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Diffusive boundary layers of the colony-forming plankton alga *Phaeocystis* sp.—implications for nutrient uptake and cellular growth

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Abstract

The impact of colony formation on cellular nutrient supply was calculated for *Phaeocystis* in a turbulent environment using a diffusion–reaction model. The model included diffusive boundary layer as predicted by Sherwood numbers in mass transfer to a sphere. Literature values for nutrient uptake (V_{max}, K_m) of single cells and colonies and the size dependence of cell numbers in colonies were used in the model. Colony formation was shown to decrease nutrient uptake by *Phaeocystis* cells because of the presence of diffusive boundary layers with concentration gradients surrounding the colonies. At diffusion limitation, this concentration gradient was reflected by an apparently higher half-saturation constants for nutrient uptake, K_M, for colonial cells compared with that for single cells. The diffusion limited supply of inorganic nitrogen and orthophosphate from the bulk water phase with concentrations of 2 and 0.2 μM, respectively, was sufficient to support nutrient demands for 1 cell doubling in colonies in 6–10 h, respectively, at a shear rate of 0.1 s^{-1}. The same nutrient concentration levels could theoretically support nutrient demands of single cells for one cell doubling within 2–3 h. It was concluded that the lower grazing pressure in the size class of colonies relative to that of single free-living cells may be more important for colony formation than nutrient concentrations.

Phaeocystis sp. is a common marine plankton alga that exists as free-living cells and as colonies with up to thousands of cells. The cells are primarily positioned at the surface of a mucus matrix, which can be several millimeters in diameter (Rousseau et al. 1994). The extracellular matrix mainly consists of exopolymeric gelatinous material, the synthesis of which was negatively correlated to the mineral nitrogen concentration in the Southern Bight of the North Sea (Lancelot 1983). *Phaeocystis* blooms often occur at <0.5 μM orthophosphate in the sea (Veldhuis et al. 1986). Thus, colony formation appear to be of competitive advantage under nutrient limitation (Lancelot 1995). Uptake studies with ^{32}P, however, have shown that the maximum uptake rate of orthophosphate is similar for single, free-living cells and colonial cells, whereas the half-saturation constant was one order of magnitude larger for colonial cells than for free-living cells (Veldhuis et al. 1991). Colonial cells grow significantly slower than free-living cells under phosphate limitation (Veldhuis and Admiraal 1987). These results are consistent with limitation of nutrient uptake by the presence of a diffusive boundary layer (DBL) surrounding *Phaeocystis* colonies.

DBLs around *Phaeocystis* colonies have been directly demonstrated by the detection of steep gradients of oxygen and pH levels in the DBL due to the photosynthetic activity of *Phaeocystis* cells in the mucus matrix (Lubbers et al. 1990; Ploug et al. in press). The measured DBL thickness surrounding sinking aggregates has shown to be in good agreement with the DBL thickness predicted by Sherwood numbers (Ploug et al. 1997; Ploug and Jørgensen 1999). *Phaeocystis* colonies, however, are often neutrally buoyant or sink at low velocities of a few meters a day. Turbulence with shear rates of 0.1–1.0 s^{-1} decreases the DBL thickness and may thus increase nutrient uptake in *Phaeocystis* colonies in the natural environment (Ploug et al. in press).

In the present study, we used a model to analyze the quantitative impact of DBLs on nutrient supply and limitation for colonial cells as compared with single free-living cells in a turbulent environment.

Materials and methods

Turbulence and shear—The impact of turbulence on mass transfer to a single free-living cell or a colony is dependent

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on its size relative to the viscous length scale in the ocean, L_ν (Mann and Lazier 1991):

$$L_\nu = (\nu^3/\epsilon)^{1/4} \quad (1)$$

where ν is the kinematic viscosity of sea water and ϵ is the energy dissipation rate. The kinematic viscosity of seawater is ca. 10^{-2} cm^2 s^{-1}. The energy dissipation rate in the sea normally ranges between 10^{-2} and 10^{-6} cm^2 s^{-3} (Mann and Lazier 1991; MacKenzie and Leggett 1993 and references therein). The viscous length scale thus ranges from 1 mm ($\epsilon = 10^{-2}$ cm^2 s^{-3}) to 10 mm ($\epsilon = 10^{-6}$ cm^2 s^{-3}) in the sea. Above the viscous length scale, the flow is turbulent, while below it, viscosity dominates resulting in a laminar shear. It should be noted that Eq. 1 may underestimate L_ν since most of the shear energy is present in eddies with length scales of $>L_\nu$ (Lazier and Mann 1989).

