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Microbial degradation of *Phaeocystis* material in the water column

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Abstract

Observational evidence shows that the large amounts of mucilaginous substances produced by blooms of *Phaeocystis* colonies largely resist rapid microbial degradation in surface waters of most *Phaeocystis*-dominated ecosystems. In this paper the biodegradability of *Phaeocystis* colony-derived material is analysed with respect to current knowledge and novel data on the chemical nature of *Phaeocystis* material in relationship with specific bacterial enzymatic activities. Particular emphasis is given to the chemical nature of *Phaeocystis* colony matrix which constitutes more than 80% of total colony biomass at maximum development. This analysis gives evidence of the potential biodegradability of this mucilaginous material made of nutrient-deprived polysaccharides. Other factors controlling microbial degradation as the production of antibacterial substances by *Phaeocystis* colonies, cold temperature and lack of inorganic nitrogen and phosphate are further considered. It is concluded that nutrient limitation currently observed at the senescent stage of *Phaeocystis* blooms might well explain the low biodegradability of *Phaeocystis* material. However the lack of bacteria attached to colonies during the exponential phase of *Phaeocystis* bloom development are not clearly understood and needs further investigations.

1. Introduction

One of the characteristic features of *Phaeocystis* blooms is the production of large amounts of mucoid colonial material which may represent up to 90% of total algal biomass (Rousseau et al., 1990). One of the more spectacular consequences of this input of organic material to the marine environment is the formation of large amounts of foam on the beaches during blooms of this algae (Lancelot et al., 1987). Of greater ecological significance is, however, the fate within the marine ecosystem of the organic material produced by these blooms. As for any other algal bloom, the organic material produced may either be ingested by zooplankton or degraded by bacteria. The relative magnitude of these two rates will determine whether the material is channeled into the food web at the microbial, or at the mesozooplankton level, presumably with large consequences for the relative importance of "classical" versus "microbial" parts of the ecosystem (Fig. 1). The rate of these two processes relative to the sinking rate of the material from senescent blooms will determine whether the primary degradation takes place (1) in or close to the photic zone, (2) in the aphotic part of the water column or (3) reaches the bottom. Bacterial degradation of the colony may thus be important, not only as a clean-up process in areas like the southern part

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of the North Sea where the blooms of *Phaeocystis* may be a nuisance, but also as a potential structuring process in the marine food webs of this (Joiris et al., 1982) and in other areas sustaining large commercial fisheries such as the Barents Sea (Wassmann et al., 1990; Thingstad and Martinussen, 1991). A model eventually aiming at assessing the role of *Phaeocystis* in the marine ecosystem therefore requires a proper understanding, not only of how bacterial degradation of the mucilaginous material is controlled, but also of the relative importance of this process compared to the rates of other processes moving and transforming the material.

In bacterial degradation, macromolecules have to pass through a two-step process (Billen and Servais, 1989), with an initial hydrolysis followed by a subsequent bacterial uptake and utilization of the monomers. A variety of factors such as temperature, antibiotics, nutrient concentrations, competition and predation, will in a consortium influence these processes and thereby regulate the rate of degradation.

The importance of various mechanisms in regulating the degradation process is reflected in three observations on bacterial growth related to *Phaeocystis*:

– Colonies in early stages of blooms are almost entirely free of attached bacteria (Lancelot and Rousseau, 1994; Thingstad, pers. obs. Barents Sea). (See Fig. 2).

– Late stationary phases of cultures (Davidson and Marchant, 1987) and late bloom stages in natural environments (Veldhuis et al., 1986; Billen

