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Aerobic and Anaerobic Degradation and Mineralization of ^{14}C-Chitin by Water Column and Sediment Inocula of the York River Estuary, Virginia

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Potential rates of chitin degradation (C_d) and mineralization (C_m) by estuarine water and sediment bacteria were measured as a function of inoculum source, temperature, and oxygen condition. In the water column inoculum, 88 to 93% of the particulate chitin was mineralized to CO_2 with no apparent lag between degradation and mineralization. No measurable dissolved pool of radiolabel was found in the water column. For the sediment inocula, 70 to 90% of the chitin was degraded while only 55 to 65% was mineralized to CO_2. ^{14}C label recoveries in the dissolved pool were 19 to 21% for sand, 17 to 24% in aerobic mud, and 12 to 21% for the anaerobic mud. This uncoupling between degradation and mineralization occurred in all sediment inocula. More than 98% of the initial ^{14}C-chitin was recovered in the three measured fractions. The highest C_d and C_m values, 30 and 27% day^{-1}, occurred in the water column inoculum at 25°C. The lowest C_d and C_m values were found in the aerobic and anaerobic mud inocula incubated at 15°C. Significant differences in C_d and C_m values among water column and sediment inocula as well as between temperature treatments were evident. An increased incubation temperature resulted in shorter lag times before the onset of chitinoclastic bacterial growth, degradation, and mineralization and resulted in apparent Q_{10} values of 1.1 for water and 1.3 to 2.1 for sediment inocula. It is clear that chitin degradation and mineralization occur rapidly in the estuary and that water column bacteria may be more important in this process than previously acknowledged.

Billions of tons of chitin are produced annually by the myriad of invertebrates that inhabit the waters and sediments of the oceans and estuaries of the world (9). After molting or death, this exoskeletal material serves as a C, N, and energy source for bacteria and fungi (5). The contribution of this chitin to the total C and N budget of an estuary or coastal system has never been determined because of methodological problems in measuring both chitin production and decomposition.

Montgomery et al. (14) developed a method for measuring chitin content in the water column whereby very fine particulate chitin is quantified by fluorescent-labeled lectin binding. They found that standing stock chitin concentrations of the >0.2-μm fraction in Delaware Bay ranged from 4 to 21 μg liter^{-1}. Chitin sinks rapidly as it is molted and is also present in the many animals that inhabit the benthos. It is possible that low water column concentrations are a direct result of this physical and behavioral partitioning. However, chitin from small zooplankton, such as copepods, may remain in suspension for days. These abundant animals are the most probable source of chitin found in the water column (9).

Boyer and Kator (3) showed that particulate ^{14}C-labeled chitin incubated with York River water at 20°C was mineralized to $^{14}CO_2$ at a rate of 21% day^{-1}, with <4% recoverable in the dissolved pool at any time. If we assume that the York River estuary possesses chitin concentrations comparable to those of the Delaware Bay and that these concentrations reflect only 4% of true production, then annual production of chitin in the York River estuary would be on the order of thousands of metric tons year^{-1}. It seems clear now that low concentrations of chitin in the water column does not necessarily imply that chitin production is small and of minor importance to the ecosystem. On the contrary, low chitin concentrations are most probably due to rapid recycling of this important C and N source.

It has been assumed that chitin degrades rapidly in sediments because it does not accumulate to any significant extent; however, actual rates of chitin mineralization in sediments have not been accurately determined because of methodological limitations. Previous studies of chitin degradation rates in sediments were performed by using gravimetric or litterbag techniques, where rates of degradation were based on the disappearance of particulate chitin (4, 6, 7, 10, 12, 13, 17, 18). In addition to being subject to sediment interference, this approach provides no information as to the fate of chitin C.

An important variable affecting degradation rate of particulate matter is surface area or particle size. The smaller the particle, the greater the surface area and, hence, the greater accessibility of the substrate to biological attack. The size of chitin particles in nature ranges from the macroscopic, as in horseshoe crabs, to the microscopic, found in fungi and diatoms. It is not unrealistic to think of degradation accelerating as particle size decreases. Therefore, it is important that the initial particle size used be reported to facilitate comparison between experiments. It is also possible that chitin from different species as well as from distinct body parts of the same organism will degrade at different rates because of the different levels of the basic constituents of the carapace, i.e., chitin, protein, and calcium carbonate (2), and to the variation in the chemical nature of the chitin itself (5).