The shear rate, E, due to turbulence in the ambient water is dependent on the energy dissipation rate and the kinematic viscosity of seawater (Karp-Boss et al. 1996):

$$E = \left(\frac{\epsilon}{\nu}\right)^{1/2} \quad (2)$$

The shear rate due to turbulence in the mixed layer thus normally ranges from 0.01 to 1.0 s^{-1}.

The energy in turbulent eddies decreases with decreasing size of the eddies because the viscous forces are strong at small scale, and the flow is considered to be statistically steady below this scale. The Reynolds number describes the relative importance of inertial and viscous forces acting at solid boundaries, i.e., in the vicinity of organisms or colonies of organisms in the sea. The Reynolds number, *Re*, due to shear in a turbulent environment is defined as (Karp-Boss et al. 1996)

$$Re = \frac{r_0^2 E}{\nu}, \quad (3)$$

where r_0 is the radius of the organism or colony and E is the shear rate due to turbulence in the ambient water. For organisms and colonies of 3.5–500 μm radii and at shear rates of 0.01–1.0 s^{-1}, *Re* ranges from 1.22×10^{-7} to 0.25.

The relative importance of turbulence and diffusion on mass transfer of nutrients to phytoplankton cells and colonies is dependent on cell or colony size, the shear rate, and the diffusion coefficient of the chemical species, which is described by the Peclet number, *Pe* (Karp-Boss et al. 1996),

$$Pe = \frac{r_0^2 E}{D}, \quad (4)$$

where D is the diffusion coefficient of the chemical species. The diffusion coefficient for nitrate is 1.17×10^{-5} cm^2 s^{-1}at 10°C, and it deviates less than 5% from that of ammonium (Li and Gregory 1974). The diffusion coefficient for orthophosphate is 0.48×10^{-5} cm^2 s^{-1} at 10°C (Li and Gregory 1974). For organisms and colonies of 3.5–500 μm radii, and at shear rates of 0.01 – 1.0 s^{-1}, *Pe* ranges from 1.0×10^{-4} to 520 for nutrients with diffusion coefficients ranging from 0.48×10^{-5} to 1.17×10^{-5}.

Diffusive boundary layers—Microscopic organisms and colonies smaller than the Kolmogorof length scale in the sea are surrounded by a DBL through which the exchange of solutes between the organisms or colonies and the surrounding water occurs by molecular diffusion. The radial diffusive flux of a chemical solute through the DBL is given by Sherwood et al. (1975) and Ploug et al. (1997):

$$J = D\frac{C_\infty - C_0}{\delta_{\text{eff}}} \quad (5)$$

where D is the molecular diffusion coefficient of the chemical species, C_∞ is the bulk water concentration, C_0 is the concentration at the sphere surface, and δ_{eff} is the effective DBL thickness.

The area-integrated flux, Q, is:

$$Q = 4\pi r_0^2 D\frac{(C_\infty - C_0)}{\delta_{\text{eff}}} \quad (6)$$

Shear reduces the effective thickness of the DBL. The relative increase in mass transfer due to flow and turbulence compared to pure diffusion is described by the Sherwood number, *Sh* (Sherwood et al. 1975; Karp-Boss et al. 1996). In a stagnant fluid, the effective DBL thickness, δ_{eff}, extends to a distance in the surrounding waters that is equal to the radius of the sphere, i.e., $2r_0$ from the center of the sphere (Sherwood et al. 1975; Ploug et al. 1997):

$$Sh = \frac{r_0}{\delta_{\text{eff}}} = 1 \quad (7)$$

The effective DBL thickness decreases and the Sherwood number increases with increasing shear rate. The area-integrated flux in a turbulent environment with shear can thus be described by

