Vol. 14: 39–48, 1998 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published January 2

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

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ABSTRACT: Microbial degradation of *Phaeocystis globosa* colonies and their derived organic matter by free-living and attached bacteria was investigated in Belgian coastal waters during the spring development of diatom-*Phaeocystis* colonies in 1994. Results obtained show concomitant evolution of hydrolytic ectoprotease and β-ectoglucosidase ectoenzymatic activities with respect to the phytoplankton bloom, suggesting that the low biodegradability of *Phaeocystis* colonies leading to transient accumulations of *Phaeocystis*-derived material in the coastal North Sea was not due to a lag phase required for the induction of β-ectoglucosidase. Up to 66% of total bacterial biomass was found attached to particles larger than 10 µm. While occurring always in low abundance compared to free-living bacteria, both the average specific biomass and growth rate of particle-attached bacteria were very high, i.e. 60 fg C $cell^{-1}$ and 0.28 h^{-1}, respectively. Similarly, specific ectoenzymatic activities of particle-attached bacteria were on average about 5 times higher than those characterising free-living bacteria. Budget calculations show a 53% contribution of *Phaeocystis*-attached bacteria to the mineralisation of *Phaeocystis*-associated production, i.e. a 53:47% role for attached and free-living bacteria, respectively.

KEY WORDS: *Phaeocystis* degradation · Free-living and particle-attached bacteria · Ectoenzymatic activity · Growth rate

INTRODUCTION

The explosive blooms of large *Phaeocystis globosa* colonies are a common spring event in the eutrophic continental coastal waters of the North Sea (Lancelot et al. 1987, Lancelot & Rousseau 1994). The successful development of *Phaeocystis* colonies in nitrate-rich marine environments (Smith 1993, Lancelot & Rousseau 1994) has been attributed to the high ability of the colony form to take advantage of large concentrations of ambient nitrate (Riegman et al. 1992), the resistance against mortality by metazooplankton grazing pressure (Weisse et al. 1994) and sedimentation due to gelling properties of their mucilaginous matrix (Lancelot & Rousseau 1994). However, little is known about the mechanisms driving the sudden termination of *Phaeocystis* blooms usually observed in *Phaeocystis*-dominated environments (Wassmann 1994). Three mechanisms possibly determine the fate of *Phaeocystis* biomass: colony disintegration producing large amounts of dissolved organic matter (DOM) and *Phaeocystis* cells; aggregation; and mass sedimentation.

Colony disruption and cell autolysis, the latter under control of nutrient depletion at *Phaeocystis* maximum density, have been suggested as the main factor controlling *Phaeocystis* decline (van Boekel et al. 1992). *Phaeocystis* colony disintegration produces free-living cells and DOM in the surrounding water. Released *Phaeocystis* cells are likely maintained at a low density due to intense protozoan grazing pressure (Weisse

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& Scheffel-Möser 1990). On the other hand, DOM derived from colony disruption, exudation and autolysis constitutes substrates to support bacterial growth. However, due to the large contribution to this pool of the *Phaeocystis* colony matrix (up to 90% of *Phaeocystis*-derived organic matter; Rousseau et al. 1990) which is an homogeneous polysaccharide composed of different monosaccharides (Thingstad & Billen 1994, Janse et al. 1996), *Phaeocystis*-derived DOM is likely deficient in N and P with respect to bacterial needs (Eberlein et al. 1985, Thingstad & Billen 1994). Accordingly, transient accumulation of DOM (Eberlein et al. 1985, Billen & Fontigny 1987) and foam (Lancelot & Rousseau 1994) have been ascribed to nutrient limitation observed at the senescent phase of *Phaeocystis* blooms rather than to a poor biodegradability of this material.

Current knowledge of the aggregation potential of *Phaeocystis* colonies during their development and their contribution to vertical flux is still limited (Riebesell 1993, Lancelot & Rousseau 1994, Rousseau et al. 1994). The mucilaginous nature of the colony matrix should confer high surface stickiness to *Phaeocystis* colonies, therefore enhancing rapid aggregation and subsequent sedimentation. In contrast, recent field (Riebesell 1993) and laboratory controlled experiments (Passow & Wassmann 1994) suggest that compared to diatoms *Phaeocystis* colonies largely resist aggregation with other suspended living and non-living particles and do not contribute significantly to the vertical flux of particles (Riebesell et al. 1995). Individual large senescent *Phaeocystis* colonies observed in both temperate and polar waters (Verity et al. 1988, Thingstad & Martinussen 1991, Putt et al. 1994, Rousseau et al. 1994) were rapidly colonized by dead and living auto- and heterotrophic microorganisms.