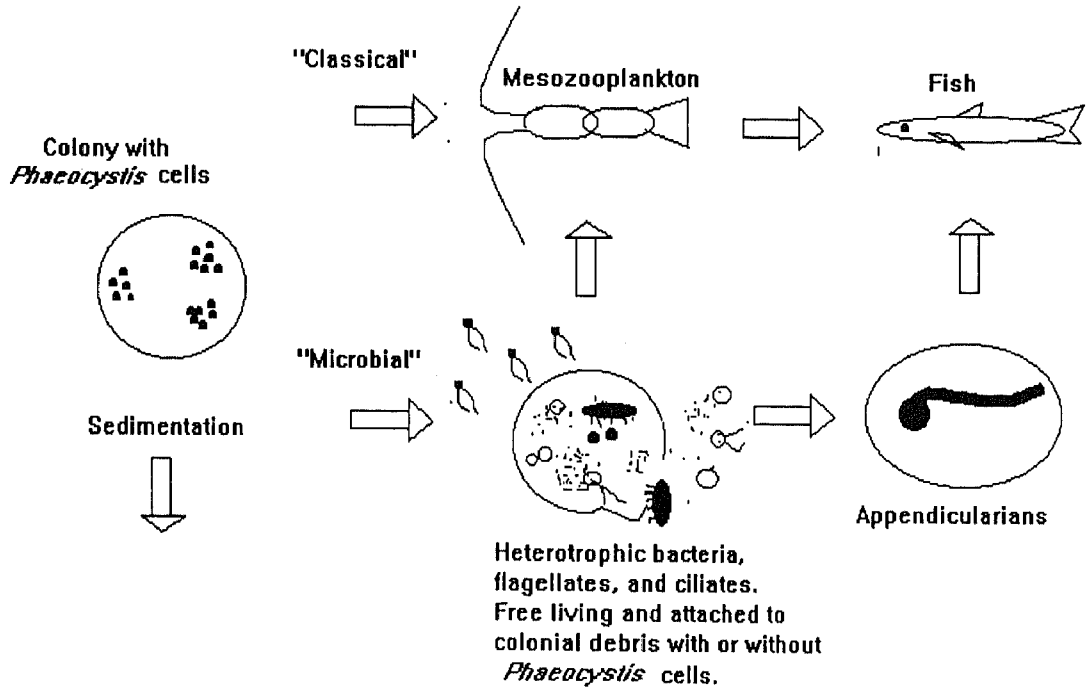

Fig. 1. Main fates of *Phaeocystis* material. A "competition" exists between the "classical" food chain where healthy colonies enter a food chain via mesozooplankton to fish, and a "microbial" food chain where a consortium of heterotrophic microorganisms (bacteria, heterotrophic flagellates, ciliates, etc.) may develop both as free living and attached to the remains of decaying colonies. The secondary production of this microbial complex may be fed back via mesozooplankton into the food chain to fish, either via filter feeders with fine meshed filters such as e.g. appendicularians, or via e.g. copepods feeding on colonized aggregates of remains of *Phaeocystis* colonies. The rates of transfer into the two food chains structures the food chain of *Phaeocystis*-dominated ecosystems and influences the depth to which particulate organic material from senescent blooms will sediment (organisms not drawn to scale).

and Fontigny, 1987, Lancelot and Rousseau, 1994) are accompanied by increased growth of free living bacteria, along with colonization of the mucus by attached bacteria (Thingstad and Martinussen, 1991). (See Fig. 3).

– Organic material may accumulate in the water during the senescent phase of blooms (Eberlein et al., 1985; Billen and Fontigny, 1987).

Together these observations seem to indicate that a substantial part of the material is of a chemical nature that makes it available to bacterial attack, but also that the degradation process may be slow both in the early and in the senescent phase of blooms. The reasons for a slow degradation may be widely different in early and senescent stages of the bloom.

2. Type of material

Organic material produced by marine microorganisms are not necessarily of a chemical nature that make them readily available to bacterial attack. Using seawater bacterial communities, Pett (1989) found 29% of the material from *Skeletonema costatum* cells to have a half-life on the order of months, and 4% to have a half-life of years, and Brophy and Carlson (1989) found glucose and leucine added to natural water to be transformed into high molecular weight compounds that persisted 6 months of incubation.

As was shown by Guillard and Hellebust (1974), the material excreted by colonial forms of *Phaeocystis pouchetii* is predominantly carbohydrates. Qualitative sugar composition of the excreted material was similar in the two strains investigated, and was dominated by glucose, mannose and rhamnose. Chromatography of the material indicated that the same sugars were present in the matrix material. Molecular weight determination showed that most of the liberated material was of high molecular weight (less than 20% had MW < 700), but with a wide spectrum in sizes. More than two-thirds of the material consisted of oligo- and poly-saccharides with molecular weights equivalent to 4–40 hexose residues. In

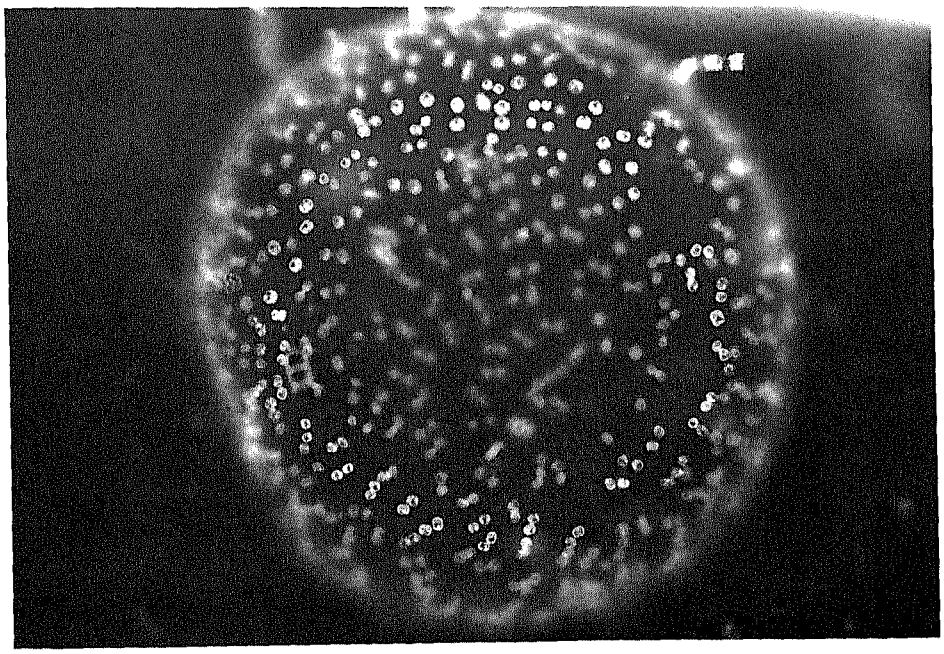

Fig. 2. Healthy *Phaeocystis globosa*-type colony sampled in the Belgian coastal zone at the early development of the spring bloom. (Inverted microscopy; photograph: V. Rousseau).

