Carbohydrate production by phytoplankton and degradation in the marine microbial food web

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Chapter 6

The carbohydrates of *Phaeocystis* and their degradation in the microbial food web – a review

Anne-Carlijn Alderkamp, Anita G. J. Buma, and Marion van Rijssel

The ubiquity and high productivity associated with blooms of colonial *Phaeocystis* makes it an important contributor to the global carbon cycle. During blooms organic matter is produced that is rich in carbohydrates. We distinguish five different pools of carbohydrates produced by *Phaeocystis*. Like all plant cells, *Phaeocystis* cells contain (1) structural carbohydrates, mainly as part of their cell wall and (2) monosaccharides, that are present as intermediates in the synthesis and catabolism of cell components. Both solitary and colonial cells produce (3) an intracellular storage glucan, whereas colonial cells of *Phaeocystis* excrete (4) carbohydrates that are the main constituent of the mucous colony matrix and (5) extracolonial dissolved organic carbon (DOC) rich in carbohydrates. In this review the characteristics of these pools are discussed and quantitative data are summarized.

When nutrients are limited, *Phaeocystis* blooms reach a stationary growth phase, during which excess energy is stored as carbohydrates. This so-called overflow metabolism increases the carbohydrate to POC ratio to 0.6. Overflow metabolism can be channelled towards both glucan and mucopolysaccharides. Summarizing the available data reveals that during the stationary phase of a bloom glucan may contribute between 0-51% to POC, whereas mucopolysaccharides contribute between 5-60%. The accumulation of carbohydrates leads to an increase in C/N and C/P ratios of *Phaeocystis* organic material.

At the end of a bloom lysis of *Phaeocystis* cells and deterioration of colonies leads to a massive release of dissolved organic matter (DOM) rich in glucan and mucopolysaccharides. Laboratory studies have revealed that this organic matter is potentially readily degradable by heterotrophic bacteria. However, observations in the field of accumulation of DOM and foam indicate that microbial degradation may be
hampered. The high C/N and C/P ratios of *Phaeocystis* organic matter may lead to nutrient limitation of microbial degradation. Upon prolonged degradation times carbohydrates self-assemble into hydrogels. This may have a profound effect on carbon cycling, since hydrogels provide a vehicle to move DOM up the size spectrum to sizes subject to sedimentation. In addition, it changes the physical nature and microscale structure of the organic matter encountered by bacteria, thereby affecting the degradation potential of the *Phaeocystis* organic matter.

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INTRODUCTION

*Phaeocystis* is a marine planktonic algal genus with a worldwide distribution. It is present from tropical to polar regions and can form blooms in areas with high nutrient concentrations, such as coastal or upwelling regions (Baumann et al. 1994; Lancelot et al. 1998; Schoemann et al. 2005). One of the characteristics of *Phaeocystis* species is the complex polymorphic life cycle. It consists of different stages of free-living cells 3-9 µm in diameter, alternating with colonial stages usually reaching several mm in diameter (Kornmann 1955; Rousseau et al. 1994; Peperzak et al. 2000), sometimes up to 3 cm (Chen et al. 2002). Although single-celled *Phaeocystis* are ubiquitous in the worlds oceans, it is the colonial form that has attracted most attention due to its massive blooms (Lancelot et al. 1987a; Schoemann et al. 2005). At present six *Phaeocystis* species have been described (Zingone et al. 1999) of which only three are reported to form blooms; *P. globosa* in temperate regions (English Channel, coastal North Sea, China), *P. pouchetii* in temperate to polar waters of the Northern hemisphere, and *P. antarctica* in (sub) polar waters of the southern hemisphere (Schoemann et al. 2005). During blooms *Phaeocystis* may contribute over 90% to the total phytoplankton abundance and can be responsible for up to 65% of the local annual primary production (Joiris et al. 1982; Veldhuis et al. 1986b; Lancelot and Mathot 1987b). The high productivity associated with blooms and its ubiquity make *Phaeocystis* an important contributor to the global carbon cycle (Smith et al. 1991; Arrigo et al. 1999; DiTullio et al. 2000; Schoemann et al. 2005). A substantial part of this carbon is produced as carbohydrates (Lancelot et al. 1987b; Rousseau et al. 1990; Fernandez et al. 1992; Mathot et al. 2000). In general, most of *Phaeocystis* organic matter is remineralized within the water column (Wassmann 1994). To understand the impact of this vast carbon injection on the ecosystem, it is important to consider the different types of carbohydrates that are produced. In the following sections we will first discuss the current state of knowledge with respect to the characteristics and quantities of different pools of carbohydrates produced during the various life cycle stages. Secondly, mechanisms of DOM release during and following a bloom will be discussed, and finally, the fate of carbohydrates in the microbial food web.
Carbohydrates in algae and plants are often classified based on methodological discrimination. The structural carbohydrates are not water soluble, whereas the other types of carbohydrates are water soluble and typically extracted in hot water. In *Phaeocystis* five different pools of carbohydrates can be distinguished. Like all plant cells, *Phaeocystis* cells contain (1) structural carbohydrates, mainly as part of their cell wall and (2) monosaccharides, that are present as intermediates in the synthesis and catabolism of other carbohydrates. Both solitary and colonial cells produce (3) an intracellular storage glucan, whereas colonial cells of *Phaeocystis* excrete (4) carbohydrates that are the main constituent of the mucous colony matrix and (5) extracolonial dissolved organic carbon (DOC), rich in carbohydrates.

