

## Diffusive boundary layers, photosynthesis, and respiration of the colony-forming plankton algae, *Phaeocystis* sp.

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### Abstract

Diffusive boundary layers, photosynthesis, and respiration in *Phaeocystis* colonies were studied by the use of microelectrodes for oxygen and pH during a bloom in the Barents Sea, 1993, and in the Marsdiep, Dutch North Sea, 1994. The oxygen microenvironment of a *Phaeocystis* colony with a mean diameter of 1.4 mm was mapped from 346 O<sub>2</sub> measurements and showed gradients of oxygen concentration in the water phase up to 1 mm distance from the colony surface. The effective diffusive boundary layer was 0.4–0.9 mm thick. Oxygen concentrations inside colonies reached 180% of the bulk water, and pH increased up to 0.4 units when measured in light at saturating intensities (>90  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). The respiration in the dark was low, resulting in a 6% lowering in oxygen concentration and 0.04 units lowering in pH inside colonies, compared to the bulk water phase. Such colonies were net heterotrophic communities at light intensities up to 10  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . A week later, colonies were net heterotrophic at light intensities up to 80  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The effective diffusion coefficient for oxygen in the gelatinous colonies was not significantly different from that in sea water.

The physical properties of sea water shift dramatically from a centimeter scale down to a micrometer scale in the ocean, as the viscous forces become predominant with decreasing length scale in the ocean. Turbulent eddies do not occur on a submillimeter scale in the ocean because of strong frictional and adhesive forces acting between the water mol-

ecules and surfaces at this scale (Lazier and Mann 1989). Slowly sinking spheres that are impermeable to flow are also surrounded by a thin stagnant water film that adheres and moves together with the sphere (Berg 1983). Diffusive boundary layers (DBLs) with concentration gradients of gases and nutrients leading to chemical microenvironments develop at small scale and at interfaces where flow and turbulence are retarded due to viscous forces, and diffusion is fast relative to advection. The length scale at which diffusive boundary layers persist around colony-forming algae and to which extent they create chemical microenvironments in the pelagic environment is relatively unknown because of few experimental studies. The chemical microenvironment of phytoplankton colonies is dependent on the DBL thickness, the diffusion coefficients of the chemical species, and the chemical/biological activities in the colonies.

The prymnesiophyte, *Phaeocystis* sp., is a common marine plankton alga of polar (Barnard et al. 1984; SooHoo et al. 1987; Wassmann et al. 1990), temperate (Eberlein et al. 1985; Veldhuis et al. 1986; Lancelot and Mathot 1987), and tropical regions of the sea (Guillard and Hellebust 1971). During its life cycle it covers a size scale ranging from a few micrometers to several millimeters in the ocean, as it occurs as both free-living flagellated cells and forms blooms in a colonial phase with up to millimeter-large gelatinous colonies with thousands of cells primarily positioned at the mucus surface (Rousseau et al. 1994) (Fig. 1). The pH in *Phaeocystis* colonies has been shown to be significantly higher than that of the surrounding water during photosynthesis (Lubbers et al. 1990). Whereas colonies in the early

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### Acknowledgments

Thanks are due to Paul Wassmann for inviting H. Ploug to join a research cruise on R/V *Jan Mayen* in the Barents Sea and for kindly providing laboratory facilities for the further work at the Norwegian College of Fishery Science, University of Tromsø, Norway. We also thank Hein de Baar, Netherlands Institute for Sea Research (NIOZ), for providing laboratory facilities at NIOZ. Jeroen de Jong, NIOZ, is thanked for practical help and Veronique Schoemann, Université Libre Bruxelles, is thanked for collaboration, discussions, and help throughout the study. The microelectrodes were constructed by Anja Eggers and Gabriele Eickert, Max Planck Institute for Marine Microbiology, Bremen. Thanks are also due to useful comments and suggestions from an anonymous referee. This work was financed by the Environment Programme of the European Commission (J: EV5V-CT94-0511) to Christiane Lancelot (organizer), Université Libre Bruxelles, and by the Danish Natural Science Research Council (J: 11-0557-1 PD), the Danish Research Academy (J: V930148), by the Nordic Research Academy (NorFA) (J: 93.30.042/00 to H.P.), and the Max Planck Society (Germany) to H.P.

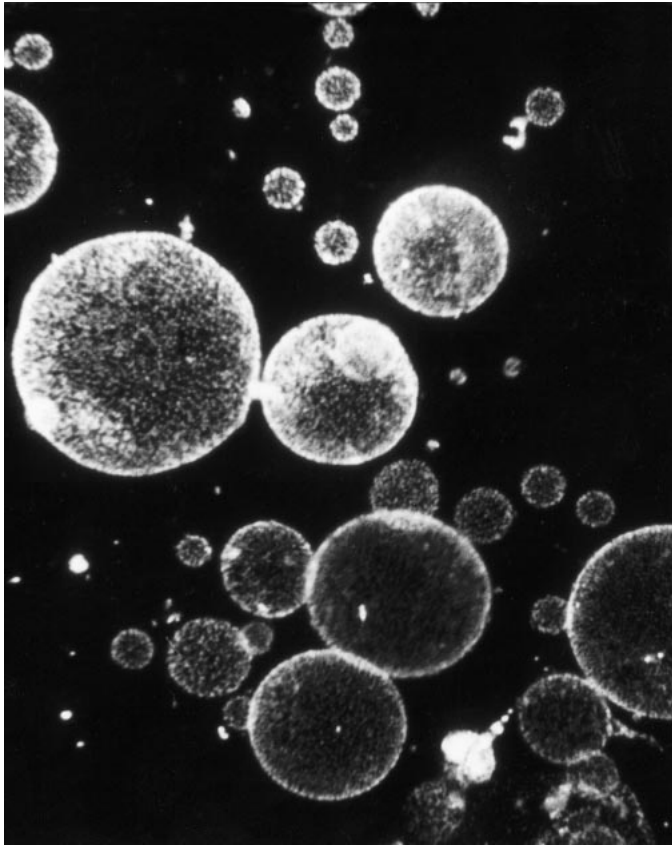


Fig. 1. *Phaeocystis* colonies.

stages of blooms are almost entirely free of attached bacteria, late bloom stages are accompanied by colonization of the mucus by attached bacteria (Davidson and Marchant 1987; Lancelot 1995). The accumulation of mucus in the water column during the senescent phase of blooms indicates that the degradation of mucus is relatively slow (Eberlein et al. 1985). The relative importance of autotrophic and heterotrophic processes in *Phaeocystis* colonies, however, is poorly known, and the diffusion coefficient of oxygen in *Phaeocystis* mucus that may also affect the biological processes in the colonies is unknown.

In the present study we analyzed gradients of oxygen and pH in *Phaeocystis* colonies by the use of microelectrodes. DBL thickness, the diffusion coefficient of oxygen, photosynthesis, and respiration were quantified from the oxygen gradients in *Phaeocystis* colonies. The measurements were performed during a bloom in the Barents Sea and at different stages of a bloom within and around the Dutch North Sea in order to determine at which light intensities and stages of the bloom such colonies create chemical microenvironments and at which light intensities they are autotrophic and heterotrophic communities, respectively.