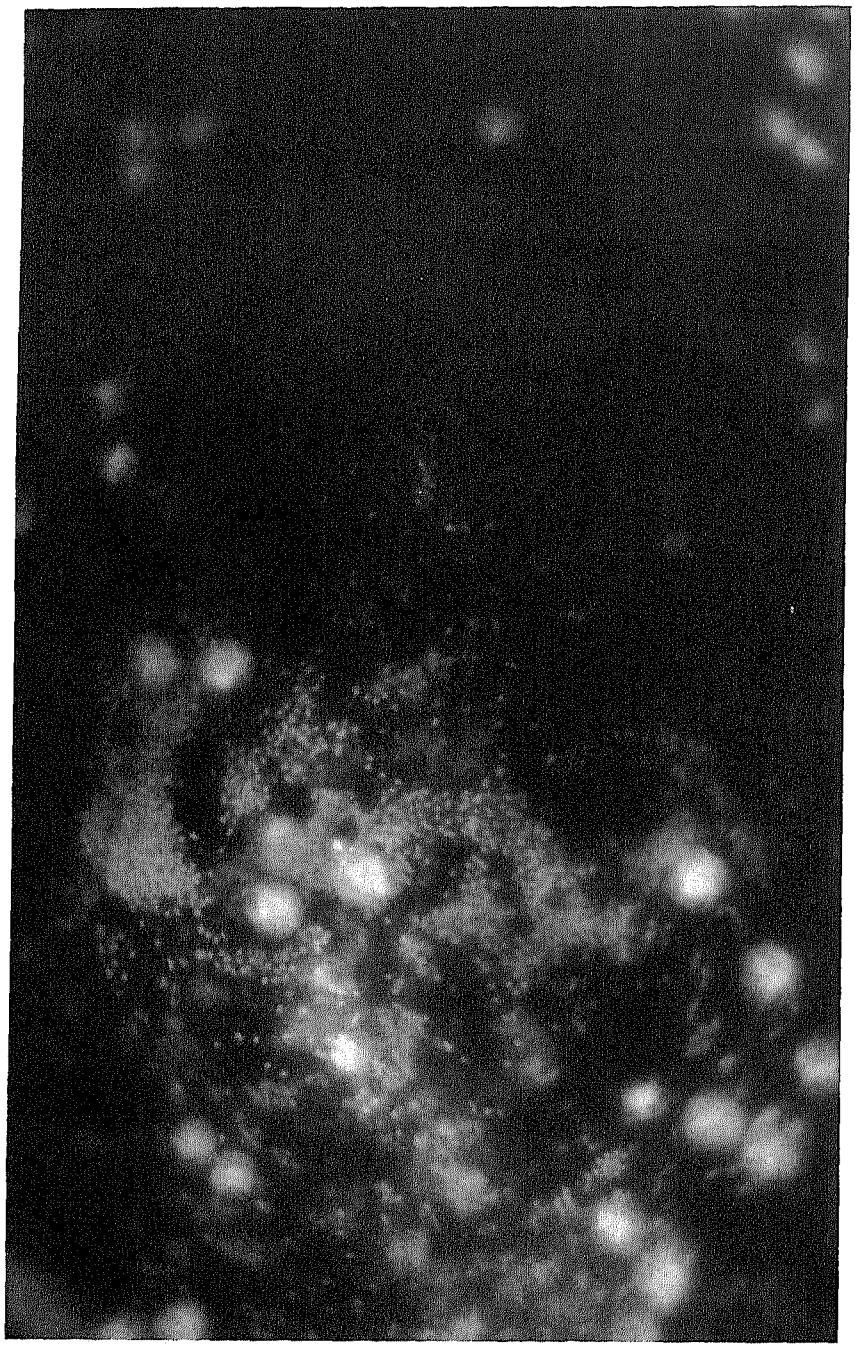

Fig. 3. Decaying colony of *Phaeocystis* from the aphotic zone in the Barents Sea. Bacteria have colonized the mucoid colonia material with some *Phaeocystis* cells (bright) still remaining in the matrix. Preparation stained by DAPI and primuline photographed under UV excitation in epifluorescence microscopy. (photograph: I. Martinussen).

recent investigations, Lancelot et al. (unpublished data) provide further indications concerning the structure of *Phaeocystis* mucus. Gas chromatography of a hydrolysate of mucus revealed a presence of 70% glucose, 15% xylose and an unidentified derivative, possibly acidic owing to the positive response to alcian blue staining.

Concentrated mucus was found to act as a competitive inhibitor to 4MUF-β-glucoside, an artificial fluorogenic substrate of β-glucosidases, indicating that at least parts of the polysaccharides are polymers linked by β-glucosidic bonds. No effects were found on the hydrolysis of 4MUF-α-glucoside. The susceptibility of polymers to enzymatic hydrolysis depends on both the presence of suitable enzymes recognizing the polymeric bond involved, and on the secondary and tertiary structure of the polymer, determining their accessibility for the active site of enzymes.