**Structural polysaccharides**

Structural carbohydrates are mainly present as cell wall constituents. Flagellate *Phaeocystis* cells are covered with body scales consisting of flat plates with a pattern of radiating ridges (Parke et al. 1971; Moestrop 1979; Hallegraeff 1983; Baumann et al. 1994). In two other haptophytes, *Pleurochrysis scherfelii* and *Chrysochromulina chiton* the scales contained acidic, sulfated pectin-like polysaccharides, cellulose and glycopeptides (reviewed by Leadbeater 1993). In a culture of *Phaeocystis* sp. in the stationary phase, structural carbohydrates in the organic matter made up for 13% of the TOC (Biersmith and Benner 1998).

In addition to intracellular structural carbohydrates, flagellate cells of *Phaeocystis* excrete filaments composed of α-chitin, forming star-like structures. In many morphological descriptions based on electron microscopic observations, five-rayed filamentosous stars were observed around flagellate cells of *P. pouchetii*, *P. antarctica*, *P. globosa*, and *P. cordata*, whereas nine-rayed stars were observed around *P. scrobiculata* (Parke et al. 1971; Moestrop 1979; Baumann et al. 1994; Chretiennot-Dinet et al. 1997; Zingone et al. 1999). The biological function of the chitin filaments remains a mystery up to now (Chretiennot-Dinet et al. 1997).

**Monosaccharides**

Monosaccharides are the first products of photosynthesis and form the precursors for biosynthesis of most molecules. Moreover, during catabolism of storage glucan monosaccharides are formed as intermediates (Granum and Myklestad 1999). In addition to intermediates in metabolic processes, they serve a role in osmoregulation (Craigie 1974; Dickson and Kirst 1987a; 1987b). The monosaccharide concentration in phytoplankton cells is quite stable; for example in diatoms monosaccharide concentration was hardly influenced by light (Vårum et al. 1986; Van Oijen et al. 2003). During a bloom of *P. globosa* in the Marsdiep, The Netherlands, the monosaccharides made up for 28-60% of water extractable particulate carbohydrates (Alderkamp, unpublished data). This ratio was similar to an Antarctic community dominated by diatoms (Van Oijen et al. 2003).
Storage glucan

Most algae contain an intracellular food reserve in the form of α- or β-glucan. The actual type of glucan is an important taxonomic characteristic. In the class of Prymnesiophyceae the reserve polysaccharides are of the laminarin type (van den Hoek et al. 1993). They can be divided into laminarins, chrysolaminaran and paramylon, distinguished mainly on the basis of chain length and presence of mannitol end groups. All have an essentially linear β-D-(1,3)-glucose backbone with some branching at position 6 (Percival 1970; Craigie 1974; Myklestad 1978; Paulsen and Myklestad 1978). Variations have been reported in the glucan structure of the prymnesiophyccean *Emiliania huxleyi*, that consists of a mainly β-D-(1,6)-linked glucose backbone with branching at position 3 (Vårum et al. 1986). The glucan of *Phaeocystis* is of the chrysolaminaran type (Janse et al. 1996b), a type of laminarin devoid of endstanding mannitol groups, and containing typically 16-38 glucose residues (Craigie 1974). In algal cells chrysolaminaran is located in vacuoles, as was observed microscopically in *Phaeocystis* (Kornmann 1955; Parke et al. 1971; Chang 1984) and using immunolocalization of 1,3 beta-D-glucan in cell sections of the diatoms *Phaeodactylum tricornutum*, *Cilindrotheca fusiformis* and *Thalassiosira pseudonana* (Chiovitti et al. 2004). Chrysolaminaran is the most common storage glucan in phytoplankton, including diatoms (Myklestad and Haug 1972; Myklestad 1978; 1988; Paulsen et al. 1978). Due to the presence in ubiquitous bloom forming phytoplankton chrysolaminaran it is considered to be one of the most abundant types of carbohydrate in the marine environment (Painter 1983).

Chrysolaminaran was characterized as principal storage glucan in a non-colony forming strain of *P. globosa* (Janse et al. 1996b). In this strain, 95% of the water extractable carbohydrates consisted of glucose and all glucose polymers were digested by the enzyme laminarinase (E.C. 3.2.1.6 from *Trichoderma* sp.). This enzyme preparation contains endo-β 1-3 glucanase activity and some chitinase, cellulase and α-amylase activity. Laminarin from the brown seaweed *Laminaria digitata* was digested to monosaccharides by this enzyme preparation. In colony forming strains of *P. globosa*, however, no more than 40-70% of glucose polymers were digested (van Rijssel et al. 2000; Alderkamp et al. 2006). The reason for this partial digestion may be differences in the type of chrysolaminaran produced by single cells and colonial cells, e.g. with respect to polymer size or number of branches. Therefore, chrysolaminaran from colonial cells may not be fully degraded by the enzyme laminarinase. Alternatively, there may be a different type of glucan produced by colonial *Phaeocystis* cells.

If there is another type of storage glucan besides chrysolaminaran, it is not clear if this is located in the cells or in the colony matrix.

In all plants photosynthetic rates exceed metabolic demands when nutrients are not limiting and irradiance is optimal. The excess energy thus produced is stored as glucan. This glucan can be used as a respiratory substrate to maintain growth when irradiance is sub-optimal, for example during the night. Chrysolaminaran is produced in the light and its production is dependent on light intensity. Nocturnal respiration of chrysolaminaran provides energy to maintain cell metabolism and its carbon skeletons can be used for protein synthesis (Cuhel et al. 1984; Lancelot and Mathot 1985; Janse et al. 1996b; Granum et al. 1999; Alderkamp et al. 2006). Diel rhythms in storage carbohydrates concentrations have been well documented in laboratory cultures of diatoms (Hitchcock 1980; Vårum et al. 1986), field populations of
pelagic marine phytoplankton dominated by diatoms (Barlow 1982; Hama and Handa 1992) and mesocosms dominated by *P. pouchetii* (Alderkamp et al. 2006). Notably the diel variations in carbohydrates can be a direct result of the variations in light intensity, or the result of an intrinsic rhythm (Post et al. 1985; Falkowski and Raven 1997). For instance, Loogman (1982) showed that the diel dynamics in carbohydrate concentrations introduced by a diel light cycle, continued for several days after the light was switched to a continuous regime.