$$Q = Sh4\pi r_0 D(C_\infty - C_0) \quad (8)$$

The Sherwood number depends on both the Reynolds number, *Re*, and the Peclet number, *Pe*. For $Re \ll 1$ and $Pe < 0.01$ (Batchlor 1979; cf. Karp-Boss et al. 1996),

$$Sh = 1 + 0.29x(Pe)^{1/2}. \quad (9)$$

For $Re \ll 1$ and $0.01 < Pe < 100$ (Karp-Boss et al. 1996),

$$Sh = 1.014 + 0.51x(Pe)^{1/2}. \quad (10)$$

For $Re < 1$ and $Pe > 100$ (Batchelor 1980; cf. Karp-Boss et al. 1996),

$$Sh = 0.55x(Pe)^{1/3}. \quad (11)$$

Because of the small difference between the diffusion coefficients for nitrate and ammonium, the diffusion coefficient for nitrate was used in the model to calculate *Sh* for inorganic N uptake (ammonium and nitrate). The impact of turbulence on the effective DBL with respect to nitrate/ammonium and orthophosphate was calculated for single free-living cells and for 0.1–1.0-mm-large colonies. *Sh* was calculated according to Eqs. 9, 10, or 11, depending on *Re* and *Pe* (cf. Eqs. 3 and 4, respectively).

Colony formation and nutrient uptake—Because of the presence of a DBL with concentration gradients around phy-

toplankton cells and colonies, a modified Michaelis–Menten equation was used to describe the nutrient uptake rate, *v*:

$$\nu = \frac{nV_{\max}C_0}{K_M + C_0} \tag{12}$$

where n is the number of colonial cells located at the colony surface, $V_{\max}$ the maximum uptake rate per cell, K_M the half-saturation constant, and C_0 is the concentration at the cell surface.

The uptake rate, *v*, by the cells in the colony can be equated to the area-integrated diffusive supply of nutrients thro ugh the effective DBL per unit time, *Q* (Eq. 9):

$$Sh4\pi r_0 D(C_\infty - C_0) = \frac{nV_{\max}C_0}{K_M + C_0} \tag{13}$$

The solution of the flux equation yields $C_0 = -0.5\alpha + \sqrt{0.25\alpha^2 + \beta}$, where $\alpha = K_M - C_\infty + nV_{\max}/(Sh4\pi Dr_0)$, and $\beta = K_M C_\infty$. Equation 13, including the modified Michaelis–Menten equation, has earlier been used to describe diffusion limited nutrient uptake for phytoplankton cells, where $Sh = 1$ and $n = 1$ (Pasciak and Gavis 1974).

The number of cells in *Phaeocystis* colonies have been determined from microscopic observations of colonies from the North Sea, Prydz Bay, and in cultures to be described by (Rousseau et al. 1990):

$$\log n = 0.51 \log(4/3\pi r_0^3) + 3.67, \tag{14}$$

where n is the number of colonial cells and $4/3\pi r_0^3$ is the colony volume (mm^3).

Veldhuis et al. (1991) found $V_{\max}$ for orthophosphate to be 0.141×10^{-6} nmol P min^{-1} $cell^{-1}$ with a corresponding K_M value of 0.31 μM for single *Phaeocystis* cells under P limitation, whereas $V_{\max}$ was 10% higher, i.e., 0.158×10^{-6} nmol P min^{-1} $cell^{-1}$, for colonial cells with an apparent K_M of 3.08 μM in the bulk water phase for the whole colony. This difference in K_M may be ascribed to a DBL surrounding the mucus matrix, whereby the concentration at the cell surface is lower than the bulk water concentration. The impact of the effective DBL thickness on C_0 was here analyzed by using $V_{\max} = 0.141 \times 10^{-6}$ nmol P min^{-1} $cell^{-1} = 2.35 \times 10^{-18}$ mol P $cell^{-1}$ s^{-1} for both single cells and colonial cells. The true K_M, as a physiological parameter, was also assumed to be the same, 0.31 μM, for free-living cells and single cells living in a colony.