## Materials and methods

*Sampling and handling of Phaeocystis colonies*—Natural *Phaeocystis* colonies with diatoms (*Chaetoceros socialis* and *Nitzschia* sp.) sticking to their surface were collected during

a cruise in May 1993 at the ice edge in the Barents Sea at 73.5°N 30.5°E. The colonies were either collected from sediment traps that had been exposed during 24 h in the upper 30 m of the water column or from a WP2 plankton net towed vertically in the upper 10 m of the water column. The salinity was 35‰ and the temperature was 3°C. The in situ light intensities ranged from 20 (midnight sun) to 1,600  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , measured as the downwelling irradiance at the sea surface. The downwelling irradiance was attenuated to 1% at  $\sim 35$  m depth. In the Dutch North Sea (Marsdiep) colonies were collected at high tide on different days during a bloom in May 1994 by the use of a bucket from the NIOZ-pier at Texel. The in situ temperature was 9°C and the salinity was 24‰. Single colonies were transferred to a vertical flow system with filtered (pore size: 10  $\mu\text{m}$ ) seawater from the same depth and analyzed with oxygen microelectrodes at in situ temperature as described in Ploug and Jørgensen (1999). An upward-directed flow through a net in the flow system was used to keep the colonies suspended immediately above the net. This arrangement is equivalent to a colony sinking down through the water column at a velocity equal to the water flow. Several colonies were, however, nearly neutrally buoyant and were thus carried away by the flow. In such cases, the flow was turned off and advection was induced inside the chamber through a pipette from above connected to an air pump that created a stream of air toward the air–water interface without formation of air bubbles while the colony was sitting on the net.

*Microelectrode measurements*—The radial oxygen distributions were measured by Clark-type oxygen microelectrodes with a guard cathode (Revsbech 1989a) mounted in a micromanipulator. The electrode current was measured by a picoamperemeter and read on a strip chart recorder. The oxygen microelectrode was calibrated in air-saturated and in  $\text{N}_2$ -flushed sea water. The stirring sensitivity of the electrodes was  $<1\%$ , and the 90% response time was 0.3 s. The electrode was slender with an outer tip diameter of 6  $\mu\text{m}$ . The surface of the colonies was determined by slowly advancing the microelectrode toward the colony, until it touched the upper surface of the mucus observed under a dissection microscope. Gross production rates were measured by the light–dark shift technique (Revsbech et al. 1981). The light source was a 150-W halogen fiberoptic lamp equipped with an infrared cut-off filter (Schott, KI 1500). The light intensity (photosynthetically active radiation) was measured inside the chamber with an underwater scalar irradiance sensor (Biospherical Instruments QLS 100).

The pH microelectrodes were made according to Revsbech and Jørgensen (1986). The sensing tip was 20  $\mu\text{m}$  in diameter and 150  $\mu\text{m}$  long. A calomel electrode was used as a reference, and the electrode potential was read on a millivoltmeter connected to a strip chart recorder. The electrode potential was calibrated at in situ temperature in buffer solutions at pH 4.00, 6.92, and 9.38 (Ingold, Mettler Toledo Prozessanalytik).

*Determination of the effective diffusion coefficient of Phaeocystis colonies*—The effective diffusion coefficient of  $\text{O}_2$  inside *Phaeocystis* colonies was determined in a diffusion

chamber according to Revsbech (1989b). The setup was slightly modified in that an oxygen sensor membrane (Yellow Springs) was used instead of a silicone rubber membrane to separate the lower from the upper chamber. A 1% agar solution was poured on top of this membrane resulting in a 3-mm-thick layer. *Phaeocystis* colonies were collected and concentrated by filtering 20 liters of Marsdiep water over a 200- $\mu\text{m}$  sieve. Biological activity was effectively eliminated by adding a buffered formaldehyde solution (pH 8.2) to a final concentration of 2%. The *Phaeocystis* biomass was applied on top of the solidified agar and covered with Parafilm to minimize evaporation during equilibration. The lower reservoir was flushed with pure oxygen to create a steep oxygen gradient across the agar and the *Phaeocystis* biomass. The setup was preincubated for 24 h at 20°C before measuring the oxygen profiles. Oxygen was measured with a commercially available Clark-type oxygen microelectrode (Diamond Electro, no. 737). The electrode had a tip diameter of 30  $\mu\text{m}$  and a stirring sensitivity of <2%. The effective diffusion coefficient was calculated from the difference in oxygen gradients across the agar and the *Phaeocystis* biomass, as the diffusion coefficients of oxygen in 1% agar and in sea water are the same (Revsbech 1989b).

*Determination of diffusive boundary layers and fluxes*—Where mass transfer to or from a sphere proceeds only by molecular diffusion at steady-state, e.g., in a DBL at the mucus–water interface of a spherical colony, the concentration  $C_r$  at the radial distance,  $r$ , within the DBL can be described by (Crank 1975)

$$C_r = \frac{C_0 r_0 (r_1 - r) + C_1 r_1 (r - r_0)}{r(r_1 - r_0)} \quad (1)$$

where  $C_0$  is the concentration at the surface of a sphere with a radius  $r_0$ ,  $C_1$  is the external concentration at the radial distance  $r_1$ , and  $r_0 < r < r_1$ .

The radial flux of oxygen is expressed by Fick's 1st law of diffusion (Crank 1975):

$$J = -D_w \frac{dC}{dr} \quad (2)$$

where  $J$  is the flux of oxygen,  $D_w$  is the diffusion coefficient of oxygen in sea water, and  $dC/dr$  the radial gradient of the oxygen concentration. The effective DBL thickness,  $\delta_{\text{eff}}$ , that yields an equal flux as Eq. 2, is determined from extrapolating the oxygen gradient at the sphere–water interface to the bulk water phase concentration (Sherwood et al. 1975). Thus,

$$J = D_w \frac{C_\infty - C_0}{\delta_{\text{eff}}} \quad (3)$$

where  $C_\infty$  is the bulk water concentration of oxygen.

The analytical solution for the oxygen concentrations in the DBL was fitted to the measured values by applying the solver routine of the spreadsheet program Excel 4.0 (Microsoft), whereby the  $\delta_{\text{eff}}$  thickness and the oxygen fluxes were determined (Ploug et al. 1997). A diffusion coefficient for oxygen of  $1.22 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  and  $1.47 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$

was used for measurements done at 3°C and 9°C, respectively (Broecker and Peng 1974).

*Photosynthesis*—The net photosynthesis and dark respiration of a colony equals the area-integrated flux of diffusing oxygen per unit time,  $Q_t$ , measured in light and dark, respectively:

$$Q_t = -4\pi r_0^2 D_w \frac{dC}{dr} \quad (4)$$

where  $4\pi r_0^2$  is the surface area of a sphere.