Under normal bloom conditions, *Phaeocystis* will receive varying light conditions during the day. On top of this, upon vertical mixing in the water column colonies may be mixed down to sub-optimal light conditions, or even below the euphotic zone (Joint and Pomroy 1993; Arrigo et al. 1999; Wassmann et al. 2000). A capability to store energy is therefore likely to be important during the daylight as much as during the night. This may especially be relevant for *P. globosa*, since blooms often occur in turbid waters with high mixing rates, such as the coastal North Sea and estuaries. During sudden transitions from high to low light levels, carbohydrates can provide energy until cells are acclimatized to the new situation, as was shown in the diatom *Thalassiosira weissflogii* (Post et al. 1985). This was, however, not confirmed during a mesocosm study of *P. pouchetii*. Carbohydrate concentrations were unaffected by placing cells at low irradiances or in the dark during the day (Alderkamp et al. 2006).

Upon nutrient limitation excess energy and carbon tend to accumulate as storage glucan because the Calvin cycle and the tricarboxylic acid (TCA) cycle are uncoupled. Carbohydrate accumulation has been observed during the stationary growth phase of a single cell culture (Janse et al. 1996b), colonial cultures (van Rijssel et al. 2000), and a bloom of *P. globosa* (Veldhuis et al. 1986a), and at the end of *P. pouchetii* blooms in mesocosms (Alderkamp et al. 2006). This so-called overflow metabolism under nutrient limitation is common in phytoplankton (Myklestad 1974; Myklestad 1988). It leads to an increased contribution of carbohydrates to the organic matter produced during bloom and an increased C/N ratio. C/N ratios up to 20.5 were reported in nutrient limited mesocosms of *P. pouchetii* (Verity et al. 1988), up to 17.5 during blooms of *P. pouchetii* and up to 30 during blooms of *P. globosa* at low ambient inorganic nitrogen concentrations (Baumann et al. 1994). During the stationary phase of a *P. pouchetii* bloom in mesocosms the ratio glucan/mucopolysaccharides increased (Alderkamp et al. 2006), whereas it decreased during the stationary phase of cultures of colonial *P. globosa* (van Rijssel et al. 2000). Therefore, it seems energy and carbon produced during overflow metabolism in *Phaeocystis* can be channeled towards both glucan and mucopolysaccharides.

From the above it may be concluded that the concentration of storage glucan in phytoplankton is highly variable; it is dependent on the time of the day, light intensity and nutrient status of the cell. To evaluate the contribution of glucan to *Phaeocystis* carbon, quantitative data on the contribution of glucose to the particulate water extractable carbohydrates were compiled (Table 1a). Assuming that mucopolysaccharides consist for 10% of glucose (Janse et al. 1999), the remaining glucose can be attributed to glucan. This leads to a contribution of glucan to the particulate water-extractable carbohydrate fraction of *Phaeocystis* colonies between 14-85% during the exponential growth phase, and between
Table 1A Monosaccharide composition of *Phaeocystis* carbohydrates

<table>
<thead>
<tr>
<th>Species</th>
<th>% total</th>
<th>% non-glucose carbohydrate</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. pouchetii</em> dominated mesocosm</td>
<td>26-40</td>
<td>1-8</td>
<td>9-30</td>
</tr>
<tr>
<td><em>P. globosa</em> colonial cultures, four strains from the North Sea</td>
<td>7-85</td>
<td>25-32</td>
<td>12-25</td>
</tr>
<tr>
<td><em>P. pouchetii</em> bloom, Balsfjord</td>
<td>45</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td><em>Phaeocystis</em> sp. culture</td>
<td>27</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td><em>P. globosa</em> bloom, North Sea</td>
<td>20-75</td>
<td>32-43</td>
<td>17-25</td>
</tr>
<tr>
<td><em>Phaeocystis</em> sp. bloom, English Channel</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Table 1B Monosaccharide composition of *Phaeocystis* extracolonial DOM

<table>
<thead>
<tr>
<th>Species</th>
<th>% total carbohydrates</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phaeocystis</em> sp. culture</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td><em>Phaeocystis</em> sp. culture</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td><em>P. pouchetii</em> cultures, 2 strains</td>
<td>4.5-30</td>
<td>0-5.2</td>
</tr>
</tbody>
</table>

Phaeocystis carbohydrates

0-85% during the stationary phase. Alternatively, glucan and mucopolysaccharides were separated based on polymer size differences by ethanol precipitation. During the growth phase of a *P. pouchetii* bloom in a mesocosm, glucan contributed 46-78% to particulate water extractable carbohydrates, depending on the time of the day. This increased to 82% during the stationary phase of the bloom (Alderkamp et al. 2006). To compute the contribution of glucan to colonial *Phaeocystis* POC, the carbohydrate/POC ratio of Alderkamp et al. (2006) was used (Table 2). This leads to a contribution of glucan to POC of 2-11% during exponential growth, dependent on the time of the day. During the stationary phase the contribution ranges between 0-51%. In a non-colony forming strain of *P. globosa*, chrysolaminaran contributed 40% to TOC during the stationary phase (Janse et al. 1996b).

