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Microbial breakdown of *Phaeocystis* mucopolysaccharides

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Abstract

Mucus from the microalga *Phaeocystis* was partially purified and used as the C source in various stable enrichment cultures wherein the mucus carbohydrates were degraded under oxic as well as anoxic conditions. The breakdown of mucopolysaccharides (initially at a rate of 50% in 11 d at 12°C) markedly slowed after about 15 d, leaving a fraction nondegraded. The carbohydrate composition of the residual fraction did not change during breakdown. It was shown that the incomplete degradation of mucopolysaccharides was not due to inherent resistance to breakdown in parts of the mucus carbohydrates but to the release of inhibitors produced during breakdown. The (potentially high) overall degradability of mucopolysaccharides has important consequences for the fate of C fixed by *Phaeocystis.* The balance between recycling of *Phaeocystis* biomass in surface waters and its accumulation, sedimentation, and burial in the sediment will greatly depend on factors such as nutrient availability, presence of inhibiting agents, and composition of the microbial community, rather than on structural impediments inhibiting mucopolysaccharide degradation.

One of the major processes in the global C cycle is the turnover of photosynthetically fixed organic C in the oceans. Part of this organic C may be ingested by grazers to form the basis of the grazing food chain, and part of it may be channeled into the microbial loop (Azam et al. 1983); thus, energy and nutrients are recycled within surface waters. Alternatively, organic C compounds may aggregate and escape from the euphotic zone by sinking to deeper layers of the ocean, where slow remineralization may exclude them from the C cycle for thousands of years (Williams and Druffel 1987). Only 1% of the primary production may reach the ocean floor in deeper waters (Wefer 1989), whereas in coastal waters, this may be up to 50% (Walsh et al. 1981). Sedimentation of biomass into deeper waters will usually follow accumulation of organic matter in the euphotic zone. An example of such an event is an algal bloom, in which primary production exceeds the combined losses due to predation and microbial degradation. The impact of net organic C accumulation in surface waters on sedimentation is modified by the chemical composition of the material, because composition determines the quality of a substrate as food source for various heterotrophs, and thereby its turnover (Moriarty and Bell 1993).

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A significant portion of the primary production enters the pool of particulate and dissolved organic matter in the form of bacterial and microalgal exudates, which may constitute up to 40% of net primary production (Gieskes and van Bennekom 1973). The microalga *Phaeocystis* sp. produces large amounts of carbohydrates, some of which form a mucus layer in which cells are embedded, resulting in the formation of hollow colonies up to 2 cm in diameter (van Boekel 1993). One-third (van Rijssel et al. 1997) or more (Lancelot 1995) of the total C present in the colonies can be attributed to carbohydrates. *Phaeocystis* colonies often dominate temperate and polar seas during dense spring blooms (Lancelot et al. 1987; Davidson and Marchant 1992) and hence, contribute substantially to the input of organic matter to these ecosystems.

The fate of the *Phaeocystis* biomass that accumulates during blooms is still unresolved. Potentially, it could be grazed, sink to the ocean floor, or be remineralized by bacteria. *Phaeocystis* cells and colonies can be ingested by zooplankton (Weisse et al. 1994); however, grazing is more likely to contribute to, rather than cause, the disappearance of blooms (Wassman et al. 1990; Riebesell et al. 1995). Although *Phaeocystis* biomass has been found in sediment traps (Wassman et al. 1990) and on the top of sediment cores (Riebesell et al. 1995), direct sedimentation of cells and colonies does not seem to contribute significantly to vertical flux of *Phaeocystis* primary production. In contrast, sedimentation of mucus flocs derived from the colonies could be a route by which C assimilated during *Phaeocystis* blooms may sink to greater depths (Passow and Wassman 1994; Riebesell et al. 1995; Hong et al. 1997). The availability of mucus as a growth substrate for heterotrophic bacteria is still unclear. Field observations suggest that although

bacteria attach to colonies during late bloom stages (e.g., Thingstad and Billen 1994), at least part of the mucus is not degraded rapidly because mucus-derived foam accumulates after blooms (Eberlein et al. 1985; Billen and Fontigny 1987; Lancelot et al. 1987). The presence of mucus in sediments also raises the interesting question of whether the material can be degraded under anoxic conditions after it is buried in the sediment. In addition, the relatively constant, complex composition of mucus carbohydrates throughout various spring blooms (Janse et al. 1996*a*) could be interpreted as an indication for the absence of significant degradation. In the only direct study on the biodegradability of *Phaeocystis,* Osinga et al. (1997) found that marine bacteria are able to degrade part of the *Phaeocystis* biomass. However, as degradation was only monitored by total organic carbon (TOC) measurements of nonpurified biomass, nothing could be concluded about the nature of the organic matter that was degraded or about the colony components it was derived from. Lysis products, such as the intracellular storage polymer laminaran (van Boekel et al. 1992; Brussaard et al. 1995; Janse et al. 1996*b*), may account for the initial rapid breakdown that was found; whether the complex extracellular mucus carbohydrates were degraded could not be determined. Therefore, data available so far neither suggest nor exclude breakdown of mucus carbohydrates.

In this study, the degradability of *Phaeocystis* mucus carbohydrates by heterotrophic bacteria has been studied directly using partially purified mucus as a substrate in oxic as well as anoxic enrichments of marine bacteria. Characterizing the potential degradability of this important fraction of *Phaeocystis* biomass is of prime interest to understand the impact of *Phaeocystis* blooms on the marine C cycle. The turnover of this material in the water column affects the transfer of organic matter to higher trophic levels and thus influences the flux of organic matter to the sediment.

Materials and methods

Mucus preparation—Phaeocystis biomass was collected by dragging a plankton net (100-μm mesh size) just below the surface through a dense *Phaeocystis* bloom ($>50 \times 10^6$ cells L^{-1}). Biomass dominated by *Phaeocystis globosa* collected during the 1994 spring bloom in the North Sea off the Texel coast (the Netherlands) was given the code "NS." When collected during the 1996 spring bloom in the Balsfjord (Norway), dominated by *Phaeocystis pouchetii,* the code was "BF." Immediately after harvest, the concentrated dark-brown to black mucous material was scraped from the net and stored in 5-liter portions at -20°C. After thawing, solubilization of the material was increased using a tissue homogenizer and heating at 80°C for 2 h. Approximately 95% of the biomass (based on TOC concentrations) was removed by centrifugation (1 h at 29,000 $\times$ *g*), and the protein present in the supernatant was digested for 3 h at 40°C at pH 7.5 using 25 μg ml^{-1} pronase E from *Streptomyces griseus* (Fluka Biochemika). This solution was dialyzed against 20 volumes of demineralized water at 4°C that was replaced eight times over 4 d, using dialysis tubing (15-mm diameter) with pore size 6–8 kD (Spectra/Por, Spectrum Medical Industries). During dialysis, 85% of the mucus (based on TOC concentrations) was retained in the dialysis tubing. The concentration of the retentate after dialysis was between 6 and 8 mM glucose equivalents (measured by the phenol-sulfuric acid method, *see below*), with the exact concentration depending on the batch that was prepared from the frozen *Phaeocystis* biomass stocks. More concentrated mucus, required for some experiments, was prepared by reducing the volume of dialyzed mucus by partial freeze drying. Before storage, mucus solutions were heat sterilized (121°C for 20 min) after the addition of 3% NaCl. Heat sterilization did not affect the monosaccharide composition (measured by gas-chromatographic analysis, *see below*). Also, the average chain length of mucopolysaccharides appeared to remain unchanged (10^6 dalton as measured by high-pressure liquid chromatography, *see* Janse et al. 1996*b*) during heat sterilization (data not shown).