The radial distribution of net photosynthesis was calculated from the oxygen profile in a sphere, regarding the sphere as consisting of concentric shells and using a diffusion coefficient for oxygen in the colony mucus equal to that of sea water. The quantity of diffusing oxygen through a spherical shell per unit time,  $Q_r$ , can be expressed by (Crank 1975)

$$Q_r = -4\pi D_m \frac{r_1 r_2}{r_2 - r_1} (C_2 - C_1) \quad (5)$$

where  $D_m$  is the molecular diffusion coefficient of oxygen in the mucus matrix,  $C_1$  is the concentration of oxygen at the radial distance  $r_1$ , and  $C_2$  is the oxygen concentration at the radial distance  $r_2$ . The net production profile was thus calculated as the difference in  $Q_r$  between adjacent shells divided by the volume of each corresponding shell:  $\frac{4}{3}\pi(r_2^3 - r_1^3)$ , where  $r_1 < r_2$ .

The time-dependent change in oxygen concentration due to diffusion alone at non steady-state was measured in the center of a colony during light–dark shifts as well as modeled for a sphere (Mathematica 2.2, software) from (Crank 1975)

$$\begin{aligned} (C - C_\infty) &= 0.5(C_0 - C_\infty) \left[ \text{erf} \frac{r_0 + r}{2\sqrt{D_m t}} + \text{erf} \frac{r_0 - r}{2\sqrt{D_m t}} \right] \\ &\quad - \frac{(C_0 - C_\infty)}{r} \sqrt{\frac{D_m t}{\pi}} \left\{ \exp[-(r_0 - r)^2/4D_m t] \right. \\ &\quad \left. - \exp[-(r_0 + r)^2/4D_m t] \right\} \quad (6) \end{aligned}$$

where  $C$  is the concentration at time  $t$  at the radial distance  $r$  from the center,  $C_0$  is the initial concentration,  $C_\infty$  is the bulk water concentration at  $t = 0$  at the radial distance  $r$  from the center,  $r_0$  is the radius of the sphere,  $D_m$  is the effective diffusion coefficient in the sphere matrix, and  $\text{erf}$  is the mathematical standard error function.

## Results

*Phaeocystis colonies from the Barents Sea*—The radial oxygen distributions in a 1.0-mm and a 1.4-mm large colony collected from a sediment trap are shown in Fig. 2. The colonies were near-spherical in shape, and *C. socialis* appeared to stick to the mucus matrix of *Phaeocystis pouchetii*. The upward flow velocity in the chamber corresponded to a sinking velocity of 10 m d<sup>-1</sup>. In the water phase immediately

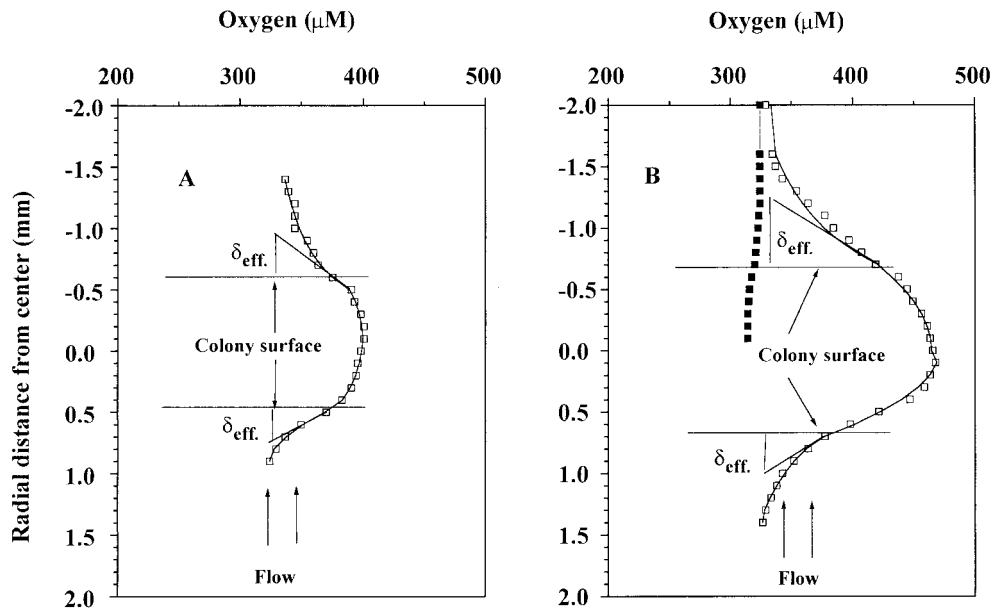


Fig. 2. The vertical, radial  $O_2$  distributions measured in light (open symbols) and dark (closed symbols) in two sinking *Phaeocystis* colonies from the Barents Sea. The curves in the DBL represent the modeled concentrations from which  $\delta_{\text{eff}}$  and the oxygen fluxes were determined.

surrounding the colonies, the oxygen concentration was asymmetrically distributed up to a distance of 1 mm from the mucus surface. The  $\delta_{\text{eff}}$  was 0.25 and 0.45 mm thick upstream (below) and downstream (above), respectively, for the smaller aggregate (Fig. 2A), and 0.33 and 0.52 mm upstream and downstream, respectively, for the larger aggregate (Fig. 2B).

The oxygen gradients inside the colonies were small and indicated that net photosynthesis occurred mainly at the surface. In light at  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , well above light-saturating intensities, the oxygen concentration inside the

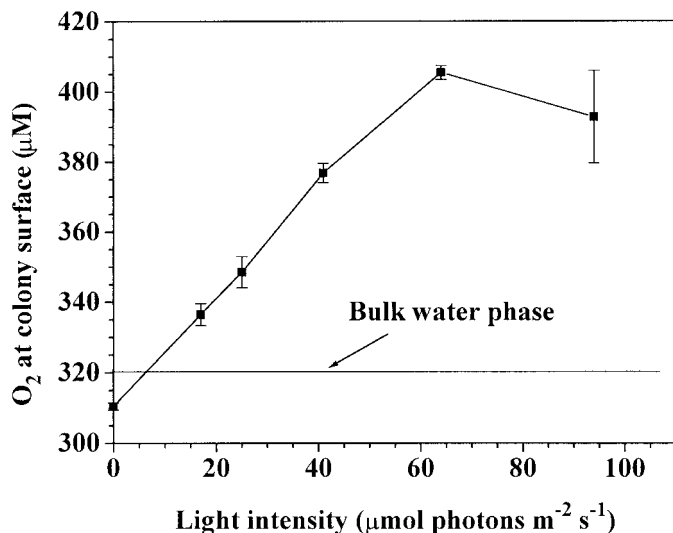


Fig. 3.  $O_2$  concentration measured at different light intensities at the surface of five colonies from the Barents Sea. Bars represent standard deviations of mean ( $n = 5$ ).

larger colony increased to 145% compared to the bulk water phase, whereas it increased to 123% in the small colony. In the dark, the oxygen concentration inside the larger colony was decreased to 97% compared to the bulk water phase. For the small colony, oxygen respiration was below the detection limit. The dark respiration was thus low, which indicated that the colonies were composed of healthy phytoplankton cells with relatively few bacteria. At saturating light intensity, the outward flux of oxygen per surface area of the small colony was  $134$  and  $114 \text{ nmol } O_2 \text{ cm}^{-2} \text{ h}^{-1}$  upstream and downstream, respectively. The flux of oxygen per surface area of the larger colony was  $119$  and  $105 \text{ nmol } O_2 \text{ cm}^{-2} \text{ h}^{-1}$  upstream and downstream, respectively. The average flux of oxygen per unit surface area was, thus, 25% higher for the small colony compared to the larger colony.