**Mucopolysaccharides**

The mucus matrix of *Phaeocystis* colonies consists predominantly of carbohydrates (Guillard and Helleburst 1971; Painter 1983; Lancelot et al. 1985; Eberlein et al. 1985; Van Boekel 1992). Qualitative staining of the colony matrix indicated the absence of lipophilic compounds and chitin, but the presence of amino groups (Hamm et al. 1999). MALDI analysis showed that N was not proteineous, but probably present in aminosugars (Solomon et al. 2003). The presence of the aminosugar sialic acid was confirmed by aminolabeling (Orellana et al. 2003). The presence of aminosugars may affect the C/N ratio of mucopolysaccharides, leading to a small difference between C/N ratios of single cells and colonial cells (Verity et al. 1988). Verity et al. (1991) concluded that the C/N ratio of the mucus constituents is lower than 9, whereas Biersmith et al. (1998) measured a C/N ratio of 15.6 in the fraction containing mucopolysaccharides.

The monosaccharide composition of *P. globosa* and *P. pouchetii* carbohydrates has been studied in bloom samples and in strains from different origins (Table 1A). The main non-glucose monosaccharides are arabinose, galactose, mannose and xylose. In addition a minor contribution of rhamnose was detected in all samples, whereas fucose, glucuronic acid, ribose, O-methylated hexoses and O-methylated pentoses were detected in some samples only. The glucosamine detected by two groups may have been derived from copepod chitin contamination in the samples. The monosaccharides detected in *Phaeocystis* material are the same as those detected in other phytoplankton extracellular carbohydrates (Hoagland et al. 1993; Biersmith et al. 1998), however the ratio of constituents seems specific for *Phaeocystis*. There were no major differences in the presence or ratio of constituents between a bloom sample from Balsfjord, Norway, dominated by *P. pouchetii* and a sample from the North Sea dominated by *P. globosa* (Janse et al. 1999). However, no data are available for other species of *Phaeocystis*, including *P. antarctica*. In five strains of *P. globosa* differences in the ratio of constituents were small and probably determined by environmental factors present in the seawater from different locations in which the algae were cultured (van Rijssel et al. 2000). Nutrient status of the cells does not seem to influence the ratio, as was shown in a batch culture of *P. globosa* (van Rijssel et al. 2000) or in bloom samples taken at different stages of a bloom (Janse et al. 1996a). This is different from the situation reported in diatoms, where upon nutrient limitation, the benthic diatom *Cylindrotheca closterium* excretes a separate extracellular polysaccharide with a different monosaccharide composition (de Brouwer et al.
2002; Underwood et al. 2004). The change in colony shape and appearance during *Phaeocystis* blooms (e.g. Verity et al. 1988; Rousseau et al. 1994) is therefore not reflected in the monosaccharide composition of the mucopolysaccharides.

*Phaeocystis* acts as a secretory cell, actively excreting mucopolysaccharides similar to animal cells and vascular plants in which the regulated export of cellular products takes place via exocytosis. While stored in secretory vesicles inside the cells, the mucopolysaccharides remain in condensed phase. Upon exocytosis, they undergo a characteristic phase transition accompanied by extensive swelling resulting in the formation of hydrated gels (Chang 1984; Chin et al. 2004). The negatively charged mucopolysaccharides become entangled and form ionic links, such as calcium bridges (Van Boekel 1992). They then form a thin, yet strong, semi permeable colony matrix, with a pore size between 1 and 4.4 nm diameter, with plastic and to a limited extent also elastic properties (Kornmann 1955; Hamm et al. 1999). Electron microscope studies of a *Phaeocystis* sp. from Tasman Bay, New Zealand, show that the matrix has a multilayered gelatinous structure, with an average thickness of 5 µm (Chang 1984). Van Rijssel et al. (1997) calculated a thickness of 7 µm based on carbohydrate measurements and the assumption of a mucous layer with a fixed thickness for various colony sizes, whereas a thickness of 10 µm was determined based on confocal laser scanning imagery. As colonial cells of *Phaeocystis* have similar (Baumann et al. 1994), or even higher growth rates (Veldhuis et al. 2005) compared to single cells, the allocation of the material needed to build the colonial matrix obviously does not hamper cell metabolism.

The contribution of mucus carbon to *Phaeocystis* organic carbon can be assessed in three different ways. In the first, cells are mechanically separated from the mucus matrix by filtration through GF/C filters. This method yields contributions of 5-80% of mucus-carbon to POC (reviewed by Riegm an and Van Boekel 1996). As many have pointed out, however, *Phaeocystis* cells are fragile and may be disrupted by filtration (Veldhuis and Admiraal 1985; van Rijssel et al. 1997; Mathot et al. 2000). By releasing their water soluble carbon the fraction of mucus-carbon may be overestimated. On the other hand, mucopolysaccharides may form gels on the filter (Chin et al. 1998) as a result of which their contribution may be underestimated. Therefore, all studies in which colonies were fractionated by filtration or centrifugation have to be interpreted with care.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth stage</th>
<th>Carb/POC ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. pouchetii</em> mesocosm</td>
<td>Exponential</td>
<td>0.13</td>
<td>Alderkamp et al. 2006</td>
</tr>
<tr>
<td><em>P. pouchetii</em> mesocosm</td>
<td>Stationary</td>
<td>0.60</td>
<td>Alderkamp et al. 2006</td>
</tr>
<tr>
<td><em>P. globosa</em> culture</td>
<td>Exponential</td>
<td>0.1</td>
<td>Van Rijssel et al. 2000</td>
</tr>
<tr>
<td><em>P. globosa</em> bloom</td>
<td>No info</td>
<td>0.19-0.35</td>
<td>Van Rijssel et al. 2000</td>
</tr>
<tr>
<td><em>Phaeocystis</em> sp. culture</td>
<td>Stationary</td>
<td>0.38</td>
<td>Biersmith and Benner 1998</td>
</tr>
<tr>
<td><em>Phaeocystis</em> sp. culture</td>
<td>All</td>
<td>0.2-0.3</td>
<td>Biddanda and Benner 1997</td>
</tr>
<tr>
<td><em>P. globosa</em> single cell culture</td>
<td>Stationary</td>
<td>0.40</td>
<td>Janse et al. 1996b</td>
</tr>
</tbody>
</table>
Table 3. Characteristics of Phaeocystis DOM