During a *Phaeocystis* bloom in the North Sea, $V_{\max}$ for ammonium uptake was found experimentally to be 300 nmol N μg chlorophyl (Chl) a^{-1} h^{-1} (Riegman et al. 1990). Assuming a Chl *a* content of 0.3 pg Chl *a* $cell^{-1}$ (Baumann et al. 1994), this corresponds to $V_{\max} = 25 \times 10^{-18}$ mol N $cell^{-1}s^{-1}$, which was used in the model. The apparent K_M for inorganic N uptake (ammonium and nitrate) for protein synthesis in *Phaeocystis* colonies have been shown to be 4 μM (Lancelot et al. 1986). K_M for inorganic N uptake (ammonium and nitrate) has not been determined in *Phaeocystis* cells. For other flagellates of similar cell size as single *Phaeocystis* cells, e.g., *Isochrysis galbana*, K_M has been measured to be 0.1μM (Eppley et al. 1969). We used $K_M = 0.1$ μM as the physiological half-saturation constant in the model.

Results

Diffusive boundary layers and turbulence—The Sherwood numbers, *Sh*, for inorganic N and orthophosphate mass transfer to a colony, and the corresponding effective DBL thickness, δ_{eff}, were calculated for colonies of different sizes in a turbulent environment with shear rates of 0.01, 0.1, and 1.0 s^{-1} (Fig. 1). DBLs develop at interfaces where the water movement is dominated by viscous forces and molecular diffusion is fast relative to the laminar advection in the vicinity of colonies, although turbulence may be high in the ambient water. *Sh* increases to a greater extent with increasing colony size for orthophosphate than for inorganic nitrogen at the same shear rate for a given colony size because the diffusion coefficient for orthophosphate is smaller than that for nitrate and ammonium (i.e., it requires less momentum to exceed the molecular diffusion coefficient of orthophosphate compared to that of inorganic nitrogen). The effective DBL thickness is therefore thinner for orthophosphate compared with that for inorganic nitrogen. The effective DBL thickness increases with sphere size, and it ranges between 0.1 and 0.4 mm for a 1-mm-large sphere in a turbulent environment with shear rates up to 1.0 s^{-1} (Fig. 1).

Diffusive boundary layers and nutrient uptake—The fluxes of inorganic nitrogen and orthophosphate, which are determined by the mass transfer resistance in the boundary layer and by the potential cellular uptake rate, were calculated (cf. Eq. 13) for a single free-living cell having a diameter of 7 μm (Baumann et al. 1994) and for a spherical colony with 3,360 cells and a diameter of 1.0 mm (cf. Eq. 14). *Sh* for the colony was calculated at a shear rate of 0.1 s^{-1}. Because of the presence of the DBL, the concentration of inorganic nitrogen and orthophosphate at the surface of both a single free-living cell and at the surface of a colony is significantly different from that of the bulk water phase at low nutrient concentrations (Fig. 2A,D). The concentration at the colony surface was significantly lower than that at the surface of a single cell because of the higher total nutrient uptake (i.e., higher number of cells) and a thicker DBL of the colony. The apparent half-saturation constant, K_M, for 1-mm-large colonies was 3.5 and 1.0 μM for inorganic N and P, respectively, or 35- and 3.2-fold higher than the respective K_M for free-living cells (Fig. 2B,E). The uptake rate per cell was thus always lower for colonial cells compared with that for single free-living cells. A Hanes plot for inorganic nitrogen and orthophosphate uptake-kinetics per cell is shown in Fig. 2C and 2F, respectively. In a Hanes plot, the slope is equal to $1/V_{\max}$, and the intersection at the y-axis is equal to $K_M/V_{\max}$ (Cornish-Bowden 1995). However, under diffusion limitation, the slope is not constant; that is, the curve bends upward at low concentrations. Whereas diffusion limitation was hardly detectable for free-living cells, it occurred for colonial cells at bulk water concentrations of <7.5 μM nitrate/ammonium and <2 μM orthophosphate, respectively. At high concentrations, the slope of the curves for single cells and colonies are similar to each other, reflecting the identical maximum nutrient uptake rate per cell.