Here I define particulate chitin as that which is retained on

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a GF/A glass fiber filter (Whatman; nominal pore size, 1.6 μm). Although the operational standard for resolving particulate from a dissolved fraction is the GF/F filter (nominal pore size, 0.7 μm), I used the GF/A filter because of the high sediment load of my experimental systems. Chitin degradation is defined as the process by which exoenzymes of chitinoclastic bacteria hydrolyze particulate chitin into a dissolved fraction consisting of microparticles, oligomers, and other small-molecular-weight constituents, which may or may not be assimilated into the bacteria themselves. Chitin mineralization is the bacterially mediated conversion of these dissolved components to CO_2 and/or CH_4 under anaerobic conditions (1). Since individual bacteria are smaller than the nominal pore size of a GF/A filter, they may be recovered as part of the dissolved fraction, whereas particle-associated bacteria will be recovered in the particulate fraction. This is a methodological limitation forced by the use of sediment systems.

With all of these problems associated with measuring chitin degradation, it is understandable why there are so few studies present in the literature (5). With the synthesis of ^{14}C-labeled chitin, we can now begin to collect some rudimentary data concerning the fate of chitin C in aquatic ecosystems. This study summarizes the results of using ^{14}C-labeled chitin to quantify potential rates of chitin degradation (C_d) and mineralization (C_m) as a function of bacterial inoculum source (water and sediment type), temperature, and oxygen conditions in the York River estuary.

MATERIALS AND METHODS

Synthesis and characterization of ^{14}C-labeled chitin. ^{14}C-labeled chitin from blue crabs, *Callinectes sapidus*, was prepared and characterized as described previously (3). Briefly, crabs were allowed to molt and then immediately injected with 2 μl of a 0.1-μCi/μl concentration of *N*-acetyl-D-[1-^{14}C]glucosamine (Amersham) per g of body weight. Blue crabs were sacrificed at 10 h postinjection, and the carapaces were cleaned and dried. Weighed amounts of ^{14}C-chitin were combusted in a LECO 521 induction furnace, and the $^{14}CO_2$ was trapped in a scintillation cocktail composed of 800 ml of toluene, 90 ml of methanol, 6 g of 2,5-diphenyloxazole, and 100 ml of Carbo-sorb (Packard). The specific radioactivity was determined with a Beckman 150 liquid scintillation counter, correcting to disintegrations per minute, with ^{14}C-toluene internal standardization.

Sampling of water and sediments. Two sampling sites in the York River estuary, a subestuary of the Chesapeake Bay, were chosen for their different sediment characteristics. The first site was a sandy shoal off Gloucester Point. Sediment composition at that site was 97.2% sand by weight (16). The other site, located in Sarah Creek, consisted of fine silts and clays. The depths of the aerobic zones determined by a platinum electrode at the sandy and muddy sites were 35 and <2 mm during summer months. In May, sediment samples from each site were collected by using 5-cm (inside diameter) Plexiglas core tubes which were closed with butyl rubber stoppers. A total of six cores were taken from each site. Water samples were taken at a depth of 1 m by using a sterile glass bottle.

Preparation of inocula and incubation flasks. Upon return to the laboratory, the cores were split into aerobic and anaerobic fractions by extruding the cores and scraping off the upper aerobic region and pooled within the station. Ten milliliters of pooled sediment was added to 90 ml of sterile, aged York River water (SYRW) and homogenized by stirring. Anoxic conditions of the anaerobic sediment was maintained during homogenization by constant purging with oxygen-free N_2. Ten-milliliter aliquots of sediment homogenate was added to each 250-ml flask containing 90 ml of SYRW and 10 mg of UV-sterilized ^{14}C-chitin. Ten-milliliter aliquots of anaerobic mud homogenate were transferred to 250-ml flasks containing 90 ml of N_2-sparged SYRW, 10 mg of UV-sterilized ^{14}C-chitin, and 0.1 g of NaS. Ten-milliliter aliquots of raw York River water were added to 250-ml flasks containing 90 ml of SYRW and 10 mg of UV-sterilized ^{14}C-chitin. Sterile controls were also incorporated into the experimental design by autoclaving the flasks after the inoculum was added. All flasks were sealed with butyl rubber stoppers, placed on an orbital shaker table (100 rpm), and incubated in the dark at an ambient temperature of 15°C. The experiment was repeated in September at the ambient temperature of 25°C.