Media—Medium composition was adapted from Veldhuis and Admiraal (1987). For growth under oxic conditions, a buffered basal salts medium was used containing the following (concentrations in grams per liter deionized water): NaCl (24.5); $MgCl_2 \cdot 6H_2O$ (9.8); Na_2SO_4 (3.2); $CaCl_2 \cdot 2H_2O$ (0.53); K_2SO_4 (0.85); Na_2HPO_4 (0.04); NH_4Cl (0.5); $NaHCO_3$ (0.2); minor salt solution (2 ml L^{-1}); trace element solution (2 ml L^{-1}); vitamin solution (1 ml L^{-1}); and tris(hydroxymethyl)methylamine (Tris) HCl pH 7.5 (5 ml L^{-1}). Ammonium, phosphate, bicarbonate, and minor salt and trace element solutions were autoclaved separately and added as small, concentrated volumes to the autoclaved basal salts after cooling to room temperature. The vitamin solution was filter sterilized and added separately. The minor salt solution contained the following (concentrations in grams per liter deionized water): KBr (22); $SrCl \cdot 6H_2O$ (6.39); $AlCl_3$ (0.028); H_3BO_3 (0.4); RbCl (0.061); LiCl (0.006); and KI (0.02). The trace element solution contained the following (concentrations in grams per liter deionized water): $FeCl_3 \cdot 6H_2O$ (3.15); $Na_2EDTA \cdot 2H_2O$ (4.82); $CoCl_2 \cdot 6H_2O$ (0.01); $MnCl_2 \cdot 4H_2O$ (0.18); $Na_2MoO_4 \cdot 2H_2O$ (0.006); $CuSO_4$ (0.007); and $ZnSO_4 \cdot 7H_2O$ (0.022). The vitamin solution was adapted from Schut et al. (1993) (concentrations in milligrams per liter deionized water): p-aminobenzoic acid (50); pyridoxine-HCl (100); thiamine-HCl (50); riboflavin (50); nicotinic acid (50); D-Ca-pantothenate (50); lipoic acid (50); nicotinamide (50); vitamin B12 (50); biotin (20); and folic acid (20). No buffer was added when the enrichment culture was sampled for TOC measurements. For anoxic medium, the concentration of $CaCl_2 \cdot 2H_2O$ in the basal salts medium was reduced from 0.53 to 0.1 g L^{-1}. An anoxic bicarbonate solution of 84 g L^{-1} $NaHCO_3$ and an anoxic sulfide solution of 39 g L^{-1} Na_2S were prepared in boiled and then cooled deionized water and were subsequently autoclaved and stored under N_2CO_2 (80/20%). The concentrated ammonium solution, resazurin (0.0001%), and yeast extract (0.01%) were added to the basal salt medium, which was flushed with N_2/CO_2 (80/20%) at 80°C and autoclaved. After cooling to room temperature, the concentrated phosphate solution, minor salt solution, trace element solution, vitamin solution, anoxic bicarbonate solution (30 ml L^{-1}), and sulfide solution (1 ml L^{-1}) were added under aseptic and anoxic conditions.

Media intended to inhibit sulfate reduction and methanogenesis were sulfate free and contained BES (2-bromoethanesulphonate). Na_2SO_4 in the major salts solution was replaced by 2.7 g L^{-1} NaCl, and BES was added to a concentration of 50 mM.

Growth and sampling—Aerobic enrichment cultures were grown in cotton-plugged Erlenmeyer flasks (medium volume = ≤20% of the maximum Erlenmeyer volume) or culture tubes (5 ml medium in 20-ml tubes) that were incubated statically at 12°C. Hungate anaerobic techniques (Hungate 1969) were used for anaerobic enrichment cultures. Partially purified mucus was added to seawater medium to obtain a starting concentration of approximately 2 mM glucose equivalents (measured by the phenol-sulfuric acid method, *see below*). This concentration was chosen to ensure sufficiently high mucopolysaccharide concentrations throughout mucus degradation for application of the various analytical procedures. At regular intervals, the cultures were vortexed, samples were withdrawn aseptically, and bacteria were centrifuged (15 min at 8,000 × *g*). The supernatant was stored at −20°C for further analysis. The occurrence of mucopolysaccharide degradation was assessed by following changes in the total carbohydrate concentration in the culture medium (using the phenol-sulfuric acid method).

Enrichment cultures from various inocula—To obtain stable mucopolysaccharide-degrading enrichment cultures, various inocula collected in 1996 were used to inoculate media that contained as the C source the partially purified mucus derived from the two sources (NS and BF) described above. From each inoculum, enrichment cultures were propagated by transferring a portion of the culture into 10 volumes of fresh medium after mucopolysaccharide degradation had come to a halt (usually 3–4 weeks after inoculation). Assessment of mucopolysaccharide degradation was as described above. Because the primary goal in these experiments was to establish enrichment cultures from different sources, these initial enrichments were not monitored in sufficient detail to enable comparison of degradation rates.

Inocula from seawater were obtained on 24 April (peak of bloom), 8 May (declining bloom), and 4 December (5 months before the following spring bloom) from the Marsdiep (Dutch Wadden Sea). On each of these dates, two replicate inocula were prepared from each of two separate samples. From the BF, two replicate inocula were prepared from each of two separate samples that were taken on 16 April, 30 April, and 21 May (all during the spring bloom). To prepare the inocula, bacteria from 100 ml seawater that had been collected in sterile flasks were harvested by centrifugation (15 min at 8,000 × *g*). After resuspension in 2.5 ml of the supernatant, they were filtered (Whatman GF/F) to minimize numbers of protozoa and transferred to 25 ml sterile seawater medium containing partially purified mucus.

Inocula for mucus-attached and for nonattached bacteria were obtained from the BF on 16 April, 30 April, and 21 May. On each of these dates, two replicate inocula were prepared from each of two separate samples, one from the surface and one from the chlorophyll maximum. To prepare inocula containing mucus-attached bacteria, mucus was collected on a 4.7-cm-diameter, 100-μm mesh-size plankton net by pouring seawater with *Phaeocystis* colonies over it until it clogged. The mucus was then removed from the net by extensively shaking the net in 10 ml sterile seawater medium. The resulting mucus suspension was filtered (Whatman GF/F), and 2.5 ml of the filtrate was used to inoculate 25 ml sterile seawater medium containing partially purified mucus. Inocula from nonattached bacteria were obtained by centrifuging (15 min at 8,000 × *g*) 100 ml of filtrate after filtration through a 10-μm mesh-size filter. After resuspension in 2.5 ml of the supernatant and filtration (Whatman GF/F), the bacterial suspension was used to inoculate 25 ml sterile seawater medium containing partially purified mucus.

Two replicate inocula for bacteria from sediment were obtained on 28 June (after bloom) from microbial mats at two distinct locations near the island Schiermonnikoog (Dutch Wadden Sea) and on 16 April, 30 April, and 2 May from the bottom sediment at one location in the BF. To obtain aerobic bacteria from sediment, approximately 10 ml of sediment was mixed with two volumes of sterile seawater medium, extensively shaken, and the resulting slurry filtered through Whatman GF/F. An aliquot of 2.5 ml of this filtrate was used to inoculate 25 ml of sterile seawater medium containing partially purified mucus. For anaerobic bacteria, 10 ml of sediment was mixed with two volumes of anoxic (autoclaved) seawater and kept under an N_2/CO_2 (80/20%) atmosphere. Without prior filtration, 1.5 ml of this slurry was used to inoculate 13.5 ml anoxic sterile seawater medium containing partially purified mucus.