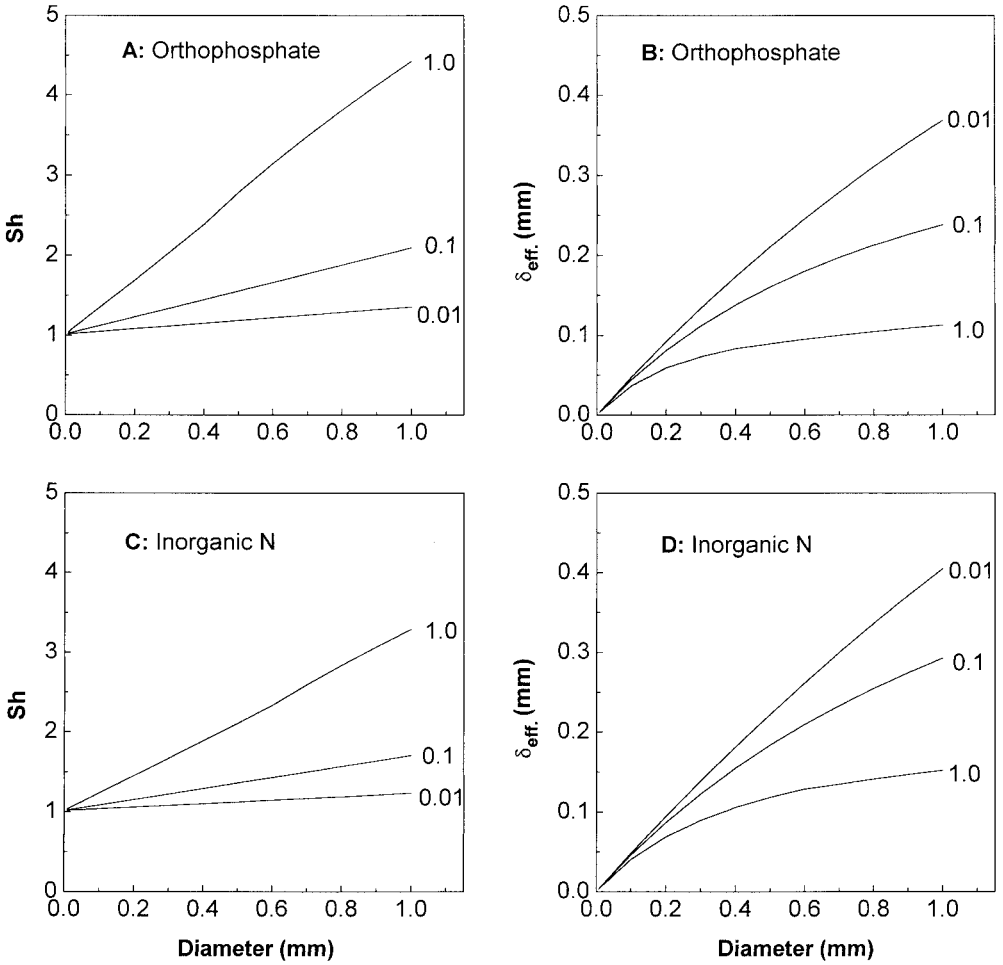

Fig. 1. The Sherwood number (*Sh*) and corresponding DBL thickness (δ_{eff}) for different colony sizes in a turbulent environment. The numbers on the curves refer to shear rate (s^{-1}). (A) *Sh* for inorganic nitrogen. (B) *Sh* for orthophosphate. (C) DBL for inorganic nitrogen. (D) DBL for orthophosphate.