Enumeration of chitinoclastic bacteria. At selected intervals, three flasks from each treatment were chosen at random. Chitinoclastic bacteria were enumerated from one flask by removing 1 ml of water with a sterile syringe and inoculating it into a most-probable-number (MPN) dilution series. The most-probable-number dilution series medium consisted of 1 liter of SYRW, 1 g of $(NH_4)_2SO_4$, 0.1 g of K_2HPO_4, and 2.5 g of ball-milled chitin (Calbiochem, San Diego, Calif.) as the sole C source. All bacterial enumerations were incubated under aerobic conditions at 20°C. Obligate anaerobic bacteria were not counted by this method since anaerobiosis was not maintained during incubation. After 2 weeks, most-probable-number dilution series tubes were assayed for bacterial growth by visual observation. Positive growth was ascertained by visible turbidity of the medium, presence of surface film on the chitin pellet, or physical clumping of the chitin particles, even after swirling of the tube.

Degradation and mineralization assays. Radiolabel was recovered as previously described (3). Each flask was injected with 2 ml of 5 N H_2SO_4 to stop all biological activity and to volatilize the dissolved CO_2. Flasks were purged with N_2 at a flow rate of 50 ml min^{-1} for 10 min, and the $^{14}CO_2$ was trapped in a scintillation cocktail and counted in the liquid scintillation counter as outlined previously. Flasks were then opened, and the contents were filtered through a Whatman GF/A glass fiber filter. The filter was air dried and combusted in a LECO induction furnace, and the resulting $^{14}CO_2$ was trapped in the scintillation cocktail and counted. Five-milliliter aliquots of each flask filtrate were added to 20 ml of Aquasol and counted as the dissolved fraction. Control flasks were analyzed at the initiation and termination of each experiment in a manner identical to that described above.

Data analysis. First-order degradation rate constants were calculated by least-squares regression of the particulate chitin remaining with time by using a modified exponential-decay equation which accounted for lag time: $\ln C_t = -k(t - L) + \ln C_0$, where C_t is the percentage of initial particulate chitin remaining at time (t) in days, C_0 is the initial amount of particulate chitin added (100%), k is the rate constant (in percent day^{-1}), and L is the lag time (in days) before onset of degradation. The slope of the regression line was k, and the y intercept was $\ln C_0$. Lag time was defined as the number of days that was required for the inoculum to initiate a 5% loss of the initial substrate. Chitin mineralization data, i.e., $^{14}CO_2$ recoveries, were analyzed by using a similar equation. Substrate half-life excluding lag time was calculated to be $0.693/k$. A modified Student's t test (11) was performed to determine differences in specific rates between

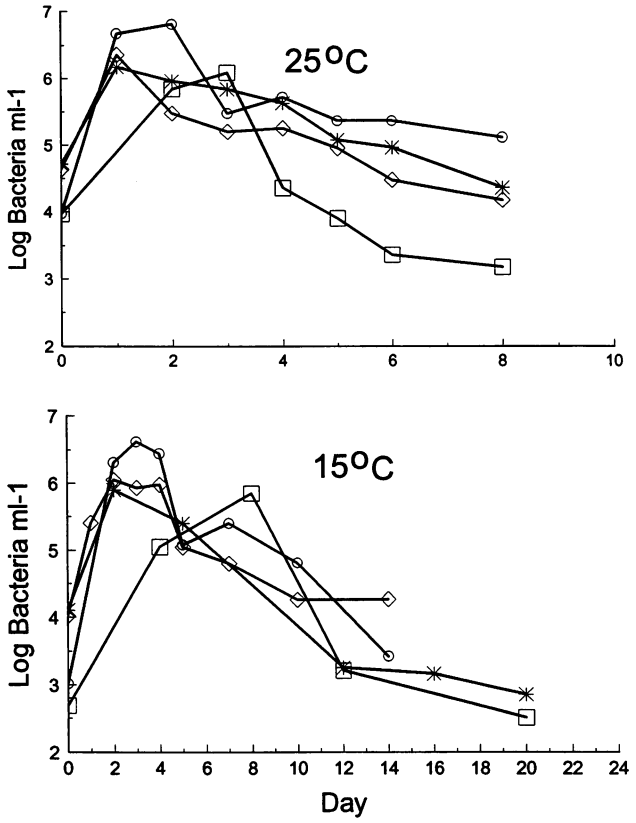

FIG. 1. Numbers of chitinoclastic bacteria ($\log_{10}$ milliliter^{-1}) from water column (○), sand (◇), aerobic mud (*), and anaerobic mud (□) inocula. Each point represents the mean of three replicates.