Mucopolysaccharide degradation in enrichment cultures—The stable enrichment culture that was used for characterization of NS mucopolysaccharide degradation was originally inoculated with bacteria concentrated from Marsdiep seawater harvested on 8 May 1996. The enrichment culture that was used for characterization of BF mucopolysaccharide degradation was originally inoculated with bacteria concentrated from BF seawater harvested on 30 April 1996. Mucopolysaccharide degradation was assessed by following changes in the total carbohydrate concentration in the culture medium (using the phenol-sulfuric acid method). For analysis of the carbohydrate composition, samples were dialyzed before carbohydrate analysis against 60 volumes of demineralized water at 4°C that was replaced six times over 2 d using dialysis tubes with pore size = 1 kD (Viskin size 1-8/32 in., Medicell International).

In experiments in which bacteria adapted to NS mucus degradation were transferred to medium containing BF mucus and vice versa, the bacteria adapted to NS degradation were originally concentrated from Marsdiep seawater harvested on 24 April and 8 May 1996, and the bacteria adapted to BF degradation were originally concentrated from BF seawater harvested on 30 April and 21 May 1996. A portion of the enrichment culture adapted to NS degradation was transferred into 20 volumes of fresh medium with BF mucus and vice versa.

Studies on anaerobic mucopolysaccharide degradation were done using enrichments originally inoculated with sediment collected on 21 May from the BF. The enrichments were incubated in 15-ml tubes, from which samples were

taken at regular intervals under aseptic and anoxic conditions (without prior vortexing of the cultures) and stored for further analysis.

Inhibition experiments—For inhibition experiments, bacteria in enrichment cultures were originally concentrated from Marsdiep seawater harvested on 8 May 1996. A stable enrichment culture in which degradation of mucus from the NS location (*see above*) had come to a halt after 50 d was centrifuged (15 min at 8,000 × *g*), and the pellet was resuspended in a small volume of seawater medium for later use as inoculum. The spent medium was divided into three equal portions. One portion was dialyzed against 60 volumes of seawater medium (4°C), replaced two times, using dialysis tubes with pore size = 1 kD (Viskin size 1-8/32 in., Medicell International), and subsequently, all three portions were autoclaved. Standard amounts of nutrients and vitamins used for medium preparation were added to the dialyzed spent medium portion and to one of the untreated spent medium portions. All three portions were inoculated with the resuspended pellet, and the total carbohydrate concentration was monitored during incubation over a period of 48 d.

In a second experiment, a stable enrichment culture in which degradation of mucus from the NS location had come to a halt after 50 d was centrifuged (15 min at 8,000 × *g*), and the pellet was resuspended in a small volume of seawater medium for later use as inoculum. The first treatment consisted of spent medium (5 ml), concentrated mucus (1 ml of a 22-mM glucose equivalents stock), and standard amounts of nutrients and vitamins. The second treatment consisted of the same solutions, except that 5 ml seawater medium was used instead of the spent medium. Treatments were inoculated with the resuspended pellet, and the total carbohydrate concentration was monitored for 35 d. The inhibition experiments were carried out in triplicate.

Mucopolysaccharide degradation using inocula directly from a natural bloom—Inocula used in experiments to compare the rate of mucopolysaccharide degradation during different bloom stages were obtained on 26 March (prebloom), 29 April (peak bloom), and 22 May (declining bloom) 1997 from the Marsdiep (Dutch Wadden Sea). To prepare the inocula, bacteria from 100 ml seawater that had been collected in sterile flasks were harvested by centrifugation (15 min at 8,000 × *g*). After resuspension in 2.5 ml of the supernatant, they were filtered (Whatman GF/F) to minimize protozoa and transferred to 25 ml sterile seawater medium containing partially purified mucus. This was carried out in triplicate. Culture conditions and assessment of the occurrence of mucopolysaccharide degradation were as described above.

Analytical procedures—Algal cells were counted as described by Cadée (1996). Total carbohydrate concentrations were routinely determined as glucose equivalents by the modified phenol-sulfuric acid method described by Liu et al. (1973) using glucose as a standard. In this colorimetric method, the response of different types of monosaccharides varies. As a result, the concentration of mucus measured using this method (expressed in glucose equivalents) is an underestimation of the actual concentration. The underestimation was determined to be ≤40% of the added concentration in preliminary experiments with defined mixtures of polysaccharides known to be present in the mucus, depending on the exact mixture of the monosaccharides. The sugar composition was determined according to Janse et al. (1996*a*); samples were subjected to methanolysis (2.0 M Methanolic HCl, 24 h, 85°C) prior to analysis of the trimethylsilylated (N-reacetylated) methyl glycosides on a Varian 3600 gas chromatograph equipped with an Econocap SE 30 capillary column, 0.32-mm diameter, 30-m length (Alltech) and a flame ionization detector. Identification and calculation of the concentration of sugar derivatives were performed by comparison with known standards. O-methylated pentoses and hexoses are not available as reference sugars, so their concentration was estimated by assuming a calibration factor similar to the mean of those of hexoses and pentoses. TOC was measured on a Shimadzu TOC-500 analyzer (Shimadzu Benelux) with sodium biphtalate as a standard.

Eurosequence BV carried out quantification of proteins. Samples were hydrolyzed (2 h in the gas phase with 5.7 N HCl at 166°C), and the resulting amino acids were analyzed on an HP 1090 Aminoquant using an automated two-step precolumn derivatization with OPA (ortho-phtalic-aldehyde) and FMOC (9-fluorenyl-methyl-chloro-formate).

Sulfide was measured colorimetrically (Visscher and van Gemerden 1991). Methane and hydrogen were measured on a Shimadzu gas chromatograph (GC-14B) equipped with an Alltech econo-cap EC-1000 (FFAP) column (0.53-mm diameter, 15-m length) and a flame ionization detector, using a column temperature of 40°C and a detector temperature of 300°C. Fatty acids were analyzed according to van der Maarel et al. (1996).

Curie-point pyrolysis gas chromatography-mass spectrometry analyses were carried out as described by van Heemst et al. (1996). The column used was a CP Sil-5 (25 m × 0.32 mm, film thickness = 0.32 μm), and the m/z was 50–800.

Results

Mucus preparation—A preparation of mucus relatively free of contamination by intracellular compounds was needed for assays to monitor mucopolysaccharide breakdown. *Phaeocystis* biomass was harvested during spring blooms dominated by this alga. Attempts to separate the mucus from cells without cell disruption were not successful, due to the fragility of *Phaeocystis* cells. Therefore, proteins and small molecules in the complex mixture of soluble cellular compounds and mucus were removed. To determine the efficiency of the purification procedure, the overall composition was analyzed before and after purification of mucus (collected at the North Sea; NS batch). The carbohydrate content increased from 60 to 80% of the TOC measured in the mucus, and protein decreased from 20 to 5%. The carbohydrate composition of the material remained virtually unaltered and was similar to the composition reported previously (Janse et al. 1996*a*). Hence, the resulting partially purified mucus was enriched in its mucus carbohydrate content, and a significant fraction of the proteins (of cellular origin) had been removed.