The extent to which attached bacteria contribute to the degradation and trophic fate of *Phaeocystis*-derived material is not known. Current knowledge on the dynamics of attached and free-living bacteria indicates that by colonizing phytodetritus bacteria give rise to highly dynamic microenvironments which form and transform through microbial processes. These microaggregates (phytoplankton colonized by microorganisms) in turn actively interact with free-living bacteria and particle feeders by providing them with labile substrates (Azam & Cho 1987, Herndl 1988) and particles with high nutritional value (Chróst 1991).

Despite the presence of attached bacteria on senescent *Phaeocystis* colonies, information is lacking about the growth characteristics of *Phaeocystis*-attached bacteria and their contribution to the degradation of *Phaeocystis*-derived material. Yet the question is of prime ecological and biogeochemical importance: a close association between *Phaeocystis* colonies and bacteria enhances remineralization in the water column, increases the transfer of organic matter to higher trophic levels, and thus reduces the flux of organic matter to the sediment.

This work specifically addresses the question of microbial degradation of *Phaeocystis*-derived material and its contribution to *Phaeocystis* bloom decline. It reports first field evidence of the significant contribution of attached bacteria in the degradation process of *Phaeocystis*-derived material. The study was conducted in spring 1994 in Belgian coastal waters of the North Sea where *Phaeocystis* colony blooms are observed each year in spring. The dynamics of free-living versus particle-attached bacteria (biomass and number; ectoenzymatic activity; growth rate) was investigated at a fixed station in Belgian coastal waters during a seasonal cycle.

MATERIALS AND METHODS

Study site. Sampling was carried out at Stn 330 (51° 26.05' N, 2° 48.50' E), *Phaeocystis* reference station in Belgian coastal waters, regularly monitored since 1988. This site is under the influence of the Scheldt river, with salinity ranging between 30 and 35‰. The average depth is 22 m and the water column is permanently well mixed resulting from very strong tidal currents. Sampling covered the period from February till end of June 1994. Sampling frequency was daily to weekly during spring and monthly during winter and summer. All water samples were carefully collected at the surface with a bucket, in order to avoid damaging or disrupting *Phaeocystis* colonies.

Phytoplankton and aggregate biomass. Phytoplankton biomass (diatoms, free-living *Phaeocystis* cells and colonies) was calculated on the basis of number and biovolume determination under an inverted microscope (Leitz Fluovert) according to the Utermöhl method (Hasle 1969). 10 to 100 ml samples were preserved with acid Lugol's solution (1% final concentration) and allowed to settle for 12 h in counting chambers (Hydrobios). A maximum of 500 cells was counted in total, with at least 100 cells of the most abundant genus or species. Diatoms were enumerated at a magnification of 100 or 200×. Diatom carbon biomass was calculated for each species or genus using a specific average cellular biovolume. The latter was established from biovolumes measured on 1 population along the whole period of its development. Biovolumes were then converted using a carbon content factor of 0.11 pg C μm^{-3} of plasma volume as recommended by Edler (1979) for diatoms. *Phaeocystis* colony and *Phaeocystis*-derived aggregate enumeration and biomass determination were performed according to the method described in Rousseau et al. (1990). *Phaeocys-*

tis-derived aggregates were defined as senescent *Phaeocystis* colonies invaded by auto- and/or heterotrophic microorganisms. Estimates of the carbon content of *Phaeocystis*-derived aggregates do not include the biomasses of microorganisms attached to the aggregates.

Phytoplanktonic production. Daily primary production rates were calculated from phytoplankton biomass, nutrient concentrations, light availability, ambient temperature using the AQUAPHY set of equations of Lancelot et al. (1991) supported by experimentally determined photosynthesis and growth parameters for the diatoms and *Phaeocystis* communities.

Bacterial number and biomass. Water samples were preserved with formaldehyde (2% final concentration). A 10 µm pore size Nuclepore membrane was used to filter 5 to 10 ml of sample in order to separate free-living bacteria from bacteria attached to *Phaeocystis* colonies and their derived particles. Accordingly, 10 µm is the minimum size of recorded *Phaeocystis* colonies (Rousseau et al. 1990). Numbers and biomasses of *Phaeocystis*-attached bacteria were estimated directly over *Phaeocystis* colonies or *Phaeocystis*-derived aggregates. Possible overestimation of attached bacteria due to scavenging of the free-living bacteria over *Phaeocystis* colonies during the filtration was considered. 10 µm prefiltered samples (1 ml aliquots) were filtered through 0.2 µm Nuclepore membrane to estimate abundance and biomass of free-living bacteria.