Intuitively, one would expect that degradability is correlated to the functional role of the polysaccharide with storage polymers being easy to hydrolyze, while polymers with a structural function need to be more resistant. It has been shown that at least part of the colony material has a storage function allowing energy requiring processes such as protein synthesis (Lancelot and Mathot, 1985; Lancelot et al., 1986) and phosphate uptake (Veldhuis et al., 1991) to continue in the dark. That part of the mucus should therefore be easily mobilizable, at least by the algae themselves. Other parts may however be more resistant. A high degree of side chain formation, or other "irregularities" in the polymer are known to cause considerable loss of degradability. No information is, however, available on the degree of side chain structure of *Phaeocystis* mucus. Lack of water solubility and close stacking of individual chains caused by hydrophobic bonds between them is another cause for low biodegradability, as e.g. in the case of cellulose (β-1,3-glucan). *Phaeocystis* mucus appears like as a gel-like material, with high degree of hydration. Electron microscopic studies of the colony material (Chang, 1984) revealed a multilayered mucilaginous envelope without any signs of structurally supporting fibers. The basis for a low biodegradability of *Phaeocystis* seems therefore not to be found in its structure. β-glucosidase activity is present among the bacterial communities of marine waters, including those were *Phaeocystis* are present (Thingstad and Martinussen, 1991), but apparently at low levels when compared to e.g. proteolytic activity (Lancelot et al., unpubl. data). These enzymes are susceptible to rapid induction (Chrost, 1991) so that the lack of ability to produce this enzyme is not an explanation for low biodegradability of mucus.

3. Antibiotics

Colonies of *Phaeocystis* may be stained for fluorescence microscopy such that both the algal cells with their chloroplast and nucleus, the mucoid material, and any bacteria attached to the mucus may be observed (Martinussen and Thingstad, 1991). Using this technique, we have found colonies from early stages of blooms in the Barents Sea to be amazingly free of bacteria. In the *Phaeocystis pouchetii*-type, where algal cells are grouped in patches on the colonial surface, the protective mechanism would seem to extend to parts of the colony not in immediate contact with the vegetative cells. The acrylic acid produced by *Phaeocystis* (Sieburth, 1961) is a potential candidate as the anti-bacterial agent. As shown by Sieburth (1961), however, the concentrations of acrylic acid required to give significant bacteriostatic/bacteriosidic effects are high (order of $g \cdot l^{-1}$).

In non-axenic batch cultures of *Phaeocystis* sp. originating from the Southern Ocean, the correlation between acrylic acid concentration and bacterial numbers was negative but low (-0.4) (Davidson and Marchant, 1987). Correlation with growth rate was not reported in these experiments.

Grossel and Delesmont (1984) reported the presence of acrylic acid during *Phaeocystis* blooms in the eastern Channel. The maximum concentration recorded averaged 13 $\mu g \cdot l^{-1}$, at least three orders of magnitude below the concentrations reported to inhibit marine bacteria. Acrylic acid is produced in equimolecular amounts with

dimethyl-sulphide (DMS) during the enzymatic splitting of β-dimethylpropiothetin (DMPT) (See Liss et al., 1994). DMPT has been suggested to be important in the osmoregulation of algae (Vairavamurthy et al., 1985). Production of DMS during bloom (not *Phaeocystis*) simulations in tanks has been found to be 7–26 times higher during the senescent phase than during the growth phase (Nguyen et al., 1988). This pattern seems difficult to reconcile with the hypothesis that acrylic acid is predominantly protecting young and healthy colonies.

Another hypothetical mechanism for colony protection could be a frequent peeling of the outermost membrane of the colonies. To our knowledge, this has not been specifically tested, but such a mechanism would be expected to give ample growth of bacteria outside the colonies in the exponential growth phase of *Phaeocystis* cultures. This does not seem to fit with the experimental results either in batch cultures where bacterial growth was associated with the stationary and not the exponential phase of *Phaeocystis* (Davidson and Marchant, 1987), or in the field, where a distinct lag is observed between the development of *Phaeocystis* blooms and that of planktonic bacteria (Laanbroek et al., 1985; Veldhuis et al., 1986; Billen and Fontigny, 1987).

4. C:N:P-ratios of *Phaeocystis*

When blooms of *Phaeocystis* collapse due to depletion of either available N- or available P-sources from the water, supply of N or P for subsequent formation of bacterial biomass is restricted to the content of these elements in the organic matter degraded, and to remineralized

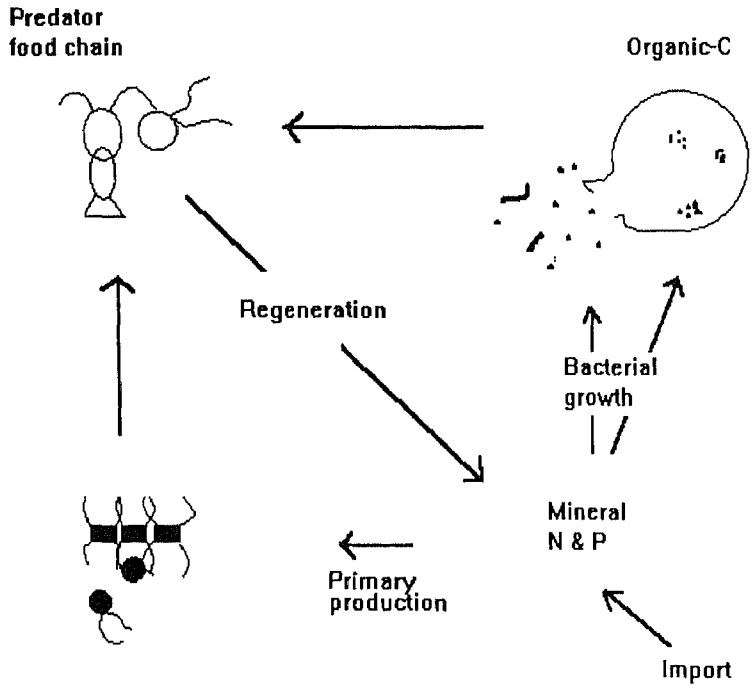

Fig. 4. Trophodynamic interactions presumably controlling the degradation of *Phaeocystis* material in the photic zone of waters where blooms collapse due to N or P-depletion. Formation of bacterial biomass on C-rich carbohydrates from mucus requires the supply of dissolved sources of N and P for which the bacteria must compete with phytoplankton. The rate of supply of these nutrient by import is controlled by hydrographic processes, and the internal regeneration by the processes in the predator food chain (organisms not drawn to scale).