Diffusion limited nutrient supply and nutrient demand for cellular growth—The inorganic N and P demand for one cell doubling was calculated for different colony sizes (Eq. 14) for a cellular C : N : P ratio of 106 : 16 : 1 (Redfield ratio) equal to 1.12 pmol C $cell^{-1}$/6.6 mol C : mol N = 1.69×10^{-1}, pmol N $cell^{-1}$ and 1.12 pmol C $cell^{-1}$/106 mol C : mol P = 1.05×10^{-2} pmol P $cell^{-1}$. The time required for doubling of cellular N and P through the uptake of these nutrients across the DBL of single cells and colonies at different bulk water concentrations was calculated from the cellular nutrient demand divided by the nutrient supply at shear rates of 0.1 and 1.0 s^{-1} (cf. Eq. 13.). The N and P demands for one cell doubling can be covered during <12 hours at bulk water concentrations of >1 μM nitrate and >0.20 μM orthophosphate for all colony sizes, even with a low shear rate of 0.1 s^{-1} (Fig. 3A,C). Free-living cells would meet their N and P demand in 2.1 and 3. 8 h, respectively, at the same bulk water concentrations. Diffusion limitation is reflected in the impact of shear on the time required to cover nutrient-demands for one cell division. By increasing the shear rate from 0.1 to 1.0 s^{-1}, the N demand for one cell doubling in 0.5–1-mm-large colonies could be covered in 15 h instead of 22–27 h at a bulk water concentration of 0.5 μM (Fig. 3A,B), and the P demand could be covered in 24 h instead of 40–42 h at a bulk water concentration of 0.05 μM (Fig. 3C,D). Free-living cells would meet their N and P demand in 3 and 12 h, respectively, at the same bulk water concentrations. Free-living cells would thus potentially grow faster than colonial cells when the N or P concentrations is the growth limiting factor. Interestingly, the time required for doubling of cellular N and P through the uptake of these nutrients across the DBL was rather independent of colony size for colonies larger than 200 μm at a shear rate of 1.0 s^{-1}, which suggests an intrinsic relation between number of cells per colony size and DBL thickness, as the total nutrient demand of a colony is proportional to the number of cells in the colony.

Diffusive fluxes and cell numbers in colonies—The nutrient uptake rate of the colonies and the concentration differ-