Bacteria numbers and sizes were determined by epifluorescence microscopy after DAPI (4',6-diamidino-2-phenylindole) staining following the procedure of Porter & Feig (1980). Bacteria were measured and classified according to shape and size allowing calculation of biovolume for each size and shape (Garnier et al. 1992). Biomass was estimated from abundance and biovolume distribution using the carbon/biovolume factor depending on biovolume as proposed by Simon & Azam (1989).

Bacterial production. Bacterial production was estimated by incorporation of ^{3}H-leucine into bacterial proteins (Kirchman et al. 1985, Simon & Azam 1989) and by incorporation of ^{3}H-thymidine into bacterial DNA (Fuhrman & Azam 1982). Reverse filtration as proposed by Dodson & Thomas (1978) using a 10 µm Nuclepore membrane was chosen to distinguish between the free-living bacteria and the total bacterial community. The radioactivity incorporated by the total bacterial community was measured on the unfiltered water sample. The radioactivity incorporated by free-living bacteria was measured in the filtrate. The radioactivity incorporated by attached bacteria was then estimated by the difference. The procedures were as follows:

Incorporation of ^{3}H-leucine was measured at 4 concentrations of L-(3,4,5-^{3}H(N))-leucine (Amersham, 120 to 140 Ci mmol^{-1}) between 2 and 77 nM (2 nM ^{3}H-leucine in each experiment but enriched with 0 to 75 nM non-radioactive leucine). For each experiment, two 20 ml aliquots of spiked sample were incubated for 30 min in the dark at *in situ* temperature. Incubation was stopped by adding formaldehyde (final concentration 1%) and reverse filtration was applied on 1 set of incubation flasks. Radioactivity incorporated into the protein of the unfiltered community and of the 10 µm prefiltered sample was measured after protein precipitation. Protein was precipitated with cold (0°C) trichloroacetic acid (TCA) (5% final concentration) followed by heating at 85°C for 30 min. The reaction was stopped by cooling and the precipitated protein was collected by filtration on 0.2 µm pore size cellulose acetate membrane (Sartorius). The radioactivity of the precipitated protein was measured with a Packard Tri-Carb scintillation counter. The incorporation rate was estimated as described in Servais (1990).

Incorporation of ^{3}H-thymidine was measured at 20 nM of methyl-(^{3}H)-thymidine (Amersham, 40 to 50 Ci mmol^{-1}). Two 20 ml samples were incubated in the presence of ^{3}H-thymidine for 60 min (Servais 1992). The incubation was stopped by adding formaldehyde (final concentration 1%). The same post-incubation procedure described for leucine incorporation was applied to distinguish radiotracer incorporation by free-living bacteria from total bacteria. The samples were then filtered onto a 0.2 µm pore size cellulose acetate membrane (Sartorius) and macromolecules were precipitated by adding three 5 ml aliquots of ice cold TCA (10%). The radioactivity associated with the filters was measured with a Packard Tri-Carb scintillation counter.

Leucine and thymidine incorporation were converted into bacterial production using conversion factors of Servais (1990), established for the North Sea bacterial communities, i.e. 3950 g C produced mol^{-1} leucine incorporated into proteins and 2.66×10^{18} bacteria produced mol^{-1} thymidine incorporated in the cold TCA insoluble material. The conversion factors for attached bacteria are assumed to be the same as those estimated for free-living bacteria.

Ectoenzymatic activity. Potential ectoenzymatic activity was measured at 20°C after adding to the sample a saturating concentration of artificial substrates that produce fluorescent products when hydrolysed by ectoenzymes present in the sample. Two ectoenzymatic activities were tested: ectoprotease and ecto-β-glucosidase. The former was measured because it has been suggested that it is a constitutive property of bacteria in aquatic environments (Billen 1991), the latter as an indicator of the degradation of *Phaeocystis*-

colony-derived material, through the specific cleavage of β-glucoside links characteristic of the *Phaeocystis* gel-producing polysaccharides (Lancelot & Rousseau 1994, Thingstad & Billen 1994). Activity of free-living bacteria was distinguished from that of particle-attached bacteria by measurement in unfiltered samples and comparing with the activity after reverse filtration through 10 µm pore size membranes (Nuclepore).

Ectoprotease activity: L-Leucyl-2β-naphthylamide hydrochloride (LLβN, Sigma, St. Louis, USA) was used as a substrate for proteolytic ectoenzymes. It produces fluorescent naphthylamine after hydrolysis of the peptide-like bond. The experimental procedure was that of Somville & Billen (1983). 2 ml water samples were transferred into a sterile quartz fluorimeter cell kept at 20°C; 50 µl of a sterile 40 mM solution (1000 µM final concentration) of L-leucyl-2β-naphthylamide was added, and the increase of fluorescence at 410 nm under 340 nm excitation (KONTRON SFM 25 fluorimeter) was measured as a function of time over 25 to 50 min. Enzyme activity was estimated from the initial slope. Fluorescence of a standard naphthylamine solution was used for calibration.