The net photosynthesis and dark respiration of a colony equals the area-integrated flux of oxygen per unit time,  $Q_n$ , measured in light and dark, respectively. The net photosynthesis, calculated from the mean gradients of oxygen at the aggregate surface upstream and downstream, was accordingly  $3.9$  and  $6.9 \text{ nmol } O_2 \text{ h}^{-1}$ , for the smaller and the larger aggregate, respectively. Hence, the total flux was 77% larger for the large colony compared to the smaller colony. The respiration in dark of the larger aggregate was  $0.57 \text{ nmol } O_2 \text{ h}^{-1}$  or 7.6% of the gross photosynthesis that was calculated as net photosynthesis plus dark respiration.

The oxygen concentration at the aggregate surface downstream of five colonies, collected from plankton net samples, increased linearly with light intensity up to  $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , where the oxygen concentration stabilized at  $\sim 80 \mu\text{M}$  above the oxygen concentration of the bulk water (Fig. 3), due to saturation of photosynthesis. These colonies had diameters of 1.5–2.0 mm. The oxygen concentration at the colony surface was exactly equal to the oxygen concentra-

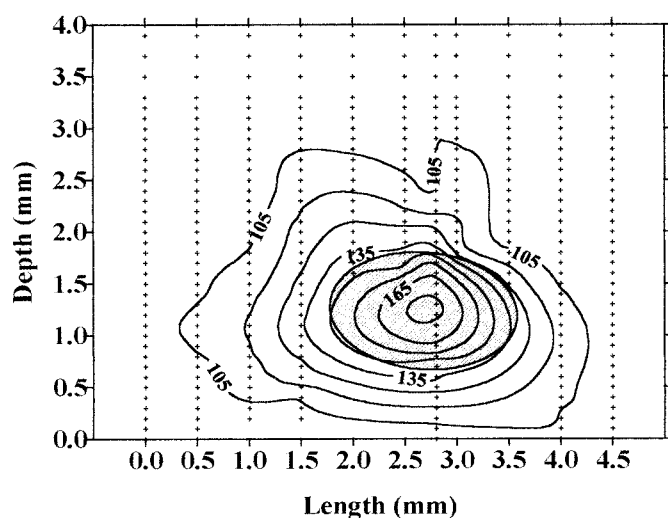


Fig. 4. Isopleths of  $O_2$  (in % of air saturation) within and around a *Phaeocystis* colony (shaded area) measured in light at saturating light intensity.

tion of the bulk water phase at a light intensity of  $7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Thus, at this light intensity (compensation point) there was no net exchange of oxygen between the colonies and the surrounding water, and the heterotrophic  $O_2$  consumption was balanced by the autotrophic  $O_2$  production.

*Phaeocystis* colonies from the Dutch North Sea—The oxygen distribution in the surrounding water in a neutrally buoyant colony sitting on the net was measured in a vertical section comprised of 11  $O_2$  profiles at 0.1-mm depth increments spaced 0.5 mm apart. The light intensity was  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  that was above saturation of photosynthesis. This colony was spheroid in shape, the shortest axis being 1.2 mm and the longest 1.9 mm (Fig. 4). From

these measurements isopleths of oxygen concentrations were calculated (Winsurfer, minimum curvature, maximum error: 0.001). The hyperoxic microenvironment of the colony was significantly larger than the colony itself. The oxygen isopleths followed the colony shape close to its surface, whereas irregularities were found in the outer boundary layer at oxygen concentrations  $<115\%$  of the air-saturated water phase. This was probably due to advection, convection, and thus, hydrodynamic instability in the outer boundary layer.

Radial distributions of oxygen measured in light at  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and the oxygen distribution in dark are shown for two different neutrally buoyant colonies that were sitting on the net during measurements (Fig. 5A,B). In both examples oxygen was symmetrically distributed through the colonies in the light. The presence of the nylon net thus had little effect on the measured profiles. In the colony shown in Fig. 5B, gradients of oxygen were steepest in the water phase up to a distance of 0.7 mm from the colony surfaces. This colony was near-spherical in shape with a diameter of 1.4 mm. The  $\delta_{\text{eff}}$  was  $\sim 0.40$  mm along the vertical axis for the colonies shown in Fig. 5A and B, and no significant differences between the upper and the lower surfaces were observed. Figure 5A shows measurements along the vertical axis through the center of the colony also shown in Fig. 4. The  $\delta_{\text{eff}}$  along the horizontal axis through the center of the same colony (Fig. 4), similarly determined from the isopleths, were 0.99 and 0.54 mm on the left and the right side, respectively.

The distributions of oxygen and pH in a sinking *Phaeocystis* colony measured at saturating light intensity or in dark are shown in Fig. 6. The colony was spheroid in shape and had a brownish color. The shortest axis was 0.8 mm and the longest axis was 2.3 mm. The upward flow velocity corresponded to a sinking rate of  $3 \text{ m d}^{-1}$ . Gradients of oxygen and pH along the shortest axis were asymmetrically distributed upstream and downstream. The  $\delta_{\text{eff}}$  was 0.3 and 0.5 mm

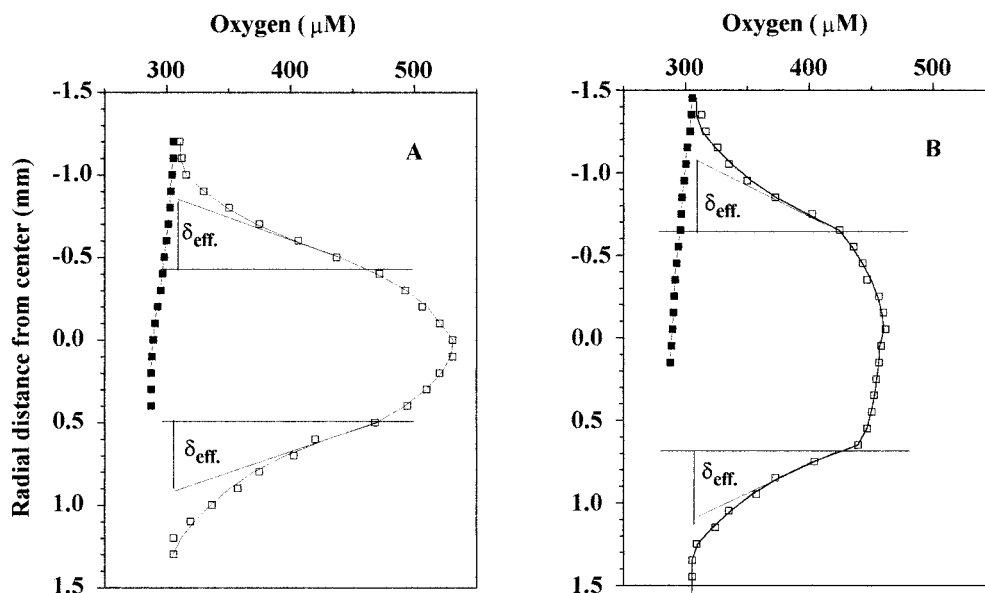


Fig. 5. The vertical, radial  $O_2$  distributions measured in light and dark in two neutrally buoyant *Phaeocystis* colonies. Symbols as in Fig. 2.