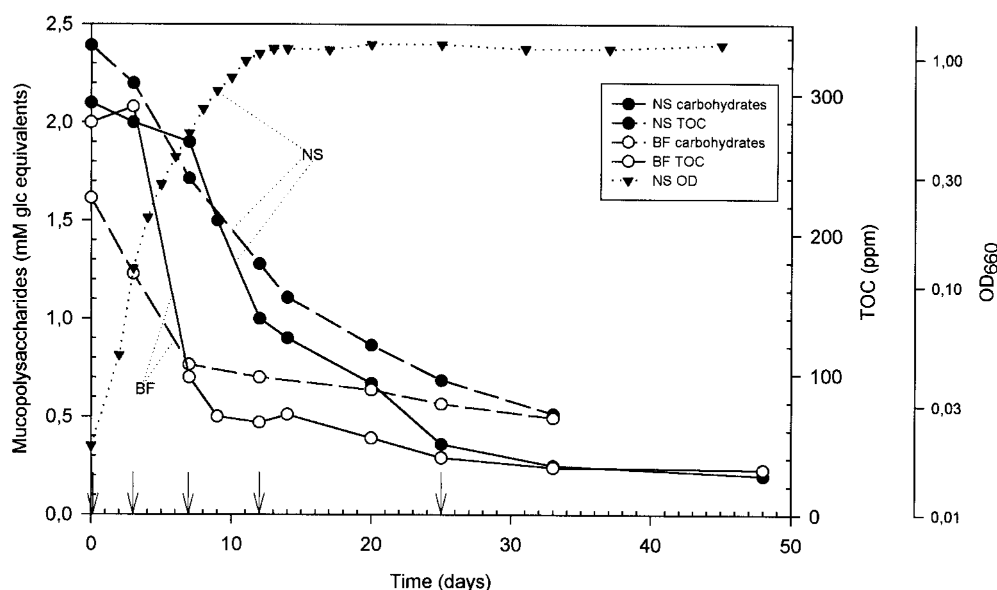

Fig. 1. Breakdown of semipurified *Phaeocystis* mucus. Carbohydrate concentration (solid lines) and total C concentration (dashed lines) were measured in enrichments with NS or BF mucus and inoculum. Optical density at 660 nm was measured in the NS enrichment culture. The arrows indicate when samples were withdrawn for analysis of mucus carbohydrate composition (*see Fig. 2*).

Aerobic mucus degradation in stable enrichment cultures—To obtain stable, mucopolysaccharide-degrading enrichment cultures, artificial seawater medium with the partially purified mucus as a sole C source was inoculated with samples with different origins. Enrichment cultures were considered stable if transfer of the bacteria in the culture yielded new enrichments degrading partially purified mucus. Degradation was monitored by measuring the total carbohydrate concentration in the supernatant. After the concentration had declined to <50% of the original value, 10% of the culture was transferred to fresh medium.

When partially purified mucus obtained from the BF was used as the C source, all inoculations that were used resulted in stable enrichment cultures. When mucus collected in the NS was used, however, the initial inoculation in most cases was followed by a small decrease in carbohydrate concentration, but only 12 out of the 50 inocula taken during the spring blooms gave rise to stable enrichment cultures in which the mucus-degrading capacity could be transferred repeatedly. Such stable enrichments were derived from all of the different sources, that is, inocula concentrated from seawater, mucus (“attached” bacteria), 10-μm filtered seawater (“free bacteria”), and sediment. Four inocula derived from seawater harvested 5 months before the start of the bloom did not yield stable mucus-degrading enrichment cultures (using NS mucus as the C source).

Mucopolysaccharide degradation in typical enrichment cultures with NS and BF mucus substrate was characterized by rapid degradation in the beginning, followed by the breakdown leveling off and a small fraction of the mucus remaining undegraded (Fig. 1). In the NS enrichment, the bacteria had a growth rate of 0.23 d^{-1}, and the stationary growth phase was reached after 10 d (OD_{660} = 1). The maximum (initial) degradation rate was lower in the NS enrichments (50% decrease in 11 d) than in the BF enrichments (50% in 4 d). When the concentration had dropped below 0.5 mM glucose equivalents, the breakdown decreased significantly. In these enrichment cultures, typically between 0.2 and 0.5 mM glucose equivalents mucus substrate remained undegraded (which was confirmed by GC analysis of the residual substrate). The trends that were based on total carbohydrate analyses were confirmed by TOC measurements (Fig. 1).

To exclude the possibility that incomplete degradation of partially purified mucus was due to the assay conditions used, the following control experiments were carried out in one representative enrichment with NS mucus. Possible limitation of a medium component was investigated by the addition of ammonium and phosphate solutions, trace elements, and vitamins in two times the quantities that were used for the preparation of standard medium. Partially purified yet nonautoclaved mucus was used to exclude an effect of heating on the degradability of the substrate. Neither affected the rate or the extent of mucus breakdown. In addition, sterilizing the inoculum completely prevented mucus breakdown. This excluded the possibility that binding of mucus polymers to bacterial cells (which were removed before carbohydrate concentration measurements) accounted for the observed carbohydrate removal.

Mucus composition during degradation—In none of the stable enrichments was the mucus completely degraded. To investigate whether the resistance to further breakdown could be explained by accumulation of a refractory residue due to selective degradation, the relative proportions of sugar moieties in mucus carbohydrates were followed during

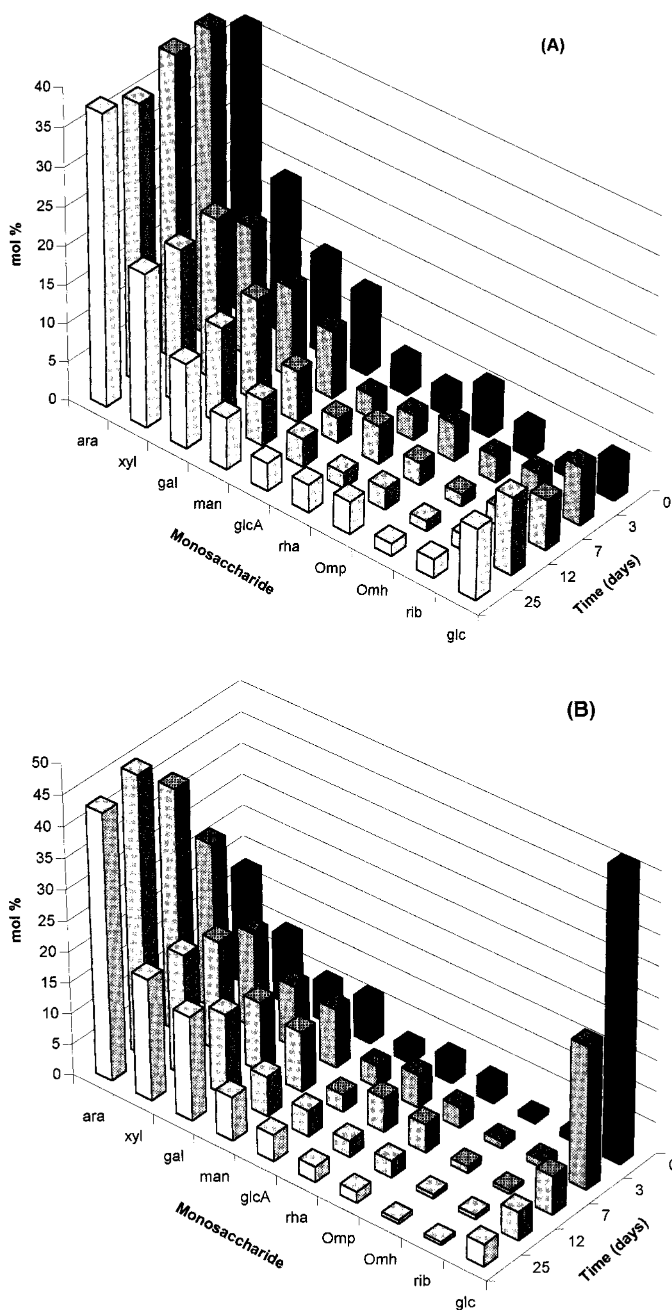

Fig. 2. Carbohydrate composition during *Phaeocystis* mucus breakdown. On the days indicated in this figure, the carbohydrates in the NS and BF enrichments (*see arrows in Fig. 1*) were analyzed. The molar percentage of the monosaccharides in the mucus is given for the NS (A) and BF (B) enrichments. Abbreviations: ara, arabinose; xyl, xylose; gal, galactose; man, manose; glc, glucose; rha, rhamnose; glcA, glucuronic acid; Omp, O-methylated pentoses; Omh, O-methylated hexoses; rib, ribose.