Ectoglucosidase activity: 4-Methylumbelliferyl-β-glucoside (MUF-GLU, Sigma, St. Louis, USA) was used as a substrate for β-glucosidase, which produces fluorescent 4-methylumbelliferone after hydrolysis of β-linked (1-2, 1-3, 1-4, 1-6) disaccharides of glucose, celluhexose, and carboxymethylcellulose (Barman 1969). The procedure was adapted from the protocol of Hoppe (1983) and Somville (1984). 2 ml water samples were transferred into a sterile quartz fluorimeter cell kept at about 20°C; 250 µl of a sterile 6 mM solution of 4-methylumbelliferyl-β-glucoside was added. The increase of fluorescence at 445 nm under 360 nm excitation was measured as a function of time over 25 to 50 min. Enzyme activity was estimated from the initial slope. Fluorescence of a standard 4-methylumbelliferone solution was used for calibration.

RESULTS

Phaeocystis colonies and *Phaeocystis*-derived aggregates

Phaeocystis colonies bloomed in late April-early May 1994, at our Stn 330 in Belgian coastal waters (Fig. 1A). The species observed in the North Sea was *P. globosa* (Baumann et al. 1994). The maximum colony biomass, 500 mg C m^{-3}, was unexpectedly low compared to the previous years (Lancelot et al. 1991) or the early spring diatom bloom (Fig. 1A). The successful development of the spring diatom bloom has been explained by the extremely high rainfall in the Scheldt river watershed in late winter 1994 (Rousseau & Lancelot 1996, Lancelot et al. 1997). *Phaeocystis*-derived aggregates appeared in the water column shortly after the appearance of *Phaeocystis* colonies and increased in biomass concomitantly (Fig. 1B). However the aggregates' carbon remained lower than the carbon of *Phaeocystis* colonies, representing a maximum of 20% of total *Phaeocystis* carbon (Fig. 1B). Both *Phaeocystis*-derived aggregates and colonies disappeared from the water column in mid-May.

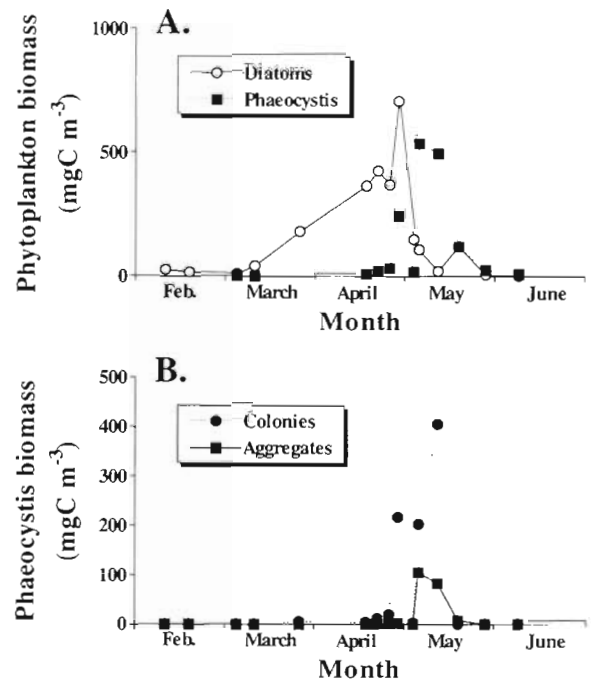

Fig. 1. Temporal evolution of (A) phytoplankton biomass (diatoms and *Phaeocystis*) and (B) *Phaeocystis* biomass (colonies and aggregates) at Stn 330 in Belgian coastal waters in 1994

Bacterial abundance and biomass

Spring variations of abundance and biomass of free-living bacteria and bacteria attached to *Phaeocystis* colonies or derived aggregates are illustrated in Fig. 2A, C.

Particle-attached bacteria were recorded at the time of occurrence of *Phaeocystis*-derived aggregates (Figs. 1B & 2A) but were always less abundant than free-living bacteria (Fig. 2A). In contrast, maximum biomass reached by both bacterial communities was equivalent, around 60 mg C m^{-3} (Fig. 2C). At the peak of the bloom, 12 to 66% of total bacterial biomass was attached to *Phaeocystis* colonies or derived aggregates

(Fig. 2C). This discrepancy between bacterial density and biomass suggests a marked difference between the specific biomass of the 2 bacterial communities. The specific biomass of free-living bacteria (15 fg C $cell^{-1}$) was low compared to attached bacteria (15 to 80 fg C $cell^{-1}$) and displayed little seasonal variation (Fig. 2B). The specific biomass of attached bacteria increased along with its growth, reaching more than 5 times the specific biomass characteristic of free-living bacteria, at their maximum development.