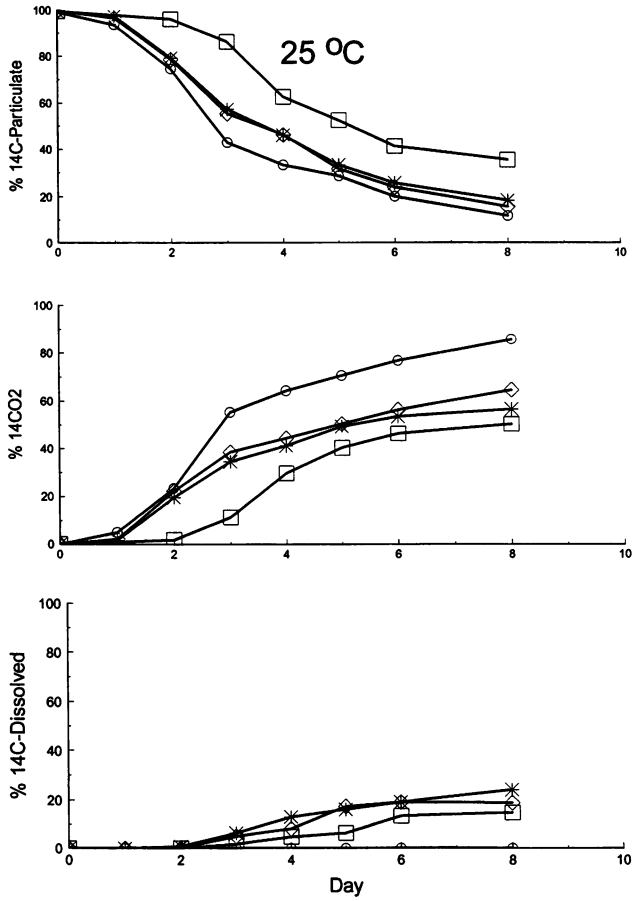

FIG. 2. Chitin degradation as percent particulate radiolabel recovered (top), mineralization as percent $^{14}CO_2$ recovered (center), and dissolved ^{14}C fraction (bottom) in water column (○), sand (◇), aerobic mud (*), and anaerobic mud (□) at 25°C. Each point represents the mean of three replicates.

treatments. Statistical significance was set at the $P < 0.05$ level.

RESULTS

Enumeration of chitinoclastic bacteria. Chitinoclastic bacterial counts ranged from an initial low of 1.0×10^3 cells ml^{-1} to peak values of 6.5×10^6 cells ml^{-1} (Fig. 1). No chitinoclastic bacteria were recovered in any of the sterile controls. The initial counts of chitinoclastic bacteria in the water column were ≈10 times higher in September than in May. The highest peak bacterial counts occurred in the water column inoculum; however, the sediments were not sampled per se but the overlying water in the flasks after some settling had occurred were. By the end of the experiment, bacterial counts in all inocula had returned to near or below initial levels. Temperature had no measurable effect on maximum chitinoclastic bacterial counts but did affect initial growth rates. At 15°C, it took about 1 day longer for the bacterial counts to reach their highest levels.

Radiolabel recoveries as a function of temperature and inoculum source. At 25°C, the water column inoculum exhibited a <1-day lag before onset of degradation compared to lags of 1 day for sand and aerobic mud and 2 days for the anaerobic mud (Fig. 2). By the end of the experiment, 11.6% ± 0.8%, 15.6% ± 1.3%, and 18.3% ± 1.6% (mean ± standard error; $n = 3$) of the initial radiolabel in the water column, sand, and aerobic mud inocula, respectively, was recovered as particulate chitin, while 30.7% ± 3.0% remained undegraded in the anaerobic mud inoculum. Percent $^{14}CO_2$ recoveries for the four inocula were 85.6% ± 5.3%, 64.6% ± 5.4%, 56.5% ± 3.9%, and 56.7% ± 3.8% for water, sand, mud, and anaerobic mud, respectively.

Sediment inocula exhibited an uncoupling of degradation from mineralization, in which both dissolved and CO_2 pools increased concurrently. Recovery of $^{14}CO_2$ accounted for 97.2% ± 6.1% of the particulate chitin degraded in the water column inoculum. No appreciable dissolved pool of ^{14}C-labeled products was found in the medium; there was no measurable intermediate between the enzymatic breakdown of chitin and its mineralization to CO_2 by water column bacteria. In the sand inoculum, 18.7% ± 3.0% of the initial radiolabel was recovered in the dissolved pool, with the remainder accounted for in the particulate and CO_2 fractions. With the aerobic mud inoculum, 24.0% ± 2.6% of the radiolabel was dissolved, while 12.3% ± 1.5% was found in the dissolved pool of the anaerobic mud inoculum.