breakdown. In the NS enrichment culture, no significant changes in carbohydrate composition occurred during the first 25 d, when almost all mucus had been degraded (Fig. 2A). However, for the BF enrichment, a dramatic decrease in the relative contribution of glucose was observed, whereas the ratios between the other monosaccharides remained unchanged (Fig. 2B). In samples taken on days 0 and 25, mucus from the NS enrichment culture was analyzed using pyrolysis GC-MS (data not shown). The pyrolysates of this *Phaeocystis* mucus were very similar, showing that no changes had occurred in the composition of the material during degradation.

At the start of the incubation, the fraction of TOC measured as carbohydrate was higher for the BF compared to the NS enrichment culture (compare the ratios between solid and interrupted lines for both enrichments in Fig. 1). After breakdown had slowed down, this ratio was identical. The decrease in carbohydrates that was measured in both enrichment cultures (1.8 mM glucose equivalents that would correspond to 10.8 mM C) was comparable with the decrease in organic C in the BF enrichment culture (12.3 mM C), but in the NS enrichment, the decrease in organic C was substantially more (19.9 mM C).

Besides the differences during breakdown of BF and NS mucus mentioned above, the enrichment cultures differed in that transfer of bacteria from an enrichment adapted to NS mucus degradation into BF-containing medium resulted in almost full degradation of the BF mucus (data not shown, but these are very similar to those shown for BF mucus in Fig. 1), whereas bacteria from a stable BF enrichment could not degrade NS mucus (data not shown).

Inhibition in the enrichment cultures—The production of inhibiting substances was investigated as a possible explanation for the fact that in none of the enrichment cultures were the mucopolysaccharides degraded completely. Mucus from an NS enrichment culture in which the breakdown had stopped was dialyzed against seawater medium, autoclaved, and reinoculated with bacteria from the same enrichment. After 48 d, 66% of this partially degraded, dialyzed mucus had been degraded. In the incubations in which the partially degraded mucus had not been dialyzed, no more than 8% degradation occurred, regardless of nutrient and vitamin addition (Table 1). Furthermore, mucus freshly added to enrichments in which the breakdown had stopped was only degraded when the medium was renewed, not in the spent culture medium (Table 2). Apparently, removal or dilution of dialyzable compounds caused resumption of the breakdown of residual mucus.

When different initial concentrations of mucus were used, degradation consistently stopped after about 2 mM glucose equivalents had been removed (Fig. 3). Comparable concentrations of laminaran (measured as TOC concentration) showed similar inhibition (data not shown). In none of the above experiments had the pH changed, even when the medium was not buffered, arguing against pH-induced inhibition. These data suggest that bacteria produce inhibiting compounds while degrading mucus.

Mucopolysaccharide degradation by inocula directly from a natural bloom—To be able to relate the data obtained for bacteria from mucus-adapted enrichment cultures to the activity of the nonenriched natural microbial community present in the sea, breakdown was also followed using inocula taken directly from seawater. Before, during, and after the 1997 *Phaeocystis* spring bloom, concentrated seawater was

Table 1. Inhibition of mucus breakdown by dialyzable substances in enrichment culture supernatant.*

	Residual mucopolysaccharide concentration		
	Spent medium (no additions)	Spent medium + trace elements, nutrients, and vitamins	Dialyzed spent medium + trace elements, nutrients, and vitamins
Day 0	1.2 mM	1.2 mM	0.9 mM
Day 48	1.1 mM	1.1 mM	0.3 mM
Percentage degraded	8%	8%	67%

* From an enrichment culture in which NS mucus degradation had stopped, bacteria were removed by centrifugation under aseptic conditions and resuspended for later use. The supernatant was not treated; supplemented with extra trace elements, nutrients, and vitamins; or dialyzed against seawater medium and supplemented with trace elements, nutrients, and vitamins before reinoculation with the centrifuged bacteria. The total carbohydrate concentrations were monitored during a 48-d incubation period. Concentrations are given in mM glucose equivalents.

transferred into medium containing partially purified mucus obtained from the NS, and the change in total carbohydrate concentration was followed (Fig. 4). In each case, breakdown of mucopolysaccharides was observed. However, the prebloom inoculum showed a longer lag phase, and the rate was somewhat lower (50% decrease in 36 d) than with inocula collected during or after the bloom (50% decrease in 25 d). These rates were always lower than those found in the stable enrichments described above.

Mucopolysaccharide degradation under anoxic conditions—To see whether *Phaeocystis* mucus was also susceptible to microbial breakdown once it had reached anoxic environments, breakdown assays with partially purified NS mucus were set up in anoxic media, and inocula were prepared from anoxic sediment samples. Stable anoxic enrichments degrading the mucus substrate were obtained. The breakdown pattern was similar to that in the oxic enrichment cultures, but the rate was lower, and a larger fraction remained nondegraded. It took at least 30 d to degrade 50% of the added mucus, and in none of the enrichments was >70% of the mucopolysaccharides degraded (both at 12°C and 25°C). Similar to what was found under oxic conditions, the carbohydrate composition did not change during breakdown (data not shown). Sulfate, methane, acetate, and traces of propionate were detected in the anoxic enrichment cultures. Inhibition of sulfate-reducing bacteria (use of sulfate-free medium) and methanogens (BES addition) did not affect breakdown (data not shown), indicating that anaerobic mucus breakdown may be a fermentative process not dependent on hydrogen transfer to methanogens or sulfate reducers.

Table 2. Inhibition of breakdown of freshly added mucus in enrichment culture supernatant.*

	Residual mucopolysaccharide concentration	
	Spent medium + mucus	Fresh medium + mucus
Day 0	3.9 mM	3.5 mM
Day 35	3.4 mM	1.5 mM
Percentage degraded	13%	57%

* A small volume of concentrated partially purified mucus solution was added either to the spent supernatant of an enrichment in which mucus degradation had stopped or to new seawater medium. After addition of trace elements, nutrients, and vitamins and reinoculation with the bacteria that had been removed from the enrichment by centrifugation, total carbohydrate concentrations were monitored during a 35-d incubation period. Concentrations are given in mM glucose equivalents.