As noted by Billen (1990), a delay of 10 d was observed between the occurrence of *Phaeocystis* maximum development and the development of free-living bacteria. In contrast, maximum biomass reached by attached bacteria coincided with maximum *Phaeocystis* development.

Bacterial production

Biomass and cell production by attached bacteria comprised 10 to 73% and 2 to 68% of total bacterial community, respectively (Fig. 3A, B). As observed for biomass, maximum production rates by attached bacteria were observed earlier in the season compared to free-living bacteria. Biomass and cell production rates of attached bacteria did not display concomitant seasonal variations because of the large range of biovolume reached by attached bacteria. More interesting was the marked difference in specific growth rate displayed by the 2 bacterial communities (Table 1). Average specific growth rate of attached bacteria (0.28 h^{-1}) was 1 order of magnitude higher than that of free-living bacteria (0.018 h^{-1}).

Ectoenzymatic activity

The seasonal evolution of potential ectoenzymatic activities was closely related to phytoplankton and bacterioplankton bloom development with maxima in April-May (Fig. 4A, B). Attached bacteria contributed to 20–60% of total ectoprotease and 10–50% of ecto-β-glucosidase. Ecto-β-glucosidase activity was generally 1 order of magnitude lower than ectoprotease activity (Fig. 4A, B). Both enzymatic activities, however, varied concomitantly as reflected in the significant correlation between them observed for both attached and free-living bacteria (Fig. 5). The slopes of the regression lines differ, however, by a factor 2 between the 2 bac-

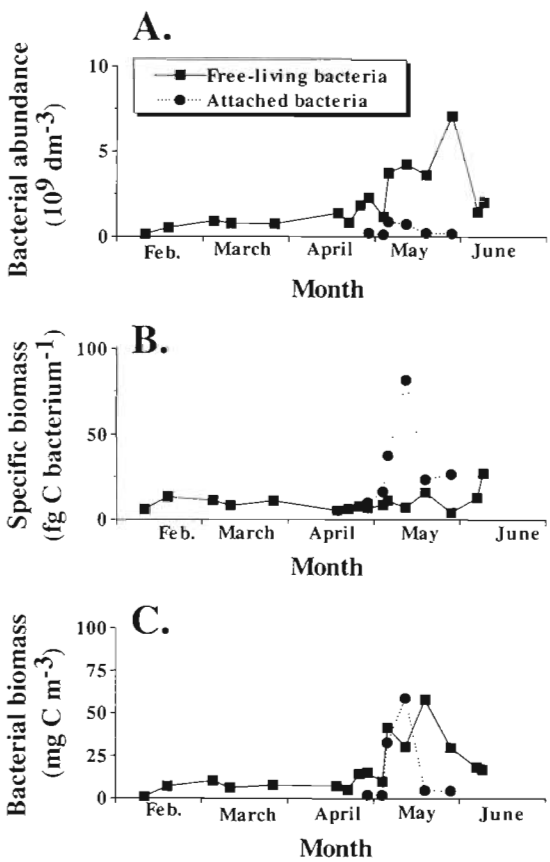

Fig. 2. Temporal evolution of (A) abundance, (B) specific biomass and (C) biomass of free-living and particle-attached bacteria at Stn 330 in Belgian coastal waters in 1994

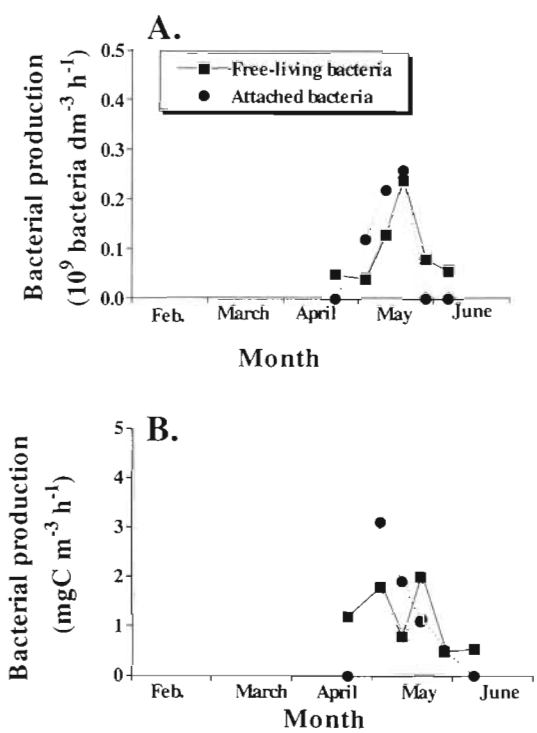

Fig. 3. Temporal evolution of (A) cell production and (B) biomass production of attached and free-living bacteria at Stn 330 in Belgian coastal waters in 1994

terial communities with free-living bacteria expressing higher β-glucosidase compared to ectoprotease. This difference is somewhat reflected in the ratio of specific activities (Table 1). More remarkable are the significantly higher specific enzymatic activities exhibited by attached bacteria compared to free-living bacteria for both ectoprotease and β-glucosidase activities (Table 1).