Similar trends as those described above, with minor differences, were found for the 15°C incubations (Fig. 3). The lag times before onset of chitin degradation for water column, sand, and aerobic mud inocula were 2 days compared to 4 days for the anaerobic mud inoculum. By the end of the experiment, 6.7% ± 0.7%, 19.4% ± 1.5%, 15.2% ±

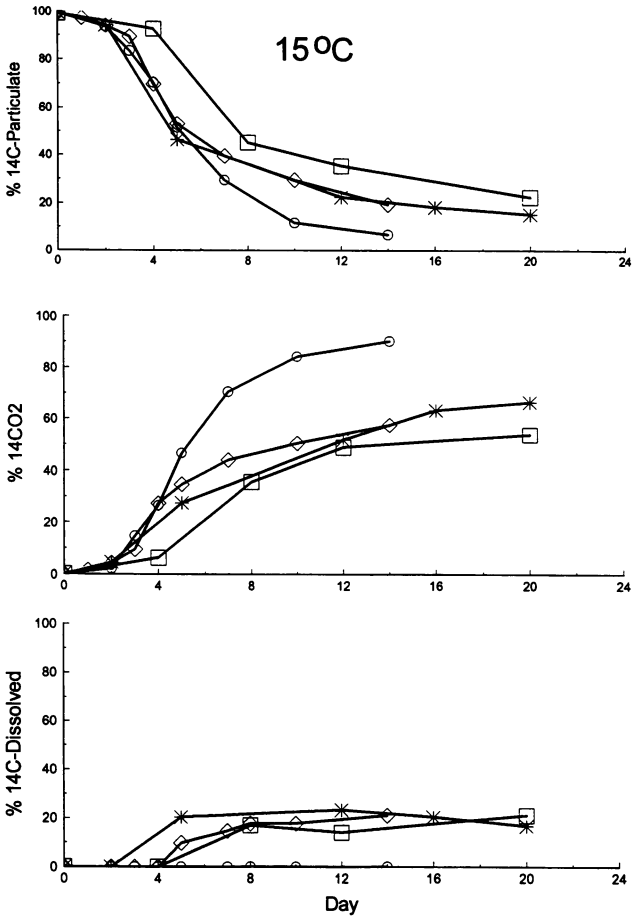

FIG. 3. Chitin degradation as percent particulate radiolabel recovered (top), mineralization as percent $^{14}CO_2$ recovered (center), and dissolved ^{14}C fraction (bottom) in water column (○), sand (◇), aerobic mud (*), and anaerobic mud (□) at 15°C. Each point represents the mean of three replicates.

1.9%, and 22.5% ± 2.3% of the initial radiolabel in the water column, sand, aerobic mud, and anaerobic mud inocula, respectively, remained as particulate chitin. However, it is important to note that the last sampling time for water and sand inocula was 14 days, and it was 20 days for aerobic and anaerobic mud inocula. CO_2 recoveries for the four inocula were 90.2% ± 4.9%, 57.4% ± 5.2%, 66.4% ± 4.1%, and 53.7% ± 4.9% for water, sand, mud, and anaerobic mud, respectively. No measurable dissolved radiolabel was found in the water column inoculum. By the end of the incubation, 21.1% ± 2.0%, 16.7% ± 0.6%, and 21.0% ± 1.0% of the radiolabel were recovered in the dissolved pool of the sand, aerobic mud, and anaerobic mud inocula, respectively.

For all treatments, 98.6% ± 3.6% (n = 192) of the initial ^{14}C-chitin added to each flask was accounted for in the three measured fractions. Sterile controls exhibited no differences in radiolabel recovery in the particulate fraction between the beginning and the end of the experiment. No radiolabel was recovered in either CO_2 or dissolved pools at the end of the trial in the sterile controls.

Chitin degradation and mineralization rates. The potential rates (in percent day^{-1}) chitin degradation and mineralization as well as substrate half-life (in days) for each treatment are shown in Table 1. All linear regressions used to determine rates possessed r^2 values of >0.90 and were highly significant ($P < 0.001$). This method of generating rates uses almost all the points of the curve; therefore, it is statistically more robust than working with only the linear portion of the curve.

TABLE 1. Potential C_d and C_m values at 25 and 15°C and relationship between C_d and C_m

Inoculum	Determinations at:					
	25°C			15°C		
	C_d[a]	C_m[a]	C_d/C_m[b]	C_d	C_m	C_d/C_m
Water	30 (2.3)	28 (2.5)	1.07	27 (2.6)	21 (3.2)	1.29
Sand	27 (2.6)	16 (4.4)	1.69	16 (4.5)	12 (6.0)	1.33
Aerobic mud	27 (2.5)	15 (4.6)	1.80	14 (3.1)	7 (10.5)	2.00
Anaerobic mud	22 (2.2)	16 (4.3)	1.38	12 (5.7)	8 (9.1)	1.50

[a] Expressed in percent day^{-1}. Substrate half-life (in days) is shown in parentheses.
[b] Relationship between degradation and mineralization (ratio).