Discussion and conclusions

Differences between mucus collected at different locations (Dutch North Sea and Balsfjord)—Differences between sites were evident in the rate of mucus breakdown and in the ability of bacteria from enrichment cultures that were adapted to degradation of mucus from one site to degrade mucus collected at the other site. These differences are likely explained by the difference in carbohydrate composition between mucus collected at the two sites. Mucus from the BF was distinguished by a relatively high glucose content, which quickly decreased during breakdown (Fig. 2B). Because only the glucose content decreased, whereas the relative proportions of the other monosaccharides remained constant, and because *Phaeocystis* is known to be capable of producing significant quantities of the intracellular storage glucan laminaran (Janse et al. 1996*b*), the high glucose content likely reflects the presence of laminaran in mucus preparations from the BF. The susceptibility of laminaran to microbial breakdown explains the more rapid initial decrease in carbohydrate concentration (Fig. 1) and the concomitant

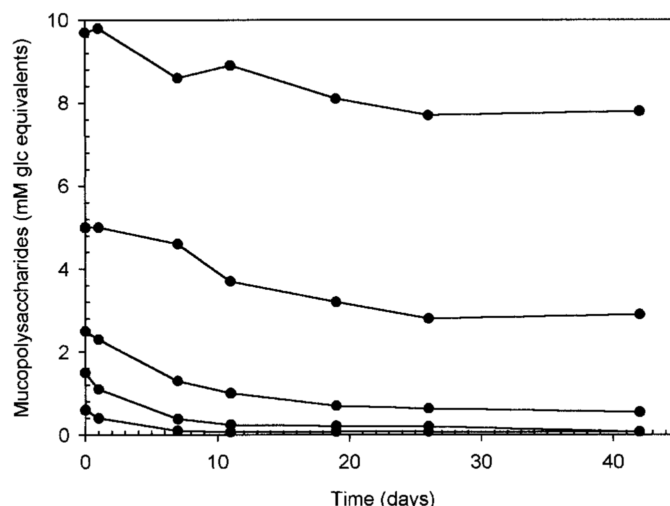

Fig. 3. Breakdown of different concentrations of *Phaeocystis* mucus. The mucus concentration is given in glucose equivalents.

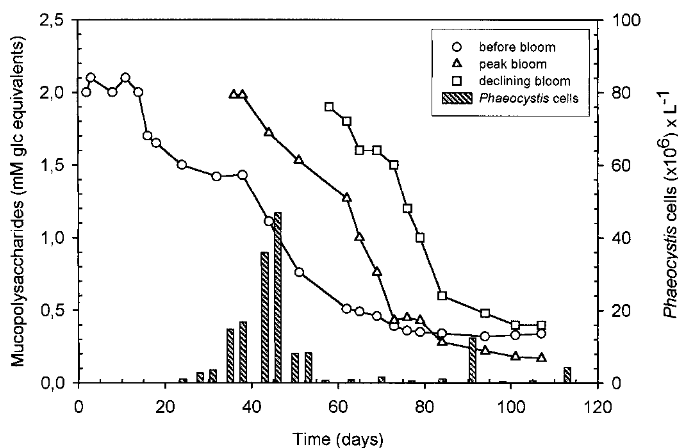

Fig. 4. Breakdown of *Phaeocystis* mucus by inocula collected from a natural bloom at three stages of development. Bars represent the number of *Phaeocystis* cells in the seawater during the 1997 spring bloom in the Marsdiep. Medium containing partially purified mucus was inoculated with bacteria collected before, at the peak, or after the spring bloom.

decrease in glucose (Fig. 2B) in these enrichments. While dominantly present in the mucus collected at the BF, laminaran constituted an insignificant fraction in the mucus collected at the NS (Fig. 2A). Species differences between *P. pouchetii* and *P. globosa* or differences in physiological parameters such as nutrient status could have been responsible for differences in laminaran production (Janse et al. 1996*b*). The activity of grazers and bacteria could have caused differences in the degree of laminaran consumption because *Phaeocystis* cells can be selectively grazed upon, and variation may occur in the degradation by bacteria (Arnosti et al. 1994; Janse et al. 1996*b*) and probably also by protozoa (Sherr 1988) of laminaran leaking from lysed cells. Thus, differences in production and consumption of laminaran between the two sites where *Phaeocystis* had been harvested may explain its absence in NS mucus.

Due to the dominant presence of easily degradable laminaran in mucus derived from the BF, selection in the stable enrichments containing this mucus as a C source would probably have favored microbes growing fastest on laminaran, which are not able to degrade complex mucopolysaccharides. In contrast, in the NS mucus-containing enrichments, the microbial community adapted to the complex mucopolysaccharides as the major substrate. This could explain the inability of inocula from enrichments adapted to mucus from the BF to degrade mucus from the NS; the ability of inocula obtained from enrichment adapted to NS mucus to degrade BF mucus is probably due to the wide spectrum of bacteria capable of degrading laminaran.

Another finding that can be explained by the high laminaran content of mucus collected at the BF is the high ratio between carbohydrate and total C concentrations measured in BF mucus compared to NS mucus before degradation and the identical ratio after degradation (Fig. 1). The colorimetric assay for measuring total carbohydrate concentrations (phenol-sulfuric acid) has a higher response to glucose than to the other monosaccharides in the mucus (Liu et al. 1973). As a result, a higher carbohydrate concentration was measured for BF mucus (due to the high laminaran content) when compared to an equal amount (based on TOC measurement) of NS mucus. After degradation, when laminaran had been selectively consumed in the BF enrichment, this difference in response to the total carbohydrate assay had disappeared. The amount of carbohydrate that was degraded in BF mucus measured as glucose equivalents and as total C matched well (1.8 mM glucose equivalents and 12.3 mM C, respectively). In the NS mucus-containing enrichment, by contrast, the amount of mucus that was degraded when measured as glucose equivalents seemed lower than when total C was measured. This could be explained by the degradation of mucopolysaccharides (with a lower response to the phenol-sulfuric acid method due to the presence of monosaccharides other than glucose) instead of laminaran. This is in agreement with the hypothesis put forward above that in the stable enrichments containing BF mucus, only laminaran and no mucopolysaccharides were degraded.

In conclusion, for studying the degradation of *Phaeocystis* mucopolysaccharides, the partially purified mucus obtained from the NS represented the most relevant substrate because of the insignificant amount of laminaran in this material relative to BF mucus. However, whether the mucopolysaccharides consist of only one or several complex heteropolysaccharides can not be concluded on the basis of our data.

Effects of mucopolysaccharide composition on its degradation—For the breakdown of complex polymers such as *Phaeocystis* mucopolysaccharides, a succession of different degradation steps involving different enzyme systems could be expected, possibly involving activities of several different bacteria (Rheinheimer 1992; Warren 1996). Pectin degradation, for example, requires the action of different enzymes, each releasing fragments of different composition. The pectin chains contain so-called "hairy regions," with varying monosaccharide ratios resulting from unevenly distributed neutral sugar side chains (van Rijssel 1992). In spite of the gradual decrease in the rate of degradation of the *Phaeocystis* (NS) mucopolysaccharides (Fig. 1), the monosaccharide composition remained remarkably constant during breakdown (Fig. 2A). Hence, there was no indication of variation in degradability of different polymer fragments, and the decrease in degradation rate after 25 d may have been caused by factors other than structural impediments in the mucopolysaccharides. The uniform carbohydrate composition during breakdown may suggest that *Phaeocystis* heteropolysaccharides have a rather uniform structure, with repeating units (Aspinall 1983) that are readily processed after they are liberated by enzyme systems. However, it can not be excluded that in the natural environment the mucus contains secondary or tertiary structures, resulting from the chemical nature and size of carbohydrates, that were lost during mucus purification (Decho 1990). Such structures may aid or impede enzymatic access to hydrolytic sites and would thus influence the breakdown pattern. As a comparison, in cellulose, there are alternating amorphous and crystalline regions, differing in the arrangement of their glucose chains. The different types of cellulose are attacked by specific cellulases

(Aubert et al. 1988), leading to different degradation rates in carbohydrates of chemically identical composition.