and "new" inputs of these elements. Consumption of readily degradable substances like glucose may under such conditions be controlled by trophodynamic processes of mineral cycling such as competition, predation and remineralization (Pengerud et al., 1987) (Fig. 4). Nutrient limitation of the degradation process would be particularly be expected when C:N and/or C:P ratios of the organic substrates are high (Martinussen and Thingstad, 1987). Veldhuis et al. (1986) found protein to be the dominant photosynthetic product during early stages, while carbohydrate synthesis was dominant during late stages of the *Phaeocystis* spring bloom in Dutch coastal waters.

C:N-ratios of *Phaeocystis* has been found to increase as the bloom progresses (Hickel, 1984) and values more than 5 times the Redfield ratio has been found at low concentrations of ambient nitrogen sources (Lancelot et al., 1991; Baumann et al., 1994). *Phaeocystis globosa*-type was found by Jahnke (1989) to have an atomic C:N:P-ratio of $568 \pm 102 : 59 \pm 9 : 1$ under phosphate deficiency, an increase in C:P of about 5 relative to the Redfield ratio.

Mineral nutrient limitation of the degradation of C-rich parts such as the carbohydrates could therefore be expected. Of interest in this respect is the observation by Eberlein et al. (1985) of high concentrations of carbohydrates following a *Phaeocystis* bloom in the German Bight. No specific information of phosphate concentrations during this particular event was reported, but due to the high nitrate : phosphate content in the river runoff from the northwestern part of the European continent to the North Sea (Lancelot et al., 1991), blooms in this coastal area may become P-limited (van Bennekom et al., 1975; Veldhuis et al., 1986), Since bacterial P-content is high relative to Redfield's ratio (Goldman et al., 1987; Vadstein et al., 1988), one may speculate that bacterial development is particularly restricted under P-limitation. In this scenario, organic matter from *Phaeocystis* blooms in the German Bight would be conserved due to P-limitation, and the probability would increase for alternative fates such as zooplankton ingestion, sedimentation or advective transport into the Jutland Current where nitrate-rich/phosphate-poor water masses are transported northwards along the western coast of southern Scandinavia (Thingstad et al., unpubl. data). In stable water columns such as the polar waters of the Barents Sea, the particulate part the organic matter would sediment from the deep chlorophyll maxima into the nutrient replete water masses below (Wassmann et al., 1990). Nutrient limitation would then be expected to be a problem mainly for degradation of dissolved components left in the cold, oligotrophic melt layer of this region.

5. Temperature

Phaeocystis is common species both in Antarctic (El-Sayed et al., 1983) and in Arctic (Skjoldal and Rey, 1989) waters where blooms may occur at water temperatures below 0°C. There has been suggestions in the literature that bacterial growth is inhibited at low temperatures (Pomeroy and Deibel, 1986; Autio, 1990). Following the arguments presented in the introduction, large differences in the temperature sensitivity of key processes such as primary production, zooplankton grazing, particle sedimentation and bacterial degradation would have the important consequence of promoting different ecosystem structures in cold and temperate waters. Other investigators have, however, found bacterial activities in polar regions comparable in magnitude to that in temperate waters (Cota et al., 1990). Possibly such apparently conflicting observations may be reconciled using a more elaborate hypothesis where higher substrate concentrations are required to compensate for lower temperatures (Pomeroy and Wiebe, 1993). Thingstad and Martinussen (1993) found high bacterial numbers and activities associated with deep chlorophyll maxima formed by *Phaeocystis* at late stages of the ice edge bloom in the Barents Sea. Similarly, differences in temperature sensitivity of the different processes of bacterial degradation (hydrolysis by extracellular enzymes, uptake, bacterial growth, etc.) could qualitatively alter the degradation at different water temperatures. The extracellular enzymatic hydrolysis of macromolecules has been suggested to be rate limiting

in temperate waters (Somville and Billen, 1983; Billen, 1991). Studies of the temperature sensitivity of extracellular proteases in natural Barents Sea water down to the freezing point (−1.9°C) did not reveal any lower limit for the functioning of these enzymes (Thingstad and Martinussen, 1991). Psychrophilic bacteria may grow well at such low temperatures on monomeric organic substrates (Harder and Veldkamp, 1971). When estimates of in-situ bacterial growth rates are compared to growth rates of natural bacterial communities to which substrates have been added, the in-situ growth rates seem to be far below the maximum obtainable for any given temperature (range investigated −1.9 to +25°C, Billen and Servais, 1989), indicating that temperature is not the major limiting factor.