Excluding lag time, C_d values in the water column, sand, and aerobic mud inocula were not significantly different from each other (30, 27, and 27% day^{-1}, respectively); however, all were significantly higher ($P < 0.05$) than the C_d of the anaerobic mud inoculum (22% day^{-1}). The substrate half-life reflected the C_d value, being in the anaerobic mud inoculum. The C_m in the water column at 25°C (28% day^{-1}) was significantly higher than it was for the other inocula ($P < 0.01$). C_m values of sand, mud, and anaerobic mud inocula were not significantly different from each other (16, 15, and 16% day^{-1}, respectively). C_d and C_m values in the water column inoculum were not significantly different but were significantly different for each sediment inoculum ($P < 0.05$).

At 15°C, the C_d in the water column was significantly higher than for any other inocula (27% day^{-1}; $P < 0.01$). The C_d values of sediment inocula were not significantly different from each other (16, 14, and 12% day^{-1}). The C_m in the water column at 15°C was significantly higher (21% day^{-1}; $P < 0.01$) than for any other treatment at that temperature (Fig. 3 and Table 1). C_m values of sediment inocula were not significantly different from each other (12, 7, and 8% day^{-1}). The C_d and C_m values in the water column and sand inocula were not significantly different but were significantly different for mud and anaerobic mud inocula ($P < 0.05$).

As expected, C_d and C_m were more rapid at 25°C than at 15°C ($P < 0.05$; Table 1); however, the effect of temperature was not the same for all inocula. Because the trials were performed at different times of year, these effects may have been due to differences in species of chitinoclastic bacteria present at the time of sampling as well as temperature. Although it is not strictly correct to compute Q_{10} values for studies originating on different dates, the apparent Q_{10} of chitin degradation in the water column was only 1.1 compared to 1.7, 1.9, and 1.8 for the sand, mud, and anaerobic mud, respectively. The apparent Q_{10} of chitin mineralization, in the same order as that described above, was 1.3, 1.3, 2.1, and 2.0.

DISCUSSION

This study has shown that chitin decomposition in estuarine water and sediments is a rapid and relatively complete process. The rates of chitin degradation shown here were much higher than those previously reported (Table 2), except for those of Hood (6) and Boyer and Kator (3). The high

TABLE 2. Chitin degradation rate comparisons

Site	Degradation rate (T)	Reference
Pure culture		
Aburatsubo Inlet	3.0 mg day^{-1} 10^9 $cells^{-1}$ (25°C)	17, 18
Puget Sound	2.1 mg day^{-1} 10^9 $cells^{-1}$ (22°C)	4
Barataria Bay	3.8 mg day^{-1} 10^9 $cells^{-1}$ (22°C)	5
Mixed culture		
Atlantic Shelf	5.6 mg day^{-1} $(4 \times 10^8$ cells$)^{-1}$ (22°C)	9
	6.0 mg day^{-1} $(3 \times 10^8$ cells$)^{-1}$ (22°C)	11
York River	21% day^{-1} (20°C)	3
York River	12–30% day^{-1}	This study
In vitro		
Aburatsubo Inlet	50 mg $month^{-1}$ (22°C)	17, 18
Barataria Bay	47 mg $month^{-1}$ (22°C)	5
Atlantic Shelf	1.6 mg day^{-1} 6×10^7 $cells^{-1}$ (12°C)	11
Simulated seabed model		
Puget Sound	18.8 mg g^{-1} day^{-1}	12
Pacific Coast	4.6 mg g^{-1} day^{-1}	12
In situ: Barataria Bay	35–118 mg g^{-1} day^{-1}	5

C_d reported in these studies was due in part to the use of native or unprocessed chitin as the substrate. Original conceptions of the recalcitrance of chitin have been perpetuated by the use of harshly purified chitin, a substrate more resistant to biological attack than the native form (6, 7). The analogy of using a piece of filter paper to determine the decay rate of a leaf is apropos.