The degradation rate of a complex substrate could also be affected by gradual depletion of nutrients associated with the substrate during degradation. However, in our experimental setup, this was precluded by the addition of a surplus of nutrients to the medium.

Effect of inhibitors on mucopolysaccharide degradation—Our data suggest that the production of inhibitors by the microbial community may explain the limited breakdown observed in enrichment cultures (Tables 1, 2; Fig. 3). The decrease in breakdown rate of high concentrations of mucopolysaccharides as shown in Fig. 3 appeared to occur when comparable concentrations of laminaran were used instead of mucopolysaccharides as well. Therefore, the inhibiting substances are not likely to be specifically related to mucus degradation per se, but rather, constitute products of the metabolism of the community that had been established in these enrichments grown with relatively high substrate concentrations. The relevance of this inhibitory effect for the situation in the natural environment remains to be seen. In the sea, only at high substrate concentrations—that is, on or in colonies or mucus flocs—could one expect the accumulation of inhibitors to be high enough to hinder breakdown because the inhibitors would readily diffuse away (unless they would bind to colony compounds).

Breakdown rates for mucopolysaccharides—The breakdown rate for mucopolysaccharides (50% in 11 d) was lower than that reported for the rapidly degradable fraction of *Phaeocystis* by Osinga et al. (1997) (50% in 2–3 d). However, it is much higher than the rate they found for a slowly degradable fraction (80–99% in 1 yr). It is likely that the rapidly degraded fraction they reported may have consisted mainly of easily degradable lysis products and that the mucopolysaccharides were part of the slowly degraded fraction. The relatively high breakdown rate of mucopolysaccharides in the current enrichment studies could be explained by several factors, including the addition of medium components such as nutrients and vitamins; the lower concentration and partial purification of mucus that may have minimized the effect of inhibiting or refractory compounds; and the adaptation of the microbial community to the substrate during repeated transfers of the enrichments. This latter factor probably had only a minor impact, because when natural microbial communities that were not specifically adapted were used, there was also considerable mucus breakdown (50% in 25–36 d; Fig. 4). The mucopolysaccharide concentrations that were used in this study are much higher than the mucus concentrations that are measured in seawater during blooms (Janse et al. 1996*a*). However, it is most likely that the microorganisms involved in mucus breakdown will face mucus concentrations in or near intact and lysing colonies that may well be at least as high as what was used in our assays.

These data suggest that, potentially, most of the mucus produced by *Phaeocystis* could be recycled in the euphotic zone within the time span of a bloom (10–140 d, Lancelot 1995), provided the conditions are favorable: a microbial community well adapted to this substrate, ample nutrients, and no inhibiting agents. No indications for the presence of structural impediments preventing breakdown were found. If, however, due to unfavorable conditions, turnover is low in the surface waters, and the mucus sinks and becomes buried in the sediment, we have now demonstrated that the material can also be degraded under anoxic conditions. The degradation rate under anoxic conditions was considerably lower than that reported for several (simple) carbohydrates by Arnosti et al. (1994), which is likely due to the complex nature of *Phaeocystis* mucus. As under oxic conditions, there were no indications for halted breakdown due to changes in mucus composition during anaerobic breakdown, because the carbohydrate composition remained unchanged. Remineralization of *Phaeocystis* mucus under anoxic conditions may have important consequences for oceanic C cycling. Sedimentation of organic C into anoxic sediments accounts for >90% of the annual burial of C (Henrichs and Reeburgh 1987), and *Phaeocystis* mucus may contribute a considerable fraction (Wassman et al. 1990; Passow and Wassman 1994; Riebesell et al. 1995; Hong et al. 1997).

Variation among different inocula in ability to yield mucopolysaccharide-degrading enrichment cultures—The inability to obtain mucus-degrading enrichment cultures from seawater collected 5 months before the *Phaeocystis* spring bloom may reflect changes in the microbial community throughout the year. Species important for mucus breakdown could be relatively more abundant in spring, and their growth may be further stimulated by high mucus concentrations. This could explain the shorter lag phase and the higher rate of mucus breakdown in enrichments inoculated with seawater from the top or end of a bloom than from before the bloom (Fig. 4). The distinct lag observed between the development of *Phaeocystis* blooms and that of planktonic bacteria (Laanbroek et al. 1985; Veldhuis et al. 1986; Billen and Fontigny 1987) may also reflect the slow adaptation of the microbial community. The source of the inocula was not important, because enrichment cultures able to degrade partially purified mucus could be obtained from particulate and soluble seawater fractions as well as from sediment. While it is possible that the bacterial community attached to mucus and the free-living community do not differ significantly, as has been found with regard to the enzymatic activity expressed by these fractions (Becquevort et al. 1998), it is equally possible that the filtration method used to distinguish between attached and free-living bacteria does not separate these two groups well enough to find a difference in ability to give rise to a mucus-degrading community.

Conclusion

The data presented in this paper show that *Phaeocystis* mucopolysaccharides can be degraded by marine bacteria under oxic as well as anoxic conditions. Furthermore, these data argue against the occurrence of structural features hampering biodegradation in (parts of) *Phaeocystis* mucus. Therefore, potentially all *Phaeocystis* mucopolysaccharides could probably be recycled before aggregation and sedimentation occurs. Conditions that appeared to have an adverse effect on mucopolysaccharide degradation, such as the ab-

sence of a well-adapted microbial community or the accumulation of inhibitors, may be encountered occasionally in the sea. However, these conditions are not inherent to the nature of the mucus and will only be temporary. Phenomena such as the absence of bacteria during early bloom stages, accumulation of foam after blooms, and sedimentation of mucous flocs must be due to other factors than inherently low degradability, such as the availability of nutrients, whose roles remain to be identified.

References

ARNOSTI, C., D. J. REPETA, AND N. V. BLOUGH. 1994. Rapid bacterial degradation of polysaccharides in anoxic marine systems. Geochim. Cosmochim. Acta **58:** 2639–2652.

ASPINALL, G. O. 1983. The polysaccharides, v. 2. Academic.

AUBERT, J-P., P. BEGUIN, AND J. MILLET. 1988. Biochemistry and genetics of cellulose degradation. FEMS Symp. **43.** Academic.

AZAM, F., T. FENCHEL, J. S. GRAY, L. A. MEYER-REIL, AND F. THINGSTAD. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10:** 257–263.

BECQUEVORT, S., V. ROUSSEAU, AND C. LANCELOT. 1998. Major and comparable roles for free-living and attached bacteria in the degradation of *Phaeocystis*-derived organic matter in Belgian coastal waters of the North Sea. Aquat. Microb. Ecol. **14:** 39–48.

BILLEN, G., AND A. FONTIGNY. 1987. Dynamics of *Phaeocystis* dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar. Ecol. Prog. Ser. **37:** 249–257.

BRUSSAARD, C. P. D., AND OTHERS. 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar. Ecol. Prog. Ser. **123:** 259–271.

CADÉE, G. C. 1996. Accumulation and sedimentation of *Phaeocystis globosa* in the Dutch Wadden Sea. J. Sea Res. **36:** 321–327.

DAVIDSON, A. T., AND H. J. MARCHANT. 1992. The biology and ecology of *Phaeocystis* (Prymnesiophyceae). Prog. Phycol. Res. **8:** 1–45.

DECHO, A. W. 1990. Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Annu. Rev. **28:** 73–153.

EBERLEIN, K., M. T. LEAL, K. D. HAMMER, AND W. HICKEL. 1985. Dissolved organic substances during a *Phaeocystis pouchetii* bloom in the German Bight (North Sea). Mar. Biol. **89:** 311–316.

