the *Riftia* tubes even after 6 months of vent exposure. The comparison of the sites suggests that resistance to degradation is independent of environmental conditions and is a function of the structural and/or chemical parameters that determine chitin accessibility to biodegraders.

### **Conclusions: Biogeochemical Implications of Chitin Biodegradation**

Chitin produced in the marine environment is unlikely to accumulate to a large extent or to be preserved in sediments. From open water to anoxic sedimentary layers, the efficiency of chitinoclastic microorganisms is very high. The microbial degradation of chitin in the marine environment is a highly complex and structured process involving a variety of microorganisms and microbial consortia. The specialized features of some of them (mode of life of microborers in skeletal remains in sediments, adaptation of anaerobic decomposers to more or less refractory compounds, etc.) lead to an optimization of recycling. This explains why chitin does not accumulate to a large extent in most marine sediments. For the general marine "economy" to maintain the steady state of geochemical cycles, chitin mineralization processes are of great ecological significance as it contributes to both carbon and nitrogen cycles.

The biodegradation and breakdown of chitin in calcified skeletons is accompanied by an increased dissolution rate of a biogenic carbonates, thus has repercussions for chemical equilibria (the organic sheaths around  $\text{CaCO}_3$  crystallites partly protect them from dissolution in thermodynamically undersaturated media, i.e. deep and/or cold environments). After extraction of the organic matter, the calcified skeletons, less "protected" from the inside, are more rapidly dissolved particularly where microborers increase the surface accessible to dissolution.

Considering the biomass of the chitin and chitinoproteic complexes produced annually in the oceans, and their ability to complex transition elements without interference of alkali nor alkali-earth ions (4, 13, 14), the amount of heavy metals ions associated with them should be taken into account when computing global geochemical cycles. While humic and fulvic substances form relatively stable complexes with metal ions, chitin-metal complexes are more unstable, particularly as chitinase is activated by some of the metals bound on chitin (13, 14). The latter process makes chitin a potentially important agent for the transport of metal in the marine environment, while humic substances should merely act as sinks for heavy

metals. In open water, metals bound on chitinoproteic complexes of suspended matter are released or chelated from/on the chitin through biological weathering (deacetylation and hydrolysis), either in the water column or in the upper layers of the sediments. Decomposition of these complexes in areas of high biological productivity, together with other vector mechanisms (clays, metal oxides and hydroxides, etc.), may be a source of localized metal rich interstitial waters and sediments in the marine environment.

Resistant biopolymers are known to be significant contributors to kerogen (176-180). Recently, new investigations have shown that not only plant remains, but also animals yield highly resistant macromolecules (176-182). However, the organic material found in most fossil invertebrates (especially Paleozoic and Mesozoic), unlike plant remains, have no clear source in the living animal, as the cuticle chemistry appears altered during diagenesis (176-184). The preservation potential of chitin in the fossil record is lower than that of other macromolecules (like lignin for example; 176). Even when chitin is cross-linked, as in thick sclerotized cuticles (178-180), or associated with proteins stabilized by disulfur bridges as in *Vestimentifera* (174), it will only be preserved in very special diagenetic environments (bituminous shales or strongly reducing bottom conditions). The primary control on chitin preservation in the geological record is not only time, but rather the nature of depositional environments inhibiting diagenetic alteration (179). Chitin is rarely detected in substantial amounts even in modern sediments (49).

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