Chitin mineralization in the water column was rapid, tightly coupled with degradation, and nearly complete. Almost all of the initial chitin was degraded and mineralized to CO_2 with no measurable dissolved radiolabel found in the medium. All of the sediment inocula exhibited a decoupling between degradation and mineralization with the formation of a significant soluble pool of ^{14}C label. There are four possible explanations regarding the uncoupling between degradation and mineralization in sediments: (i) increased grazing pressure in sediments, (ii) differential use of chitin between water column and sediment bacteria, (iii) greater exocellular chitinase production by sediment bacteria relative to uptake, and (iv) low dissolved-oxygen-related bacterial formation of soluble metabolic intermediates in sediments. It is possible that increased grazing pressure occurred in the sediments, which may have resulted in a larger dissolved organic carbon (DOC) concentration pool. This could also help explain the rapid decline in numbers of chitinoclastic bacteria after exponential growth. However, bacterial numbers declined substantially in all flasks, so grazing pressure can only partially explain the uncoupling.

It is not probable that water column bacteria rapidly decomposed chitin to CO_2 without the formation of a cellular component. One would have to assume that water column bacteria used chitin solely as an energy source, but the fact that bacterial numbers increased by 3 orders of magnitude suggests that some chitin C must have been used to build biomass. In addition, one might expect that a ^{14}C-labeled fraction of free-living water column bacteria would be recovered in the dissolved fraction because their small size and individual nature would allow them to pass through the GF/A filter. But this was not the case, possibly because rapid bacterial colonization of the chitin particles may have limited the substrate dissolution to the medium by promoting tight coupling between chitin-degrading and -respiring bacteria.

Another possibility is that sediment bacteria produce more exocellular chitinase relative to water column bacteria. Hoppe et al. (8) found that the ratio of peptide hydrolysis to uptake of released leucine by water column bacteria varied between 3 and 38 and that the "liberation of leucine increases much faster than its uptake at increasing concentrations of the model peptide substrate." The ratio of chitin degradation to mineralization (Table 1, C_d/C_m) seems to support this hypothesis. The C_d/C_m ratio of the water column inoculum was lower than those of the sediments, and this trend was consistent for both temperatures. It is possible that there is a difference in community structure between water column and sediments, with a preponderance of chitin degraders in sediments relative to mineralizers.

The other explanation is that much more low-molecular-weight DOC was produced in sediments as a result of chitin fermentation. It is very possible that there existed anoxic microzones in the sediment which allowed facultative anaerobic chitin-degrading bacteria, *Cytophaga* and *Vibrio* spp., etc. (1, 3, 15), to ferment chitin to glucose and acetate.

The effect of temperature on C_d and C_m in the sediments was consistent with the expected Q_{10}, where a doubling in rate occurred with a 10°C increase. In contrast, the apparent Q_{10}s of the water column C_d and C_m values were only 1.1 and 1.3. There are five possible explanations for this effect. The first is that bacteria in the water column are less temperature sensitive than those in the sediments. Another possibility is that the species composition of chitinoclastic bacteria in the estuary fluctuates with water temperature and season. The MPN dilution series method for enumeration of chitinoclastic bacteria lends itself to functional, not taxonomic, grouping. A third hypothesis is that the bacterial community structure of sediment and water column is different. A fourth hypothesis is that the bacterial activity became nutrient limited during the 25°C incubation. Previous studies have shown that nutrient amendments can increase the total amount of chitin degraded but not C_d (6, 12). Nutrient levels were not measured during incubations, but because of the low C/N ratio of chitin itself (8:1), nitrogen limitation would not be expected. In fact, the degradation of chitin can be a source of NH_4 to the system (3); it is more probable that bacteria would become phosphorus limited. The availability of oxygen is the fifth important factor, but care was taken to ensure that the flask headspace volume was large enough to preclude this occurrence.

A conceptual model of chitin C cycling in the York River estuary is depicted in Fig. 4. The different chitin-degrading bacterial guilds (CDB1, -2, and -3) are distinctions based on previous data (1, 2). It is probable that some species of bacteria are components of multiple guilds. For instance, Boyer (1) isolated both facultative and obligate anaerobic chitinoclastic bacteria from salt marsh sediments. Interspecific competition (actually interguild competition) between bacteria for the metabolic end products of chitin degradation has been shown to fuel both sulfate reduction and methanogenesis in estuarine sediments (1, 15). More research is needed to parameterize the model. We need to know such things as the annual production of chitin in an estuary and its distribution in terms of partitioning between water and sediment before we can ascertain the true importance of chitin cycling to ecosystem function.