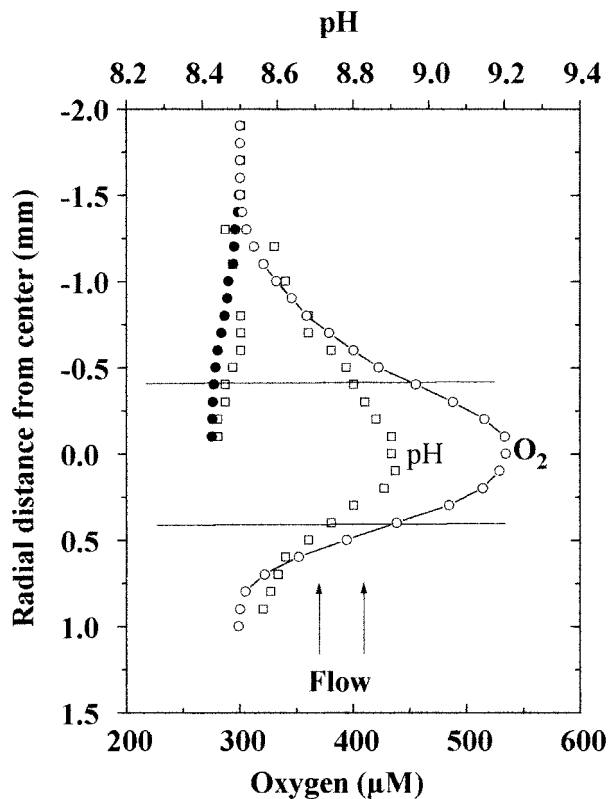


Fig. 6. The vertical, radial  $O_2$  distribution measured in light and dark and pH measured in light and dark of a sinking *Phaeocystis* colony. Each profile represents the mean of three series of measurements. Symbols as in Fig. 2.

upstream and downstream, respectively. The maximum oxygen concentrations inside the colony had increased to 180% of the bulk water concentration and pH increased by 0.4 units inside the colony, thus reaching a maximum of pH 8.9, when measured in light at  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . In the dark the oxygen concentration was lowered by 6% and pH was lowered by 0.04 units inside the colony compared to the bulk water phase.

The flux of oxygen measured at saturating light intensities for the three colonies shown in Figs. 5 and 6 was  $182 \pm 24 \text{ nmol } O_2 \text{ cm}^{-2} \text{ h}^{-1}$ , whereas the dark flux was  $11.2 \pm 2.5 \text{ nmol } O_2 \text{ cm}^{-2} \text{ h}^{-1}$ . The dark respiration thus amounted to 5.8% of gross photosynthesis at saturating light intensities. The total net photosynthesis of the spherical colony shown in Fig. 5B was  $10.6 \text{ nmol } O_2 \text{ h}^{-1}$ , and the dark uptake was  $0.51 \text{ nmol } O_2 \text{ h}^{-1}$ .

To determine the radial distribution of net photosynthesis the diffusion coefficient of oxygen in the mucus must be known. The effective diffusion coefficient for oxygen in colonies was found as the ratio of the oxygen slopes in *Phaeocystis* mucus and in agar. An example out of nine measurements is shown in Fig. 7. The mean value of the ratio was  $0.93 \pm 0.10$  (standard deviation [SD],  $n = 9$ ), i.e., the diffusion coefficient in mucus was slightly lower than that in sea water because the diffusion coefficients of oxygen in 1% agar and in sea water are the same (Revsbech 1989b).

The radial distribution of net photosynthesis (Fig. 8) was

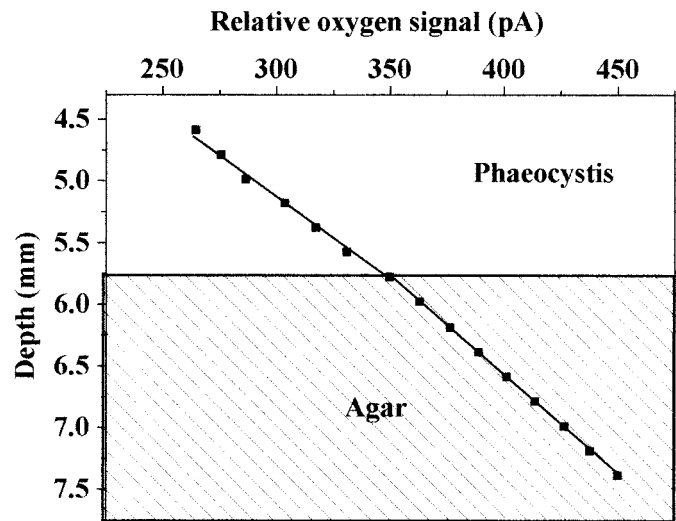


Fig. 7.  $O_2$  gradients in *Phaeocystis* mucus and in agar. The gradients were used to estimate the molecular diffusion coefficient of oxygen within *Phaeocystis* colonies.

calculated from the oxygen profile (Fig. 5B) in a sphere, regarding the sphere as an array of concentric shells and a diffusion coefficient for oxygen in the mucus equal to 93% of that in of sea water (Eq. 5). The net photosynthesis showed maxima of  $12.5$  and  $21.7 \text{ nmol } O_2 \text{ mm}^{-3} \text{ h}^{-1}$  at the colony surface, whereas net photosynthesis inside the colony was low. The volume of a sphere with a radius of  $0.7 \text{ mm}$  is  $[\frac{4}{3}\pi(0.7)^3]1.44 \text{ mm}^3$ , and 63% of the total volume is represented by the outer  $0.2 \text{ mm}$ , regarding the sphere as an

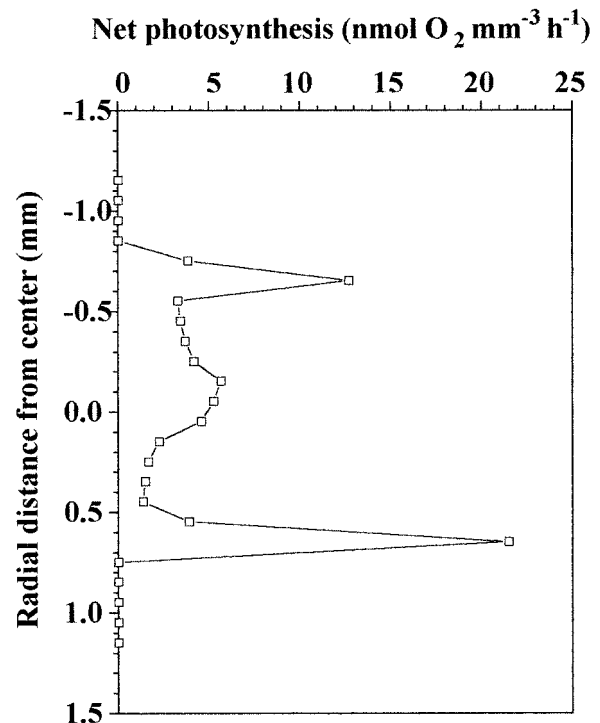


Fig. 8. Net photosynthesis profile calculated from the oxygen profile in Fig. 5B.