DISCUSSION

Biodegradability of *Phaeocystis*-derived organic matter

Although bacterial permeases recognize only monomeric or oligomeric substrates, dissolved organic matter in aquatic environments is mainly polymeric (Cole et al. 1984, Billen & Servais 1989, Münster & Chróst 1990). Biodegradability of organic matter relies thus on both the presence of specific enzymes for cleaving the polymeric bonds, and on the secondary and the tertiary structure of the polymer, determining the accessibility for the active site of the enzyme. *Phaeocystis*-derived organic matter released after colony matrix disruption has long been considered as refractory owing to the presence of foam accumulation on the beaches bordering the North Sea at the time of *Phaeocystis* blooms (Lancelot 1995). However, the transient nature of this organic matter accumulation on the one hand, and the simple sugar composition of the colony matrix (70% glucose, 15% xylose, 15% acidic sugar linked by β-glucosidic bonds; Thingstad & Billen 1994) on the other hand, would suggest that *Phaeocystis*-derived organic matter is readily biodegradable. The observed slow biodegradability of *Phaeocystis* colony-derived organic matter in a *Phaeocystis*-dominated ecosystem would possibly result from a delay required for the induction of a nonconstitutive β-glycosidase (ectoenzymes hydrolysing the polymers of mucus) and/or by nutrient shortage due to the N and P deficiency of *Phaeocystis*-derived material with respect to bacterial needs.

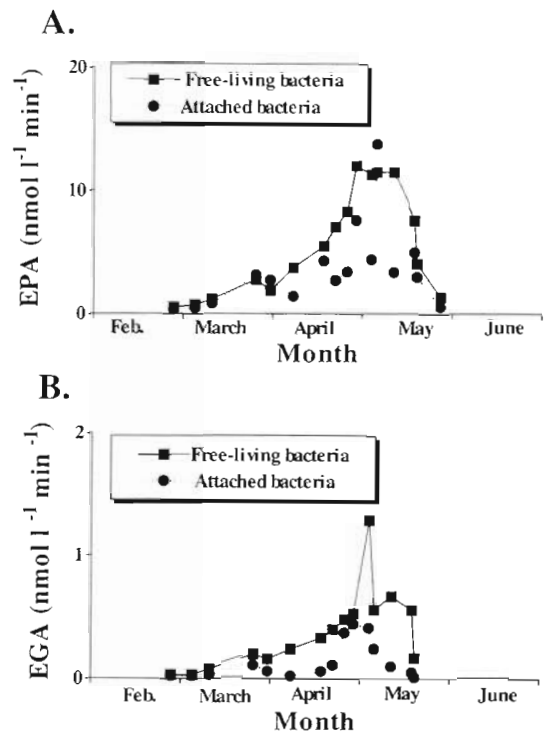

Fig. 4. Temporal evolution of (A) ectoprotease and (B) ecto-β-glucosidase activities (free-living and attached bacteria) at Stn 330 of the Belgian coastal waters in 1994

Data presented in this paper on the concomitant evolution of ectoprotease and β-glucosidase activities with respect to diatom and *Phaeocystis* development and the strong correlation between the enzymatic activities suggest that β-glucosidase activity is constitutive as ectoprotease activity and is not induced by the presence of *Phaeocystis* colonies. Consequently, transient foam accumulation observed at the time of *Phaeocystis* blooms does not reflect a lag phase required for the induction of β-glucosidase. Furthermore, the experiments on the biodegradability of various organic matter derived from *Phaeocystis* (cells, colonies and mucilaginous matrix) do not exhibit significant differences when nutrients are not limiting (Tallier 1994).