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth stage</th>
<th>% diss. carbs. of DOC</th>
<th>C/N</th>
<th>% DOC of TOC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaeocystis sp.</td>
<td>Exponential through stationary</td>
<td>25-50%</td>
<td>19.3 (HMW)</td>
<td>27%</td>
<td>Biddanda and Benner 1997</td>
</tr>
<tr>
<td>(culture)</td>
<td>Stationary</td>
<td>56%</td>
<td>6.3 (LMW)</td>
<td>14%</td>
<td>Biersmith and Benner 1998</td>
</tr>
<tr>
<td>Phaeocystis sp.</td>
<td>Late exponential</td>
<td>&gt;67% (HMW)</td>
<td>12 (HMW)</td>
<td></td>
<td>Aluwihare and Repeta 1999</td>
</tr>
<tr>
<td>(culture)</td>
<td>Late exponential</td>
<td></td>
<td>8.3 (axenic)</td>
<td></td>
<td>Solomon et al. 2003</td>
</tr>
<tr>
<td>P. globosa</td>
<td>Late exponential</td>
<td></td>
<td>7.4 (xenic)</td>
<td></td>
<td>Solomon et al. 2003</td>
</tr>
<tr>
<td>(culture)</td>
<td></td>
<td></td>
<td>6.9 (axenic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. antarctica</td>
<td></td>
<td></td>
<td>6.2 (xenic)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When assuming that carbohydrates are the only significant source of carbon in the mucus matrix, the second method consists of determination of the contribution of mucopolysaccharides to the water extractable carbohydrates. Mucopolysaccharides consist of all non-glucose constituents and for 10% of glucose. Based on the monosaccharide composition the contribution of mucopolysaccharides to the water extractable particulate carbohydrates is between 15-86% during the exponential growth phase, and 15-100% during the stationary phase (table 1A and references therein). Alternatively, glucan and mucopolysaccharides were separated based on polymer size differences (Alderkamp et al. 2006). During the exponential growth phase, mucopolysaccharides contributed 38% to the water extractable carbohydrates, decreasing to 9% during the stationary phase. When using carbohydrate to carbon ratios of Alderkamp et al. (2006) (Table 2), the contribution of mucopolysaccharides to POC is computed to be between 2-11% during exponential growth and 5-60% during the stationary phase.

The third method to estimate the carbon of colony matrices is based on conventional conversion factors for determining the carbon content of the average cell biovolume of Phaeocystis cells and an additional carbon content of the mucus matrix. In P. antarctica, Mathot et al. (2000) found on average 14% of POC devoted to matrix formation, increasing to a maximum of 33%. In P. globosa Rousseau et al. (1990) calculated that up to 90% of POC may be devoted to matrix formation in large colonies. Van Rijssel et al. (2000), assuming a hollow colony surrounded by a thin mucus layer, calculated a contribution of 10-35%. Comparisons of estimates obtained by these authors, however, show similar estimates for colony sizes predominantly observed in the field (reviewed by Schoemann et al 2005).

In addition to a structural function in the mucous matrix, mucopolysaccharides were also reported to serve as storage polysaccharides as nocturnal consumption of extracellular polysaccharides was reported in P. globosa (Lancelot et al. 1985; Veldhuis et al. 1985). In both studies cells were separated from mucous matrix by filtration under pressure. Since this treatment may cause cells to break thus releasing intracellular substances (see above), nocturnal consumption of intracellular chrysaminaran may have been classified as
consumption of extracellular substances. Using the same method to separate extracellular material from cells, Matrai et al. (1995) report no nocturnal utilization of extracellular polymers in an Antarctic strain of Phaeocystis. Also, when mucopolysaccharides and glucan were separated based on polymer size differences, no utilization of mucopolysaccharides was reported when a culture of P. globosa was placed in the dark for prolonged times (Alderkamp et al. 2006). Given the size and structure of mucopolysaccharides it seems unlikely that they serve as storage carbohydrate. Degradation and uptake of these complex polysaccharides would require a complex enzymatic cleavage and transport system in the cells. Moreover, since the gel matrix is such a definite structure stabilized by ionic bridges, it will be hard to “disentangle” polymers from the gel.

Extracolonial DOM

Extra-colonial DOM rich in carbohydrates was reported in cultures of colonial Phaeocystis and blooms of Phaeocystis (Table 3 and references therein). Carbohydrates similar in monosaccharide composition and ratio to that of mucopolysaccharides form a major contribution to this DOM (Table 1b). There seem to be two different pools of DOM; a high molecular weight (HMW)-DOM pool with a high C/N ratio of over 12 and a carbohydrate content of over 67% and a low molecular weight (LMW)-DOM pool with a low C/N ratio of 6.3. The overall C/N ratio of the DOM is low, around 7 (Biddanda and Benner 1997; Solomon et al. 2003). Similar to mucopolysaccharides, extra-colonial polymers self assemble in hydrated polymer gels on a time-scale of approximately two days (Solomon et al. 2003).