GIESKES, W. W. C., AND J. VAN BENNEKOM. 1973. Unreliability of the ^{14}C method in estimating primary productivity in eutrophic Dutch coastal waters. Limnol. Oceanogr. **18:** 414–496.

HENRICHS, S. M., AND W. S. REEBURGH. 1987. Anaerobic mineralization of marine sediment organic matter: Rates and the role of anaerobic processes in the oceanic carbon economy. Geomicrobiol. J. **5:** 191–237.

HONG, Y., W. O. SMITH, JR., AND A-M. WHITE. 1997. Studies on transparent exopolymer particles (TEP) produced in the Ross Sea (Antarctica) and by *Phaeocystis antarctica* (Prymnesiophyceae). J. Phycol. **33:** 368–376.

HUNGATE, R. E. 1969. A roll tube method for cultivation of strict anaerobes, p. 117–132. *In* J. R. Norris and D. W. Ribbons [eds.], Methods in microbiology. Academic.

JANSE, I., M. VAN RIJSSEL, J. C. GOTTSCHAL, C. LANCELOT, AND W. W. C. GIESKES. 1996*a.* Carbohydrates in the North Sea during spring blooms of *Phaeocystis:* A specific fingerprint. Aquat. Microb. Ecol. **10:** 97–103.

———, ———, P-J. VAN HALL, G. J. GERWIG, J. C. GOTTSCHAL, AND R. A. PRINS. 1996*b.* The storage glucan of *Phaeocystis globosa* (Prymnesiophyceae) cells. J. Phycol. **32:** 382–387.

LAANBROEK, H. L., J. C. VERPLANKE, P. R. M. DE VISSCHER, AND R. DE VUYST. 1985. Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, The Netherlands. Mar. Ecol. Prog. Ser. **25:** 1–11.

LANCELOT, C. 1995. The mucilage phenomenon in the continental coastal waters of the North Sea. Sci. Total Environ. **165:** 83–102.

———, AND OTHERS. 1987. *Phaeocystis* blooms and nutrient enrichment in the continental coastal zone of the North Sea. Ambio **16:** 38–46.

LIU, D., P. T. S. WONG, AND B. J. DUTKA. 1973. Determination of carbohydrate in lake sediment by a modified phenol-sulphuric acid method. Water Res. **7:** 741–746.

MORIARTY, D. J. W., AND R. T. BELL. 1993. Bacterial growth and starvation in aquatic environments, p. 25–53. *In* S. Kjelleberg [ed.], Starvation in bacteria. Plenum.

OSINGA, R., K. A. DE VRIES, W. E. LEWIS, W. VAN RAAPHORST, L. DIJKHUIZEN, AND F. VAN DUYL. 1997. Aerobic degradation of phytoplankton debris dominated by *Phaeocystis* sp. in different physiological stages of growth. Aquat. Microb. Ecol. **12:** 11–19.

PASSOW, U., AND P. WASSMAN. 1994. On the trophic fate of *Phaeocystis pouchetii* (Hariot). IV. The formation of marine snow by *P. pouchetii.* Mar. Biol. Prog. Ser. **104:** 153–161.

RHEINHEIMER, G. 1992. Aquatic microbiology, 4th ed. Wiley.

RIEBESELL, U., M. REIGSTAD, P. WASSMAN, T. NOJI, AND U. PASSOW. 1995. On the trophic fate of *Phaeocystis pouchetii* (Hariot). VI. Significance of *Phaeocystis*-derived mucus for vertical flux. Neth. J. Sea Res. **33:** 193–203.

SCHUT, F., E. J. DE VRIES, J. C. GOTTSCHAL, B. R. ROBERTSON, W. HARDER, R. A. PRINS, AND D. K. BUTTON. 1993. Isolation of typical marine bacteria by dilution culture: Growth, maintenance and characteristics under laboratory conditions. Appl. Environ. Microbiol. **59:** 2150–2160.

SHERR, E. 1988. Direct use of high molecular weight polysaccharides by heterotrophic flagellates. Nature **335:** 348–351.

THINGSTAD, F., AND G. BILLEN. 1994. Microbial degradation of *Phaeocystis* material in the water column. J. Mar. Syst. **5:** 55–65.

VAN BOEKEL, W. H. M. 1993. Interactions of *Phaeocystis* sp. with organic compounds and the microbial food web. Ph.D. thesis, Univ. of Groningen.

———, F. C. HANSEN, R. RIEGMAN, AND R. P. M. BAK. 1992. Lysis-induced decline of a *Phaeocystis* spring bloom and coupling with the microbial food web. Mar. Ecol. Prog. Ser. **81:** 269–276.

VAN DER MAAREL, M.J.E.C., S. VAN BERGEIJK, A. F. VAN WERKHOVEN, A. M. LAVERMAN, W. G. MEIJER, W. T. STAM, AND T. A. HANSEN. 1996. Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus,* sp. nov. Arch. Microbiol. **166:** 109–115.

VAN HEEMST, J. D. H., S. PEULVÉ, AND J. W. DE LEEUW. 1996. Novel algal polyphenolic biomacromolecules as significant contributors to resistant fractions of marine dissolved and particulate organic matter. Org. Geochem. **24:** 629–640.

VAN RIJSSEL, M. 1992. Pectin degradation by *Clostridium thermosaccharolyticum.* Ph.D thesis, Univ. of Groningen.

———, C. E. HAMM, AND W. W. C. GIESKES. 1997. *Phaeocystis globosa* (Prymnesiophyceae) colonies: Hollow structures built with small amounts of polysaccharides. Eur. J. Phycol. **32:** 185–192.

VELDHUIS, M. J. W., AND W. ADMIRAAL. 1987. The influence of phosphate depletion on the growth and colony formation of

Phaeocystis pouchetii (Hariot) Lagerheim. Mar. Biol. **95:** 47–54.

———, F. COLIJN, AND L. A. H. VENEKAMP. 1986. The spring bloom of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal water. Neth. J. Sea Res. **20:** 37–48.

VISSCHER, P. T., AND H. VAN GEMERDEN. 1991. Production and consumption of dimethylsulfoniopropionate in marine microbial mats. Appl. Environ. Microbiol. **57:** 3237–3242.

WALSH, J. J., G. T. ROWE, R. L. IVERSON, AND C. P. MCROY. 1981. Biological export of shelf carbon is a sink of global CO_2 cycle. Nature **291:** 196–201.

WARREN, R. A. J. 1996. Microbial hydrolysis of polysaccharides. Annu. Rev. Microbiol. **50:** 183–212.

WASSMAN, P., M. VERNET, G. MITCHELL, AND F. REY. 1990. Mass sedimentation of *Phaeocystis pouchetii* in the Barents Sea. Mar. Ecol. Prog. Ser. **66:** 183–195.

WEFER, G. 1989. Particle flux in the ocean: Effects of episodic production, p. 139–154. *In* W. H. Berger, V. Smetacek, and G. Wefer [eds.], Productivity of the ocean: Present and past. Wiley.

WEISSE, T., P. TANDE, F. VERITY, F. HANSE, AND W. W. C. GIESKES. 1994. The trophic significance of *Phaeocystis* blooms. *In* C. Lancelot and P. Wassman [eds.], Ecology of *Phaeocystis*-dominated ecosystems. J. Mar. Syst. **5:** 67–79.

WILLIAMS, P. M., AND E. R. M. DRUFFEL. 1987. Radiocarbon in dissolved organic matter in the Central North Pacific Ocean. Nature **330:** 246–248.

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