Table 1. Growth rate, specific ectoprotease activity (EPA), specific ecto-β-glucosidase activity (EGA) and EGA/EPA ratio for free-living bacteria and bacteria attached to *Phaeocystis* colonies or derived aggregates

	Free-living bacteria min–max (average)	Attached bacteria min–max (average)
Growth rate (h^{-1})	0.007–0.040 (0.018)	0.005–0.850 (0.280)
Specific ectoproteolytic activity (EPA) (nmol $cell^{-1}$ min^{-1})	2.11–9.9 (5.3)	17.00–40.18 (26.4)
Specific ectoglucosidasic activity (EGA) (nmol $cell^{-1}$ min^{-1})	0.02–1.11 (0.34)	0.06–3.7 (1.36)
EGA/EPA	0.06	0.05

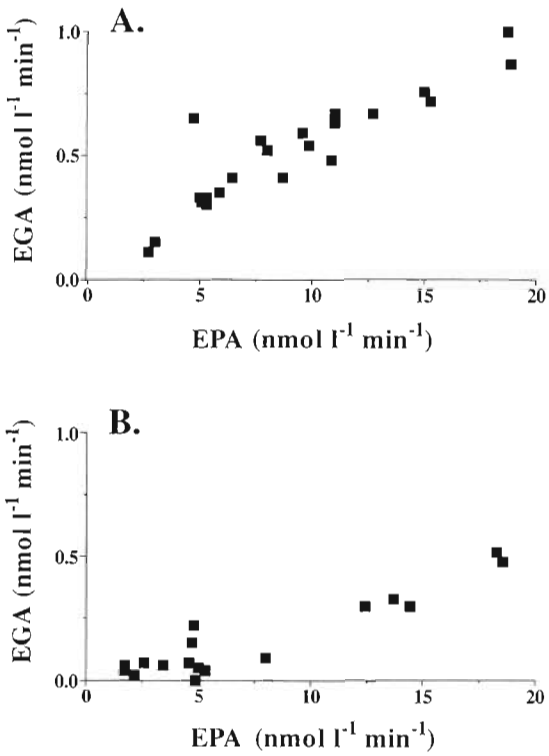

Fig. 5. Relationship between ectoprotease and ecto-β-glucosidase activities of (A) free-living bacteria $y = 0.054x$, $R^2 = 0.93$) and (B) attached bacteria ($y = 0.025x$, $R^2 = 0.85$)

Altogether, this suggests that *Phaeocystis* mucus-derived organic matter would constitute an easily biodegradable substrate for ambient bacteria, provided nutrients are not limiting. This has still to be demonstrated by laboratory-controlled experiments.

Contribution of attached bacteria to the degradation of *Phaeocystis*-derived organic matter

Like in other *Phaeocystis*-dominated ecosystems, e.g. the Ross Sea (Putt et al. 1994), bacteria were found to colonize *Phaeocystis* colonies and their derived aggregates at the peak of the bloom of the continental coastal waters of the North Sea. Early stages of actively growing *Phaeocystis* colonies were not colonized by bacteria, suggesting that attachment properties of *Phaeocystis* colonies may change according to their physiological stage. Several mechanisms have been suggested to explain the high potential of senescent phytoplankton for invasion by bacteria. The release of nutrients by senescent phytoplankton may cause a stimulation in bacterial activities which cause bacterial clumping and attachment (Albright et al. 1986). Recent biochemical and microscopic analysis of *Phaeocystis* colonies using confocal microscopy (van Ryssel unpubl.) give indirect evidence of the decrease of *Phaeocystis* colony compactness when growing in size and age. This decrease in gel compactness might well explain the high vulnerability of large senescent *Phaeocystis* colonies to invasion by bacteria, other microorganisms and/or dead particles.

In agreement with the previous studies (Biddanda 1986, Biddanda & Pomeroy 1988, Azam et al. 1993), the size of attached bacteria was significantly larger than that of free-living bacteria. According to Kanopka (1992), high values of attached bacteria biovolume could reflect the capability of bacteria to store carbon such as carbohydrates during N- and/or P-limiting conditions when cell division is no longer possible. Moreover, specific (per cell) activities (growth rates and ectoenzymatic activities) of particle-attached bacteria were significantly higher than those of free-living bacteria. Other studies have shown that bacteria inhabiting marine snow have cell-specific ectoenzymatic activities up to 3 orders of magnitude higher than bacteria in the surrounding water (Karner & Herndl 1992, Smith et al. 1992, Griffith et al. 1994, Martinez et al. 1996). Delong et al. (1993) found that particle-attached bacteria were phylogenetically distinct from free-living bacteria and were able to degrade a wide range of polymeric compounds. However, the relative contribution of free-living and particle-attached bacteria to the degradation of organic matter relies on the size and organic composition of aggregates.