High DOM concentrations have been reported in natural assemblages dominated by Phaeocystis (Eberlein et al. 1985), and high DOM-excretion rates in cultures (Guillard et al. 1971). Upon careful filtration, however, true extracolonial DOM excretion was lower, 14% of primary production in the late exponential phase of a P. globosa culture (Veldhuis et al. 1985; 1986a; Biersmith et al. 1998), and 2-14% of primary production in natural assemblages dominated by P. globosa (Laanbroek et al. 1985; Veldhuis et al. 1986a; Lancelot et al. 1987b). Similar values were observed for P. pouchetii (1-19%) (Verity et al. 1991), and during a bloom of P. antarctica (11%) (Carlson et al. 1998).

DOM production seems to be restricted to colonial Phaeocystis, since low production of DOM was reported in cultures of single cell P. pouchetii and P. globosa (Guillard et al. 1971; Janse et al. 1996b).

MECHANISMS OF DOM RELEASE DURING A PHAEOCYSTIS BLOOM

In general 10-25% of phytoplankton primary production is excreted directly as DOC (Sharp 1977; Larsson and Hagström 1979; Lignell 1990; Teira et al. 2003; Marañón et al. 2004). The three main mechanisms of DOM production are 1) release after cell lysis, e.g as a result of viral infection, protozoan sloppy feeding and egestion, 2) bacterial cleavage of exopolysaccharides (Smith et al. 1995), or 3) direct release as a physiological process of intact cells (reviewed by Nagata 2000). Two physiological mechanisms of DOC production have been advocated: a passive diffusion of small metabolites through the cell membrane (Björnsen
and an overflow mechanism whereby excess photosynthetic products are actively released when the C fixation rate exceeds the rate of macromolecular production (Fogg 1983).

During the exponential growth phase of a *Phaeocystis* bloom DOM release as a result of non-physiological processes will be low. In a *P. globosa* bloom rates of cell lysis were low in the growth phase (Van Boekel et al. 1992; Brussaard et al. 1995; 1996; 2005a), therefore DOM release as a result of cell lysis will be insignificant. Since *Phaeocystis* colonies largely escape grazing control (Breteler and Koski 2003; Nejstgaard et al in prep), sloppy feeding will not be a cause of significant DOM release either. In a diatom bloom it was shown that healthy, actively growing *Chaetoceros* and *Thalassiosira* cells were colonized by bacteria with high ectoenzyme activity. When the hydrolysis rate of phytoplankton surface material by bacteria exceeds the uptake rate, DOM will be released into the environment (Smith et al. 1995). Although the growth phase of *P. globosa* blooms are typically accompanied by an increase in bacterial numbers, bacterial production, and enzyme activity (Billen and Fontigny 1987; Van Boekel et al. 1992; Putt et al. 1994; Brussaard et al. 1996; 2005b; Becquevort et al. 1998; Arrieta and Herndl 2002), young healthy colonies are generally free of bacteria (Thingstad and Billen 1994; Lancelot and Rousseau 1994). Therefore, it is unlikely that hydrolysis of the mucous matrix by bacteria will lead to significant DOM production during the growth phase of the bloom. During all growth stages DOM excretion from healthy cells was observed in cultures of *Phaeocystis* sp. (Biddanda et al. 1997), and therefore this is probably a significant mechanism for DOM production during the growth phase of a bloom.

During the stationary phase of a bloom colonies become disrupted, and flagellate cells begin to develop from the colonial cells (Peperzak et al. 2000). Due to the overflow metabolism towards mucopolysaccharides and glucan, the ratio of carbohydrates to POC increases. During this phase of a bloom DOC production through cell lysis will be high. Cell lysis rates of up to 33% d\(^{-1}\) were observed at the peak of *P. globosa* blooms (Van Boekel et al. 1992; Brussaard et al. 1995; 1996; 2005a). In this way the carbohydrate rich cell content will be released as DOM. When the flagellate cells that develop inside colonies detach from the colony matrix, they are readily grazed upon by microzooplankton (Weisse and Scheffel-Möser 1990; Tang et al. 2001). In this way DOM may be released through sloppy feeding. During the stationary phase of a bloom, bacterial communities attach to *Phaeocystis* colonies and to ghost colonies that originate when flagellate cells that develop inside colonies detach from the colony matrix. Bacteria with high glucosidase activity are present in both free-living and particle attached communities (Billen et al. 1987; Becquevort et al. 1998). If hydrolysis rates of mucopolysaccharides from the colony matrix exceeds uptake of the polymers, mucopolysaccharides may be released as DOM.

The physiological mechanism of excess release of DOC under nutrient limitation has not been observed in *Phaeocystis*. In *Phaeocystis* sp. cultures DOC production was coupled to POC production at different growth stages (Biddanda et al. 1997). Also in enclosures of *P. antarctica*, ratios of recently produced DOC increased only slightly after nutrient depletion, (Smith, Jr. et al. 1998; Carlson et al. 1998). This indicates that nutrient limitation does not lead to increased excretion of DOC resulting from an overflow mechanism.
MICROBIAL DEGRADATION

Generally, most of the biomass produced during *Phaeocystis* blooms is remineralized in the water column (Wassmann 1994), where DOM is remineralized by heterotrophic bacteria. Occasionally, however, accumulation of DOM and foam have been reported (e.g. Eberlein et al. 1985; Lancelot et al. 1987a; Seuront et al. in prep). This seems to indicate that bacterial degradation of organic matter produced by in *Phaeocystis* blooms varies between blooms, and sometimes is hampered. There may be various reasons for slow degradation and they may be different in different stages of a bloom.