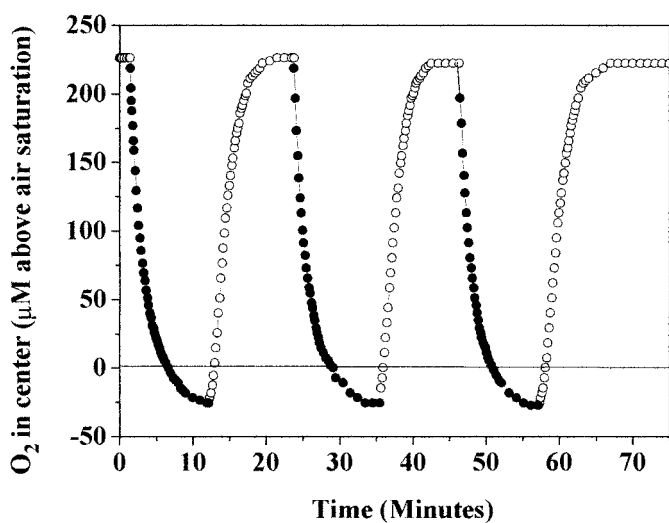


Fig. 9.  $O_2$  concentration measured in the center of a *Phaeocystis* colony during sudden shifts between light (open symbols) and dark (closed symbols). The light intensity was  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

array of concentric shells. The volume-integrated total net photosynthesis was  $11.0 \text{ nmol } O_2 \text{ h}^{-1}$  that was close to the total net area-integrated flux of  $10.6 \text{ nmol } O_2 \text{ h}^{-1}$  through the DBL. The volume-integrated photosynthesis in the outer  $0.2 \text{ mm}$  was  $9.7 \text{ nmol } O_2 \text{ h}^{-1}$  and represented 87% of the total photosynthesis. Thus, the central part of the colony represents a third of the total volume, whereas the photosynthesis in the center was only 13% of the total net photosynthesis of  $11 \text{ nmol } O_2 \text{ h}^{-1}$ .

**Dynamics of oxygen**—The oxygen microenvironment inside colonies was highly dynamic in response to changing light conditions. The oxygen concentration measured in the center of a colony during sudden shifts between light and dark is shown in Fig. 9. When measured in light at saturating intensity at steady state, the oxygen concentration in the center was  $225 \mu\text{M}$  above that of the air-saturated water phase, and the oxygen produced by photosynthesis was exactly balanced by respiration and transport of oxygen away from the measuring point. As the light was suddenly turned off, the oxygen concentration immediately decreased due to respiration and transport of oxygen away from the colony. A new steady state was established 10 min later at a concentration  $25 \mu\text{M}$  below that of the air-saturated water phase. From the steady-state concentration during light and the initial rate of decrease in oxygen concentration after the light was turned off, the turnover time of the oxygen pool was found to be 3.5 min. The diffusive transport away from the center is dependent on the effective diffusion coefficient in the mucus of the colony and on the geometry of the colony (Crank 1975). The time-dependent change in oxygen concentration after the light was turned off was modeled according to Eq. 6 for a sphere with a radius of  $0.65 \text{ mm}$  that was the mean radius of the colony. We used an effective diffusion coefficient for the mucus,  $D_e$ , equal to  $1.00 \times D_w$ ,  $0.50 \times D_w$ , and  $0.25 \times D_w$ , where  $D_w$  is the diffusion coefficient of oxygen in seawater (Fig. 10). The measured decrease in oxygen con-

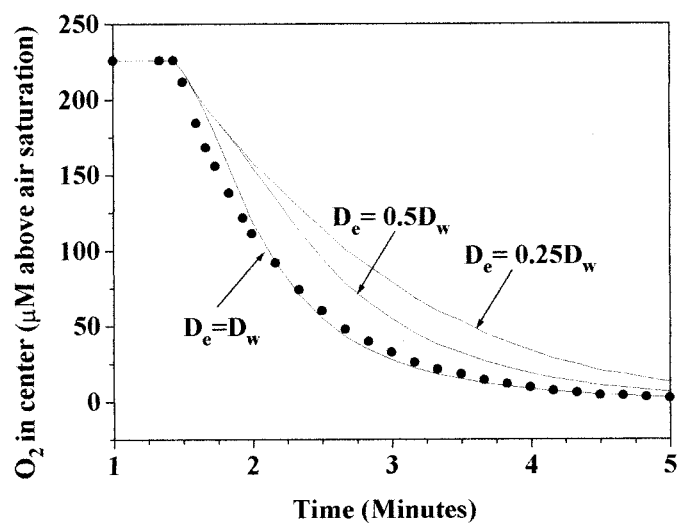


Fig. 10. The measured and modelled (Eq. 6) time-dependent change in  $O_2$  during the first minutes of darkness for different diffusion coefficients  $D_e$  in the mucus relative to that in sea water,  $D_w$ .

centration with time was similar to the model results using  $D_e = 1.0 \times D_w$ , which was close to that experimentally measured (Fig. 7). The rapid decrease in oxygen concentration was thus mostly due to diffusion loss, whereas respiration was of minor importance.

**Light and photosynthesis**—Oxygen, pH, and gross photosynthesis were measured at different light intensities at the surface of five freshly collected colonies from the Marsdiep on 29 April and on 5 May (Fig. 11A–C). All colonies had a diameter of approximately  $1 \text{ mm}$  and the measurements were based on 10–15 min exposure to each light intensity, whereby steady state was attained. The data were fitted to the hyperbolic tangent function of Jassby and Platt (1976) by a nonlinear least-square Levenberg–Marquardt algorithm (Origin 3.0, MicroCalc Software). The colonies analyzed on 29 April were all brownish in color, whereas the colonies analyzed on 5 May were pale in appearance. The light intensity, at which saturation of gross photosynthesis onsets, was calculated from the hyperbolic tangent function as  $I_k = P_{\text{max}}/\alpha$ , and was  $93$  and  $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  on 29 April and 5 May, respectively (Fig. 11A).