6. Secondary effects

Subsequent to bacterial assimilation of organic carbon originally fixed by *Phaeocystis* primary production, the fate of this material is linked to the fate of the bacterial biomass. In some environments, only a minor fraction of the bacterial biomass is apparently transferred to higher trophic levels (Ducklow et al., 1986). In the case of invaded *Phaeocystis*, the situation may be different since the attachment to floculate changes the functional size of bacteria in the predator chain. It has been shown that old colonies of *Phaeocystis* are more susceptible to mesozooplankton grazing than actively growing colonies (Estep et al., 1990). Zooplankton in the Barents Sea are also often found to concentrate in layers

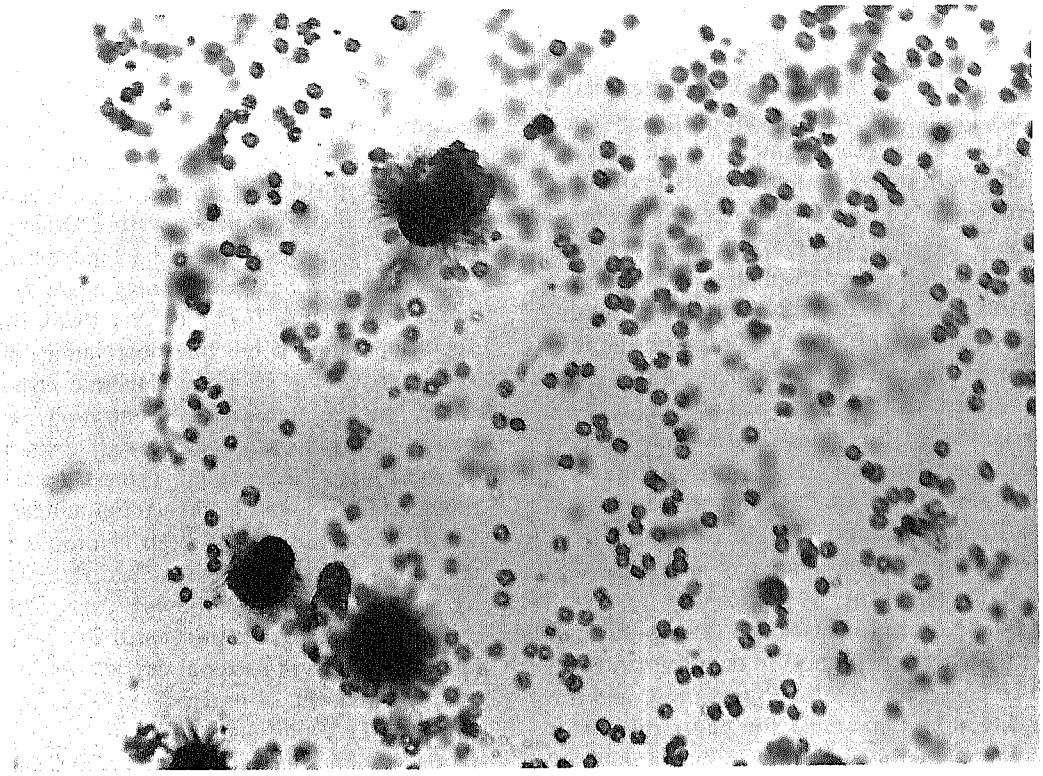

Fig. 5. Ciliates-invaded senescent *Phaeocystis* colony sampled in the Belgian coastal zone at the stationary stage of the bloom development. (Inverted microscopy; photograph: V. Rousseau).

below the deep chlorophyll maximum (Eilertsen et al., 1989). In summer situations, capelin stomach content has been found to be dominated by appendicularians (Hassel et al., 1984). With their fine-meshed filtering nets, these organisms may harvest bacteria directly (Deibel and Powell, 1987), and thus potentially constitute a short link from the microbial community of bacteria and small flagellates to commercially valuable fish stocks.

Bacteria invading the mucoid layer of *Skeletonema* cells during late spring bloom have been shown to be subject to viral attack (Bratbak et al., 1990). Increases in the count of free viruses coinciding with ageing of *Phaeocystis* colonies in Belgian coastal waters has also been observed (Heldal and Bratbak, unpubl. data). In the period following these blooms, Billen and Fontigny (1987) found, however, that the major fraction of bacterial mortality could be attributed to predation, leaving only a minor fraction to viral lysis and other causes of mortality and suggesting the predator food chain to be the major fate of bacterial biomass formed on *Phaeocystis* material.

Decaying colonies have been shown to be first invaded by ciliates and heterotrophic dinoflagellates (Fig. 5), probably grazing on liberated single *Phaeocystis* cells (Lancelot et al., 1991; Becquevort, unpubl. data). Bacterial colonization occurs later and coincides with the development of large populations of heterotrophic flagellates. Decaying aggregates formed at the wane of *Phaeocystis* blooms thus appear as microcosms with well developed grazing food chains.

7. Conclusion

Phaeocystis blooms produce an ample input of organic material to the pelagic environment.

Although the detailed chemistry of the carbohydrates produced are still not entirely known, it appears that this material should be potentially biodegradable. Despite this, it seems to largely resist rapid microbial breakdown. This is particularly the case during active growth of the colonies which are efficiently protected against bacterial colonization by mechanisms not clearly understood.

Slow microbial degradation may also be the case in the senescent phase where the skewed C:N:P-ratio of the material offered to the microbial food chain is suggested as a mechanism slowing down degradation due to nutrient limitation of bacterial growth.

When effective, such mechanisms would shift the trophic structure of *Phaeocystis* based food chains by increasing the probability of mesozooplankton grazing and/or increasing the depth to which sedimentation of colonies and mucus may occur.