**Microbial degradation of *Phaeocystis* carbohydrates**

During the growth phase of a bloom of *P. antarctica* all DOC produced was found to be rapidly degraded by bacteria (Smith et al. 1998). With time the composition and contribution of organic matter that can be utilized by bacteria will change from readily degradable freshly excreted DOC during the growth phase, to the DOC dominated by mucopolysaccharides and glucan during the senescent stage. This DOC may be more recalcitrant to bacterial degradation. In laboratory experiments, however, it was shown that carbohydrates derived from *P. globosa* and *P. pouchetii* colonies were readily degraded by bacterial communities under both oxic and anoxic conditions. In enrichment cultures of these bacterial communities on the *Phaeocystis* carbohydrates, degradation of glucan was rapid, but degradation rates slowed down markedly during degradation of remaining mucopolysaccharides (O辛inga et al. 1997; Janse et al. 1999). The monosaccharide composition and ratio of constituents remained unchanged during degradation of the mucopolysaccharides, showing no indication of refractory parts within the mucopolysaccharide fraction (Janse et al. 1999).

Identification of bacteria involved in mucopolysaccharide degradation revealed the presence of bacteria from different phylogenetic groups, such as α- and γ-Proteobacteria, Cytophaga-Flavobacter (CF) cluster of the Bacteroidetes and the Planktomycetes and Verrucomicrobiales clade. The species composition of the bacterial community remained unchanged during mucopolysaccharide degradation, indicating that degradation of mucopolysaccharides in itself does not require a succession of species (Janse et al. 2000). During a *P. globosa* bloom in a mesocosm, however, the bacterial community showed distinct changes with time. These changes were related to the growth stage of the bloom and subsequent enhanced cell lysis and concomitant release of DOM, whereas the nature of the phytoplankton growth controlling substrate (i.e. N or P) did not influence the microbial community (Brussaard et al. 2005b). Degradation of polymers requires specialized enzyme systems, and is mediated by specific extracellular enzymes that hydrolyze large molecules prior to uptake (Chróst 1991). Arrieta and Herndl (2002) revealed a succession of different types of bacterial β-glucosidases during a *P. globosa* bloom, which was related to a change in the bacterial community. Therefore, the copious amounts of glucan and mucopolysaccharides produced during a *Phaeocystis* bloom may shape the composition of bacterial communities specialized in degradation of complex carbohydrates. Bacteria appearing during the senescent stage of the blooms mainly belonged to the α-Proteobacteria and CF-cluster of the Bacteroidetes (Brussaard et al. 2005b). In the coastal North Sea, bacteria from CF-cluster of
the Bacteroidetes were dominant during and following a bloom of *P. globosa* (chapter 5). Members of the CF-cluster are chemo-organotrophic, and known for their capacity to degrade complex carbohydrates such as pectin, cellulose and chitin (Reichenbach and Dworkin 1991; Cottrell and Kirchman 2000; Kirchman 2002), therefore it may be that certain taxa of this cluster have adapted especially to degradation of complex mucopolysaccharides produced by *Phaeocystis*.

Mineral nutrient limitation of microbial degradation has been put forward as an explanation for accumulation of carbon rich DOM after a *Phaeocystis* bloom (Thingstad et al. 1994). The increase in carbohydrate to POC ratio and therefore C/N and C/P ratio due to the overflow metabolism will give rise to a substrate for bacteria with an unfavourable high carbon content. Since the C/P ratio of bacteria may be considerable lower than that of phytoplankton (Vadstein et al. 1988), especially phosphate limitation may hamper microbial degradation (Thingstad et al. 1997). In addition, since *P. antarctica* was found to remove more CO$_2$ per mole of phosphate than do diatoms (Arrigo et al. 1999), phosphate limitation during bacterial degradation of *Phaeocystis* material may be more severe than during degradation of diatom material. Indeed, while bacterial growth was carbon limited during the growth phase of a bloom of *P. globosa*, bacterial growth was also phosphate limited during the massive release of the glucan and mucopolysaccharides at the end of the bloom (Kuipers and Van Noort, in prep). This may impede degradation of carbohydrates that are otherwise easily degradable, and prolong the degradation times.

**Formation of hydrogels by *Phaeocystis* carbohydrates**

Prolonged degradation times may lead to assemblage of carbohydrates into hydrogels. As was discussed above, mucopolysaccharides spontaneously form polyanionic gels in aqueous environments. These polymer gels can than penetrate neighboring gels, and anneal them together increasing the size spectrum. In addition to gel formation by mucopolysaccharides, β-1,3-glucans are also known to form non-ionic gels in aqueous environments (Renn 1997; Kim et al. 2000). In general gelling properties of β-glucans are dependent on the concentration of the molecules, their size, primary and secondary structure and temperature (Bohm and Kulicke 1999; Lazaridou et al. 2003). Therefore, gel forming properties of laminarin in the marine environment are hard to predict. Preliminary results from our laboratory show that dissolving laminarin in seawater leads to an increase in viscosity, but to our knowledge no further investigations have been conducted to this end. Seuront et al. (in prep) showed that *Phaeocystis* derived polymeric matter could increase seawater viscosity up to two orders of magnitude. Because of the high aggregation potential of both glucan and mucopolysaccharides, the carbohydrates released during senescence of the *Phaeocystis* bloom will likely contribute to the increase in seawater viscosity (Figure 1).