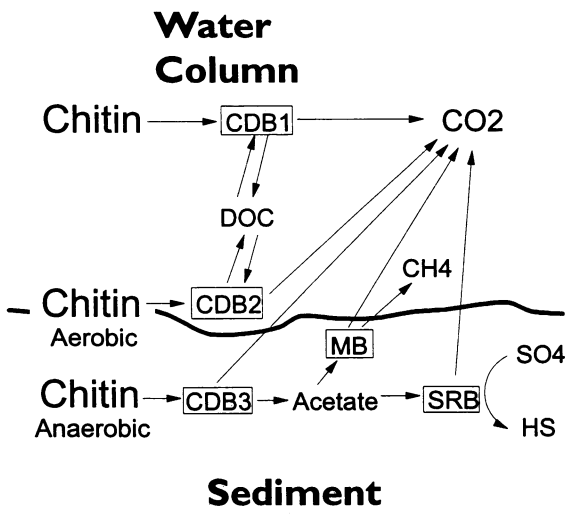

FIG. 4. Conceptual model of chitin degradation and mineralization in the York River estuary. Abbreviations: CDB, chitin-degrading bacteria; MB, methanogenic bacteria; SRB, sulfate-reducing bacteria.

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REFERENCES

1. **Boyer, J. N.** 1986. End products of anaerobic chitin degradation by salt marsh bacteria as substrates for dissimilatory sulfate reduction and methanogenesis. Appl. Environ. Microbiol. **52:** 1415–1418.
2. **Boyer, J. N.** Unpublished data.
3. **Boyer, J. N., and H. I. Kator.** 1985. Method for measuring microbial degradation and mineralization of ^{14}C-labeled chitin obtained from the blue crab, *Callinectes sapidus*. Microb. Ecol. **11:**185–192.
4. **Chan, J. G.** 1979. The occurrence, taxonomy, and activity of chitinoclastic bacteria from sediment, water, and fauna of Puget Sound. Ph. D. thesis. University of Washington, Seattle.
5. **Gooday, G. W.** 1990. The ecology of chitin degradation, p. 387–430. *In* K. C. Marshall (ed.), Advances in microbial ecology, vol. 11. Plenum Publishing Corp., New York.
6. **Hood, M. A.** 1973. Chitin degradation in the salt marsh environment. Ph. D. thesis. Louisiana State University, Baton Rouge.
7. **Hood, M. A., and S. P. Meyers.** 1977. Rates of chitin degradation in an estuarine environment. J. Oceanogr. Soc. Jpn. **33:**328–334.
8. **Hoppe, H.-G., S.-J. Kim, and K. Gocke.** 1988. Microbial decomposition in aquatic environments: combined process of exocellular enzyme activity and substrate uptake. Appl. Environ. Microbiol. **54:**784–790.
9. **Johnstone, J.** 1908. Conditions of life in the sea. University Press, Cambridge.
10. **Kator, H.** 1978. Studies on the distribution, abundance, and activities of heterotrophic and petroleum degrading bacteria from Middle Atlantic Continental Shelf waters and sediments. *In* Middle Atlantic Outer Continental Shelf environmental study, vol. IIC. Chemical and biological benchmark studies. Bureau of Land Management, U.S. Department of Interior, Washington, D.C.
11. **Kleinbaum, D. G., and L. L. Kupper.** 1978. Applied regression analysis and other multivariate methods. Duxbury Press, Boston.
12. **Lister, D.** 1979. M. A. thesis. Chitinoclastic bacteria from the Mid-Atlantic Shelf: distribution, taxonomy, and effect of oil on growth and chitin degradation. College of William and Mary, Williamsburg, Va.
13. **Liston, J., W. J. Wiebe, and B. Lighthart.** 1965. Activities of marine benthic bacteria, p. 39–41. Research in Fisheries, contribution no. 184. College of Fisheries, University of Washington, Seattle.
14. **Montgomery, M. T., N. A. Welshmeyer, and D. L. Kirchman.** 1990. A simple assay for chitin: application to sediment trap samples from the subarctic Pacific. Mar. Ecol. Prog. Ser. **64:**301–308.
15. **Pel, R., and J. C. Gottschal.** 1986. Mesophilic chitin-degrading anaerobes isolated from an estuarine environment. FEMS Microb. Ecol. **38:**39–49.
16. **Rizzo, W. R.** 1986. The community metabolism and nutrient dynamics of a shoal sediment in a temperate estuary with emphasis on temporal scales of variability. Ph. D. thesis. College of William and Mary, Williamsburg, Va.
17. **Seki, H.** 1965. Microbiological studies on the decomposition of chitin in marine environments. IX. Rough estimation on chitin decomposition in the ocean. J. Oceanogr. Soc. Jpn. **21:**253–260.
18. **Seki, H.** 1965. Microbiological studies on the decomposition of chitin in marine environments. X. Decomposition of chitin in marine sediments. J. Oceanogr. Soc. Jpn. **21:**261–269.