The contribution of attached bacteria to the degradation of organic matter derived from *Phaeocystis* was estimated from carbon budget calculations comparing the carbon requirements of attached and free-living bacteria with the spring production of organic carbon by diatoms and *Phaeocystis* colonies. Bacterial organic carbon requirement was calculated by integration of daily bacterial productions measured at Stn 330 during the spring bloom period (March to May) assuming an assimilation efficiency of 30% (Billen et al. 1990) (Fig. 6B). This calculation shows that attached bacteria require more than 53% (60 g C m^{-2}) of the carbon demand of the total bacterial community (113 g C m^{-2}). This calculation highlights the important ecological role of attached bacteria in the dynamics of a *Phaeocystis*-dominated ecosystem. Phytoplankton production was estimated by integration of daily total phytoplanktonic production during the bloom period (Fig. 6A). In 1994, *Phaeocystis* production (110 g C m^{-2}) contributed only 49% to the total spring primary production (222 g C m^{-2}) in contrast to previous years where its contribution was more than 80% (Lancelot & Mathot 1987). Assuming that bacteria attached to *Phaeocystis* colonies and derived aggregates utilize

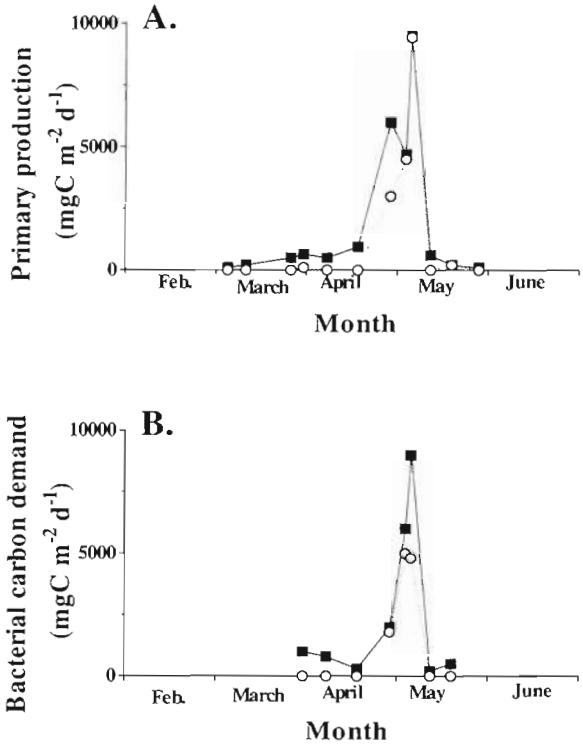

Fig. 6. Temporal evolution of (A) primary production [total phytoplankton (■), *Phaeocystis* (○)] and (B) bacterial carbon demand [total bacteria (■), attached bacteria (○)] at Stn 330 in Belgian coastal waters in 1994

only labile organic carbon derived from *Phaeocystis* colonies, the transfer of organic matter from *Phaeocystis* to attached bacteria was estimated at 60 g C m^{-2}, i.e. 55% of *Phaeocystis* colony production. Assuming that, on average, 80% of *Phaeocystis* colony production consists of carbohydrates and using an average *Phaeocystis* colony C/N ratio of 27 (Rousseau et al. 1990), it can be demonstrated that *Phaeocystis*-derived substrates available to particle-attached bacteria would be severely nutrient limited compared to nitrogen requirement of bacteria (C/N = 4; Lancelot & Billen 1985). This suggests that an additional nitrogen source is required for bacterial utilization of *Phaeocystis*-derived material. The observed concomitant development of protozoa during *Phaeocystis* blooms (Becquevort unpubl.) and their regeneration might supply the required nitrogen as well as phosphate. During years of very large *Phaeocystis* blooms, the nutrient limitation of bacterial mineralization would be more important, resulting in added accumulation of *Phaeocystis*-derived organic matter in the coastal waters of the North Sea.

In conclusion, *Phaeocystis*-attached bacteria biomass was low compared to free-living bacteria, yet it was responsible for roughly one-half of total bacterial mineralization of *Phaeocystis*-associated production in the Belgian coastal zone of the North Sea during spring-summer 1994. The fate of attached bacteria is presently unknown. They could be released from the decomposing colonies in the water column, consumed during metazoan grazing on the *Phaeocystis* aggregates, or exported to the sea floor along with sedimenting *Phaeocystis* aggregates.

Acknowledgements. The authors are indebted to the crew and scientists working on RV 'Belgica' for the weekly water sampling at Stn 330. This work is a part of a thesis by P. Thallier and F. Morri. We thank J. M. Dandois and P. Duponcheel for their technical assistance. This work was supported by EC Environment and Climate Programme (contract no. EVSV-CT94-0511) and national SSTC Impulse Programme in Marine Sciences (contract s/11/070). All our gratitude to G. Billen for helpful discussions during the course of this study and to V. Schoemann, J. deJong, F. Azam and 2 anonymous reviewers for their constructive comments on this manuscript.

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Editorial responsibility: Farooq Azam, La Jolla, California, USA

Submitted: November 11, 1996; Accepted: July 10, 1997
Proofs received from author(s): October 2, 1997