Recent discoveries have highlighted the importance of polymer gels for biogeochemical cycling of organic carbon (see the review by Verdugo et al. 2004). Gels form an abiotic vehicle to move organic molecules up the size spectrum to sizes capable of sedimentation, and may also form an intermediate in the formation of the foam often observed after *Phaeocystis* blooms (Figure 1). Large gel particles, also known as transparent exopolymeric particles (TEP) can be significant in sedimentation processes, either by direct sedimentation
(Wassmann et al. 1990; Riebesell et al. 1995; Hong et al. 1997), or by acting as glue, adhering together other materials present during a bloom and thus enhancing their contribution to a vertical flux (Passow and Wassmann 1994). Mari et al. (2005) showed that the properties of TEP produced under N or P limitation are different, as a result of which the export versus retention balance of the organic matter may be affected. It is tempting to speculate that the different N/P ratios may determine whether overflow metabolism is directed towards either glucan or mucopolysaccharide production. In this way the glucan/mucopolysaccharides ratio may influence properties of the hydrogels and TEP that is formed, and thereby the degradation potential of \textit{Phaeocystis} organic matter. In addition, the effect of iron limitation on overflow metabolism is unknown. In \textit{P. antarctica} in the Ross Sea, where iron limitation is likely to occur, only a minor increase in C/N ratio was reported upon nutrient limitation (Smith et al. 1998). An explanation would be that overflow metabolism does not occur upon iron limitation, thus increasing the degradation potential of \textit{P. antarctica} derived organic matter.

\textbf{Figure 1.} Conceptual representation of the formation of marine gels from different pools of \textit{Phaeocystis} organic matter (after Verdugo et al. 2004).
Microbial degradation of hydrogels
In general HMW-DOC and gels have a higher rate of biodegradation and turnover than LMW-DOC (Amon and Benner 1996). Gels may serve as nutrient and/or attachment surfaces and offer hot-spots of high substrate concentrations for bacteria. They enclose microenvironments of substrate for bacteria to colonize, release their extracellular enzymes and efficiently take up the degradation products at high local concentrations (Azam 1998; Azam and Long 2001). Indeed, following a P. globosa bloom bacteria attached to particles were more active than the free-living community (Becquevort et al. 1998). On the other hand, assembly of free polymers into gels may complicate degradation. In chapter 4 it was shown that degradation of carbohydrate polymers that form insoluble gels was approximately 50 times slower than the degradation of polymers that do not produce a gel structure, but otherwise contained the same type of monomer linkages. If this difference is exemplary for the difference in degradation potential of “free” polymers and polymers embedded in a gel structure, turnover times may be slowed down from days to years.

Figure 2. Conceptual representation of biotic and abiotic processes altering the size and recalcitrance of marine gels.
LMW products will be released from the hydrogels when hydrolysis rates exceed the carbon demand of the bacteria (Smith et al. 1992) (Figure 2). This may also be a result form the kinetic characteristics of the enzyme systems of marine bacteria. In chapter 4 it was shown that during degradation of polymers to monomers, intermediates accumulate when polymer concentrations are high. This situation may be comparable to the high substrate concentrations in hydrogels. In addition, polymer photocleavage by ultraviolet radiation can shorten polymer chains making tangled networks unstable (Edwards 1986) and can readily disperse marine polymer gel matrices (Orellana and Verdugo 2003). These two processes produce a random fragmentation yielding both LMW products that may be readily incorporated and metabolized by bacteria (Mopper et al. 1991; Moran and Zepp 1997; Mopper and Kieber 2001), thereby enhancing degradation rates of the gels. On the other hand, polymers will be released that are too large to permeate the bacterial membrane, yet too small to assemble into stable networks that bacteria can colonize and efficiently degrade with their extracellular enzymes. These polymers may contribute to the formation of refractory DOM. This is in accordance with the high concentrations of carbohydrates found in the oceans DOM (Benner et al. 1992).

Besides diminishing gels, bacterial activity may also increase aggregation of gels (Figure 2). Studies on bacterial exopolymer production suggest that a significant portion of assimilated carbon is incorporated into exopolymer capsular envelopes (Stoderegger and Herndl 1998). These capsular exopolymers increase aggregation probabilities of phytoplankton and other particles and act as stabilizers of already existing aggregates (Allison and Sutherland 1987; Decho 1990; Heissenberger and Herndl 1994). In addition, bacterial exopolymers are likely to be resistant to bacterial enzymatic degradation. Since a considerable fraction of bacterial extracellular enzymes are embedded in the exopolymer capsule, the exopolymers will be resistant to these enzymes. Therefore, release of bacterial derived exopolymers decreases the degradation potential of hydrogels. In addition, selective degradation of readily degradable compounds may alter the composition of the gels, and decrease the degradation potential of the remaining gel (Sannigrahi et al. 2005).

**CONCLUSIONS**

*Phaeocystis* produces at least five different pools of carbohydrates, each with their own characteristics. Since the overflow metabolism during the stationary phase of a bloom can be channeled towards glucan and mucopolysaccharides, these two pools are quantitatively the most important when the impact on the ecosystem is considered. A conceptual representation of their contribution to marine hydrogels is presented in Figure 1. At this stage, however, it is difficult to predict the contribution of either of the two pools.

*Phaeocystis* derived organic matter appears to be readily degradable, however, the high C/N and C/P ratios may give rise to mineral nutrient limitation of bacteria and therefore hamper degradation. Subsequent assembly into gels could have a dramatic effect on bacterial degradation of *Phaeocystis* derived organic matter (Figure 2). Gels may provide an easy microhabitat to colonize and degrade with extracellular enzymes, thereby enhancing degradation rates of organic matter. On the other hand bacterial activity may reduce the
degradation potential of gels by selective degradation of labile organic matter in the gels, and by incorporation of carbon into bacterial capsular material.

Gels may offer microhabitats with different biogeochemical characteristics, thereby enhancing the microbial diversity. Indeed the bacterial species composition in seawater varies at the millimeter scale (Long and Azam 2001). In research on the role of bacteria in degradation of organic matter derived from *Phaeocystis* blooms, a clear distinction has been made between the role of “attached” bacteria versus “free” bacteria (Becquevort et al. 1998). With the concept of marine gels as intermediates in the DOM-POM continuum, this distinction may not be so clear.

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