The colonies analyzed on 29 April showed a higher gross photosynthesis as well as a higher degree of supersaturation of the oxygen concentration at the colony surface at saturating intensities compared to the colonies analyzed on 5 May (Fig. 11B). In the dark, the oxygen concentration at the colony surface was 6% and 15% undersaturated, respectively, on 29 April and 5 May. The colonies analyzed on 29 April were net heterotrophic communities at light intensities below  $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , whereas the colonies analyzed on 5 May were net heterotrophic at light intensities up to  $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

The pH in the colonies is dependent on  $\text{CO}_2$  produced by respiration and  $\text{CO}_2$  assimilated through photosynthesis. The pH at the colony surface (Fig. 11C) showed a similar dependence as oxygen on light intensity. The pH was equal to

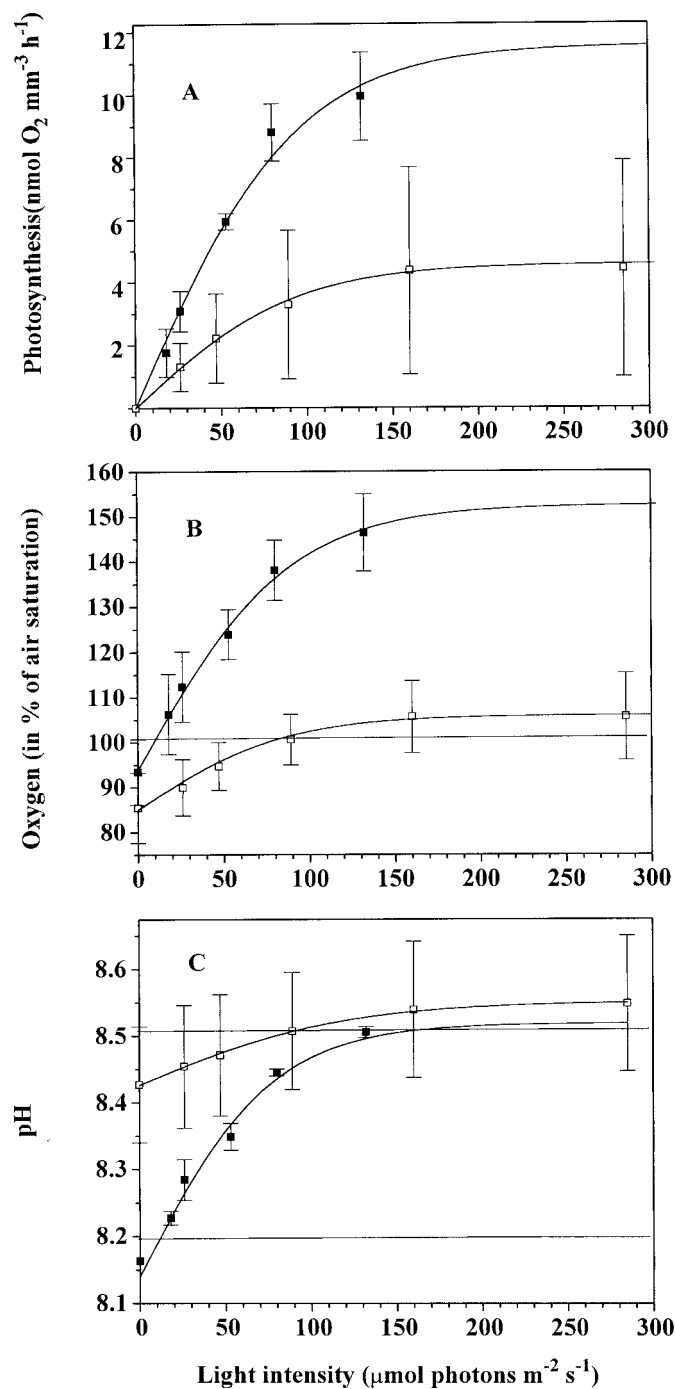


Fig. 11. Gross photosynthesis (A),  $\text{O}_2$  concentration (B), and pH (C) measured at different light intensities at the surface of five colonies on 29 April (closed symbols) and on 5 May (open symbols). Bars represent standard deviations of mean ( $n = 5$ ). The pH of the bulk water phase was 8.19 and 8.5 on 29 April and 5 May, respectively, as indicated by the horizontal lines (C).

that in the bulk water phase at light intensities of 10 and 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , which was also the compensating light intensities where respiration was balanced by photosynthesis on 29 April and 5 May, respectively (Fig. 11B). On 29 April, the pH increased by 0.33 units at the surface

at saturating light intensities, whereas in dark it was 0.05 unit lower than in the bulk water phase. On 5 May, pH was only increased 0.05 units at the surface of colonies in the light and decreased 0.07 units in the dark compared to the bulk water phase.

## Discussion

*Mass transfer in Phaeocystis colonies*—DBLs with concentration gradients of gases and nutrients surrounding *Phaeocystis* colonies are dependent on the relative importance of inertial to viscous forces in the vicinity of the colonies. The relative importance of inertial to viscous forces is described by the Reynolds number (White 1974):

$$\text{Re} = Ud_0/\nu \quad (7)$$

where  $U$  is the sinking velocity,  $d_0$  is the diameter, and  $\nu$  is the kinematic viscosity of seawater. The Reynolds numbers for the aggregates were  $6.14 \times 10^{-2}$  (Fig. 2A),  $8.6 \times 10^{-2}$  (Fig. 2B), and  $2.1 \times 10^{-2}$  (Fig. 6), and the water moving in the vicinity of the colonies was, thus, highly dominated by the viscous forces acting at the colony-water interface.

In the natural environment as the euphotic zone of the ocean, turbulence may imply thinner DBLs surrounding *Phaeocystis* colonies. The shear rate in the euphotic zone of ocean can be calculated from the energy dissipation rate, and it is normally in the range of  $0.01$ – $1.0 \text{ s}^{-1}$  (Karp-Boss et al. 1996; MacKenzie and Leggett 1991; Mann and Lazier 1991, and references herein). The average DBL thickness is inversely proportional to the Sherwood number,  $Sh$ , that describes the relative increase in mass transfer due to sinking and/or due to turbulence in the environment (Sherwood et al. 1975; Karp-Boss et al. 1996).

The Sherwood number and, thus,  $\delta_{\text{eff}}$  can be calculated from (Sherwood et al. 1975; Batchelor 1980; cf. Karp-Boss et al. 1996)

$$Sh = \frac{r_0}{\delta_{\text{eff}}} = 0.52 \left[ 2.25 \left( \frac{r_0 U}{D} \right)^2 + 1.84 \left( \frac{r_0^2 E}{D} \right)^2 \right]^{1/6} \quad (8)$$

where  $E$  is the shear rate. Equation 8 is valid when  $r_0 U/D$  and  $r_0^2 E/D$  are  $\gg 1$ .  $Sh$  for oxygen for a 1.4-mm *Phaeocystis* colony with a sinking velocity of  $10 \text{ m d}^{-1}$  at a shear rate of 0.1 and  $1.0$  are 2.36 and 4.28, respectively, which implies that the average  $\delta_{\text{eff}}$  is 0.30 mm and 0.16 mm, respectively. The colony shown in Fig. 2B had a diameter of 1.4 mm and a sinking velocity of  $10 \text{ m d}^{-1}$ . The DBL thickness was 0.33 mm upstream and 0.52 mm downstream. In a turbulent environment, DBLs surrounding *Phaeocystis* colonies will, thus, also persist on a scale close to that measured in the present study, and the chemical microenvironments in *Phaeocystis* colonies will, therefore, also persist at shear rates up to  $0.1 \text{ s}^{-1}$  in the ocean. At higher shear rates, the DBL thickness will decrease and the mass transfer due to turbulence will increase.