References

Autio, R.M., 1990. Bacterioplankton in filtered brackish water cultures: some physical and chemical parameters affecting community properties. Arch. Hydrobiol., 117: 437–451.

Baumann, M.E.M., Lancelot, C., Brandini, F.P., Sakshaug, E. and John, D.M., 1994. The taxonomic identity of the cosmopolitan prymnesiophyte *Phaeocystis*: a morphological and ecophysiological approach. In: C. Lancelot and P. Wassmann (Editors), Ecology of *Phaeocystis*-dominated Ecosystems. J. Mar. Syst., 5: 81–100.

Billen, G., 1991. Protein degradation in aquatic environments. In: R. Chrost (Editor), Microbial Enzymes in Aquatic Environments. Broch/Springer Verlag Series in Contemporary Bioscience, Berlin, pp. 123–143.

Billen, G. and Fontigny, A., 1987. Dynamics of *Phaeocystis*-dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar. Ecol. Progr. Ser., 37: 249–257.

Billen, G. and Servais, P., 1989. Modélisation des processus de dégradation bactérienne de la matière organique en milieu aquatique. In: M. Bianchi, D. Maty, J.-C. Bertrand, P. Caumette and M. Gauthier (Editors), Micro-organismes dans les Écosystèmes Océaniques. Masson, Paris, pp. 219–245.

Bratbak, G., Heldal, M., Norland, S. and Thingstad, T.F., 1990. Viruses as partners in spring bloom microbial trophodynamics. Appl. Environ. Microbiol., 56: 1400–1405.

Brophy, J.E. and Carlson, D.J., 1989. Production of biologically refractory dissolved organic carbon by natural seawater microbial populations. Deep-Sea Res., 36: 497–507.

Chang, F.H., 1984. The ultrastructure of *Phaeocystis pouchetii* (Prymnesiophyceae) with special reference to the production of new mucilaginous envelope. N.Z.J. Mar. Freshwater Res., 18: 303–308.

Chrost, R., 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: R. Chrost (Editor), Microbial Enzyme in Aquatic Environments.

Brock/Springer Verlag Series in contemporary bioscience, Berlin, pp. 29–59.

Cota, G.F., Kottmeier, S.T., Robinson, D.H., Smith, W.O. Jr and Sullivan, C.W., 1990. Bacterioplankton in the marginal ice zone of the Weddell Sea: Biomass, production and metabolic activities during austral automn. Deep-Sea Res., 37: 1145–1167.

Davidson, A.T. and Marchant, H.J., 1987. Binding of manganese by Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release. Mar. Biol., 95: 481–487.

Deibel, D. and Powell, C.V.L., 1987. Comparison of the ultrastructure of the food-concentrating filter of two appendicularians. Mar. Ecol. Progr. Ser., 39: 81–85.

Ducklow, H.W., Purdie, D.A. and Williams, P.J.L., 1986. Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science: 232: 865–867.

Eberlein, K., Leal, M.T., Hammer, K.D. and Hickel, W., 1985. Dissolved organic substances during a *Phaeocystis pouchetii* bloom in the German Bight (North Sea). Mar. Biol., 89: 311–316.

Eilertsen, H.C., Tande, K. and Taasen, J.P., 1989. Vertical distributions of primary production and grazing by *Calanus glacialis* Jaschnov and *C. hyperboreus* Krøyer in Arctic waters (Barents Sea). Polar Biol., 253–260.

El-Sayed, S.Z., Biggs, D.C. and Holm-Hansen, O., 1983. Phytoplankton standing crop, primary productivity, and near-surface nitrogen fields in the Ross-Sea, Antarctica. Deep-Sea Res., 30: 871–886.

Estep, K., Nejstgaard, J.L., Skjoldal, H.R. and Rey, F., 1990. Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. Mar. Ecol. Progr. Ser., 67: 235–249.

Goldman, J.C., Caron, D.A. and Dennett, M.R., 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr., 32: 1239–1252.

Grossel, H. and Delesmont, R., 1984. Le microflagellé *Phaeocystis* et la production hétérotrophe bactérienne marine. Coll. Int. Bactériol. Mar., Brest.

Guillard, R.R.L. and Hellebust, J.A., 1974. Growth and the production of extracellular substances by two strains of *Phaeocystis pouchetii*. J. Phycol., 7: 330–338.

Hassel, A., Loeng, H., Rey, F. and Skjoldal, H.R., 1984. Preliminære resultater fra tokt med F/F G.O. Sars i Barentshavet, 28.5–18.6.1984. Rep. Mar. Res. Inst. Bergen, Norway, 31 pp.

Hickel, W., 1984. Seston in the Wadden Sea of Sylt (German Bight, North Sea). Neth. Inst. Sea Res. Publ. Ser., 10: 113–131.

Jahnke, J., 1989. The light and temperature dependence of growth rate and elemental composition of *Phaeocystis globosa* Scherffel and *P. pouchetii* (Har.) Lagerh. in batch cultures. Neth. J. Sea Res., 23: 15–21.

Joiris, C., Billen, G., Lancelot, C., Daro, M.H., Mommaerts, J.P., Bertels, A., Bossicart, M., Nijs, J.A., 1982. A budget of carbon cycling in the Belgian coastal zone: relative roles of zooplankton, bacterioplankton and benthos in the utilization of primary production. Neth. J. Sea Res., 16: 260–275.

Laanbroek, H.L., Verplanke, J.C., De Visscher, P.R.M. and De Vuyst, R., 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. Progr. Ser., 25: 1–11.