The effective diffusion coefficient in the mucus matrix as measured from the steady-state gradients in biologically inactivated colonies was on average  $93 \pm 10\%$  of that in seawater (Fig. 7). The effective diffusion coefficient as modeled from the dynamic changes in oxygen concentration in pho-

tosynthetically active colonies during light–dark shifts was also rather similar to that in seawater (Fig. 10). An effective diffusion coefficient for oxygen similar to that in water has also been found in aggregates of the fungi, *Candida lipolytica*, embedded in 0–8% (w/w) agar (Sato and Toda 1977, in Libicki et al. 1988) and in aggregates of the yeast, *Hansenula polymorpha*, embedded in a barium alginate gel (2–40 g L<sup>-1</sup>) (Hiemstra et al. 1981, in Libicki et al. 1988). A lower diffusion coefficient in the mucus matrix would imply steeper chemical gradients in colonies that may not favor the biological processes inside the colonies.

**Photosynthesis**—The direct measurements of oxygen gradients in the DBL of *Phaeocystis* colonies enabled quantification of photosynthesis and respiration and compensating light intensities in single colonies. Dark respiration of the colonies from the Barents Sea and of young, brownish colonies from the Dutch North Sea was 5.8–7.6%, only, of gross photosynthesis. The oxygen profiles measured in light show the net oxygen production of the community including both autotrophic and heterotrophic processes. These colonies were, thus, highly autotrophic communities, which showed similar oxygen distributions at saturating light intensities and in the dark and a low compensating light intensity of 7–10  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The ratio of dark respiration and gross photosynthesis is, however, dependent on the stage of the bloom, as reflected by the increase in compensating light intensity from 29 April to 5 May in the Dutch North Sea (Fig. 11B,C). By the use of the <sup>14</sup>C-technique, dark respiration has earlier been shown to comprise 18% of photosynthesis for *Phaeocystis* colonies (Lancelot and Mathot 1985). Photoassimilated <sup>14</sup>C in *Phaeocystis* colonies has been fractionated into intracellular and extracellular metabolites (mucus and smaller carbon metabolites). The latter comprised up to 80% of the carbon assimilation (Lancelot 1983; Lancelot and Mathot 1985, 1987). As oxygen production is related to carbon assimilation, the net photosynthesis in the present measurements must include both carbon assimilated for growth and carbon assimilated for synthesis of excretion products such as mucus and smaller carbon metabolites. Assuming a carbon content of 800 ng C colony<sup>-1</sup> (Rousseau et al. 1990), and a photosynthetic or respiratory quotient of 1.2:1 mol O<sub>2</sub>:mol CO<sub>2</sub>, the carbon content of a colony could be doubled within 800 ng/(12 g mol<sup>-1</sup> × 9.16 nmol C h<sup>-1</sup>) 6 h at saturating light intensities or be degraded within 800 ng/(12 g mol<sup>-1</sup> × 0.475 nmol C h<sup>-1</sup>) = 138 h or ~6 d.

The gross photosynthesis is dependent on the density of photosynthetic cells in the colony. Thus, the high photosynthetic rates of brownish colonies and the high increase in pH and oxygen at the colony surface may be due to higher densities of photosynthetic cells or a higher chlorophyll (Chl) *a* cell<sup>-1</sup> in these colonies compared to the pale colonies at the later stage of the bloom (Fig. 11). Due to photosynthesis, pH reached values up to about 8.5 at the colony surface in general (Fig. 11), and in one case it reached a value of 8.9 inside the colony (Fig. 6). The pH of *Phaeocystis* colonies has previously been analyzed by the use of micro-electrodes (Lubbers et al. 1990) and shown to reach values up to 9.1 at saturating light intensities when the pH of the bulk water was 8.6. The pH inside colonies at saturating light

intensities is dependent on the buffer capacity and pH of the bulk water, as well as the colony size,  $\delta_{\text{eff}}$ , and density of photosynthetic cells and heterotrophic microorganisms in the colony.

The light saturation values,  $I_k$ , of *Phaeocystis* colonies have been found to be in the range of 45–250  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Baumann et al. 1994). The  $I_k$  of ~60  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in colonies with diatoms being attached to the mucus surface from the Barents Sea were similar to those obtained using the <sup>14</sup>C-technique on the same cruise (M. Vernet, pers. comm.). The  $I_k$  of *Phaeocystis* colonies in the Marsdiep were somewhat higher (90  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ).

Whereas the steady-state O<sub>2</sub> concentration and the pH at the surface are dependent on DBL thickness, the measured compensating light intensity will be unaffected by  $\delta_{\text{eff}}$  because there are no net fluxes of O<sub>2</sub> or CO<sub>2</sub> at this light intensity. The shift in compensating light intensities from 29 April to 5 May (Fig. 11B,C) from net autotrophic toward net heterotrophic communities indicates an increase in bacterial biomass relative to Chl *a*. This is consistent with results obtained by Davidson and Marchant (1987), who showed that colonies in the early stages of a *Phaeocystis* bloom are almost entirely free of attached bacteria, whereas during senescence Chl *a* declines and colonies are colonized by bacteria. The specific biomass and the growth rates of bacteria attached to *Phaeocystis*-derived material in the North Sea are on average very high, i.e., 60 fg C cell<sup>-1</sup> and 0.28 h<sup>-1</sup>, respectively, and specific ectoenzymatic activities of bacteria attached to *Phaeocystis* material are fivefold higher compared to that of free-living bacteria (Becquevort et al. 1998). Studies of the aerobic degradation of phytoplankton debris in cultures dominated by *Phaeocystis* sp. have shown a rapid increase in bacterial numbers accompanied by a rapid decrease of particulate organic carbon during the first 4 d of the experiment. However, such debris consisted of a labile, rapidly degradable fraction and a refractory, slowly degradable fraction, of which the labile fraction represented approximately 50%, only, of the debris (Osinga et al. 1997). The carbon turnover time of 6 d as measured in the present study may therefore only represent the initial degradation process of labile carbon.

DBLs with gradients of solutes, as demonstrated for oxygen in the present study, imply that the exchange of solutes between the colonies and the surrounding water occurs by molecular diffusion at the colony–water interface. Gradients of nutrients will, therefore, also occur in the DBL of *Phaeocystis* colonies. The growth conditions for *Phaeocystis* cells and attached bacteria are then significantly different from those in the surrounding water. The concentration will be lower inside the colony compared to that of the surrounding water, when the nutrient fluxes from the surrounding water are the main source for nutrient supply to the colonies. There is, however, no simple correlation between photosynthesis, nutrient uptake, and cellular growth in *Phaeocystis* colonies because of the production of mucus (Lancelot and Mathot 1987). DBLs have been considered to limit nutrient uptake in *Phaeocystis* colonies (Veldhuis et al. 1991). The concentration levels at which the DBL thickness limits nutrient fluxes from the surrounding water to *Phaeocystis* colonies and cellular growth in the colonies is poorly known but these

have been analyzed through model calculations (Ploug et al. 1999).

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Received: 16 September 1996

Accepted: 4 November 1998

Amended: 10 August 1999