Lancelot, C. and Mathot, S., 1985. Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short-and long-term incubations with ^{14}C-bicarbonate. II. *Phaeocystis pouchetii* colonial population. Mar. Biol., 86: 227–232.

Lancelot, C., Mathot, S. and Owens, N., 1986. Modelling protein synthesis, a step to an accurate estimate of net primary production: the case of *Phaeocystis pouchetii* colonies in Belgian coastal water. Mar. Ecol. Progr. Ser., 32: 193–202.

Lancelot, C., Billen, G., Sournia, A., Weisse, T., Colijn, F., Veldhuis, M., Davies, A. and Wassmann, P., 1987. *Phaeocystis* blooms and nutrient enrichment in the continental coastal zone of the North Sea. AMBIO, 16: 38–46.

Lancelot, C., Billen, G. and Barth, H., 1991. The dynamics of *Phaeocystis* blooms in nutrient enriched coastal zones. Water Pollut. Res. Rep., 23, 106 pp.

Lancelot, C. and Rousseau, V., 1994. Ecology of *Phaeocystis*-dominated ecosystems: The key role of colony forms. In: B. Leadbeater and J. Green (Editors), The Prymnesiophyte Algae. Syst. Assoc., spec. vol. Oxford University Press.

Liss, P.S., Malin, G., Turner, S.M. and Holligan, P., 1994. Dimethyl sulphide and *Phaeocystis*: a review. In: C. Lancelot and P. Wassmann (Editors), Ecology of *Phaeocystis*-dominated Ecosystems. J. Mar. Syst., 5: 41–53.

Martinussen, I. and Thingstad, T.F., 1987. Utilization of N, P and organic C by heterotrophic bacteria. II. Comparison of experiments and a mathematical model. Mar. Ecol. Progr. Ser., 37: 285–293.

Martinussen, I. and Thingstad, T.F., 1991. A simple double staining technique for simultaneous quantification of auto- and heterotrophic nano- and picoplankton. Mar. Microb. Food Webs, 5: 5-11.

Nguyen, B.C., Belviso, S., Mikhalopoulos, N., Gostan, J. and Nival, P., 1988. Dimethyl sulfide production during natural phytoplankton blooms. Mar. Chem., 24: 133–141.

Pengerud, B., Skjoldal, E.F. and Thingstad, T.F., 1987. The reciprocal interaction between degradation of glucose and ecosystem structure. Studies in mixed chemostat cultures of marine bacteria, algae and bacterivorous nanoflagellates. Mar. Ecol. Progr. Ser., 35: 111–117.

Pett, R.J., 1989. Kinetics of the microbial mineralization of organic carbon from detrital *Skeletonema costatum* cells. Mar. Ecol. Progr. Ser., 52: 123–128.

Pomeroy, L.R. and Deibel, D., 1986. Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science, 233: 359–361.

Pomeroy, L.R. and Wiebe, W.J., 1994. Energy sources for microbial food webs. Mar. Microb. Food Webs, in press.

Rousseau, V., Mathot, S. and Lancelot, C., 1990. Carbon biomass of *Phaeocystis* sp. from microscopic observations. Mar. Biol., 107: 305–314.

Sieburth, J.M., 1961. Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. Science, 132: 676–677.

Skjoldal, H.R. and Rey, F., 1989. Pelagic production and variability in the Barents Sea ecosystem. In: K. Sherman and L. Alexander (Editors). Proc. Symp. Biomass and Geography of Large Marine Ecosystems. Chicago, 16–17 February 1987, pp. 243–288.

Somville, M. and Billen, G., 1983. A method for determining exoproteolytic activity in natural waters. Limnol. Oceanogr., 28: 190–193.

Thingstad, T.F. and Martinussen, I., 1991. Are bacteria active in the cold pelagic ecosystem of the Barents Sea? Polar Res.

Vadstein, O., Jensen, A., Olsen, Y. and Reinertsen, H., 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. Limnol. Oceanogr., 33: 489–503.

Vairavamurthy, A., Andreae, M.O. and Iverson, R.L., 1985. Biosynthesis of dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. Limnol. Oceanogr., 30: 59–70.

van Bennekom, A.J., Gieskes, W.W.C. and Tijssen, S.B., 1975. Eutrophication of Dutch coastal waters. Proc. R. Soc. (B), 189: 359–374.

Veldhuis, M.J.W., Colijn, F. and Venekamp, L.A.H., 1986. The spring bloom of *Phaeocystis pouchetii* (haptophyceae) in Dutch coastal water. Neth. J. Sea Res., 20: 37–48.

Veldhuis, M.J.W., Colijn, F. and Admiraal, W., 1991. Phosphate utilization in *Phaeocystis pouchetii* (Haptophyceae) P.S.Z.N.I. Mar. Ecol., 12: 53–62.

Veldhuis, M.J.W., Admiraal, W. and Colijn, F., 1986. Chemical and physiological changes of phytoplankton during the spring bloom, dominated by *Phaeocystis pouchetii* (haptophyceae): Observations in Dutch coastal waters of the North Sea. Neth. J. Sea Res., 20: 49–60.

Wassmann, P., Vernet, M., Mitchell, B.G. and Rey, F., 1990. Mass sedimentation of *Phaeocystis pouchetii* in the Barents Sea. Mar. Ecol. Progr. Ser., 66: 183–195.