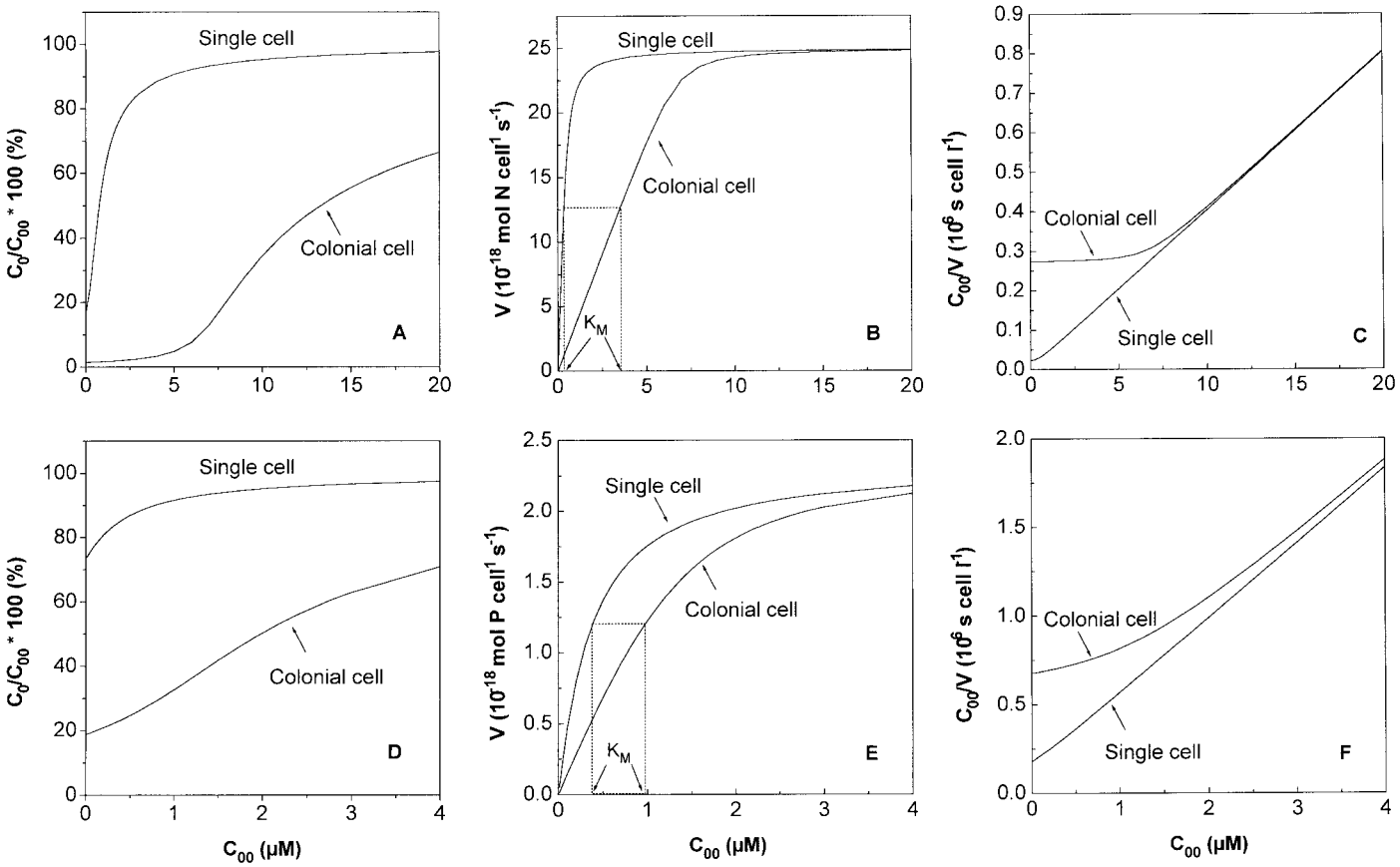

Fig. 2. Uptake kinetics of inorganic nitrogen (upper panels) and orthophosphate (lower panels) for a single free-living cell (ø: 7 μm) and a cell living in a 1-mm-large colony in a turbulent environment with a shear rate of 0.1 s^{-1}. (A, D) The concentration at the cell surface or colony surface in the percentage of the bulk water concentration at different bulk water concentrations. (B, E) The cellular uptake rate at different bulk water concentrations. The apparent K_M is indicated for single and colonial cells. (C, F) A Hanes plot (*see text*).

ence across the DBL increases proportional to the cell-specific maximum uptake rates, V_{max}, and the number of cells in the colonies when the nutrient uptake from the surrounding water is not diffusion limited (Eq. 13). However, as the number of cells in a colony of a given size increases, the concentration at the colony surface will gradually approach zero and the diffusive flux through the DBL cannot increase further unless the DBL thickness is reduced. As shown in Fig. 2, diffusion limitation onsets before the concentration reaches zero at the colony surface. The concentration of inorganic N and P at the colony surface at the shear rate is 1.0 s^{-1} is shown in Fig. 4 as a function of colony size, when the bulk water concentrations of inorganic N and P are 2 and 0.20 μM, respectively. The concentration of inorganic N at the colony surface was close to zero for all colony sizes. The concentration of inorganic P was also low and rather independent of colony size. The fluxes were therefore determined by the DBL thickness, e.g., a twofold increase in cell number per colony or a twofold increase in cellular uptake rate, V_{max}, could not result in proportionally higher fluxes across the DBL. The high potential growth rates even at such a low bulk water concentrations (Fig. 3) can thus be due to a balance between cell densities in the colonies and their nutrient uptake rates relative to the diffusion limited nutrient fluxes.

The cells are primarily positioned at the mucus surface in *Phaeocystis* colonies, which may be an adaptation to or result of diffusion limitation. However, the number of cells in colonies does not increase proportional to the colony surface area (r_0^2), but it increases proportional to $r_0^{1.5}$ (cf. Eq. 14). The cells per colony surface area therefore decreases with increasing colony size. At diffusion limitation of any nutrient, the area-integrated flux of the nutrient to the colonies is proportional to $Sh \times r_0$ as D and the concentration difference across the DBL is constant (Eq. 8). The number of cells for different colony sizes as observed by Rousseau et al. (1990) (cf. Eq. 14) versus the calculated size dependent diffusion limited flux of nutrients with diffusion coefficient of 1.17×10^{-5} cm^2 s^{-1} to colonies with diameters ranging from 0.2 to 1.0 mm at a shear rate of 1.0 s^{-1} (cf. Eqs. 10 and 11) is shown in Fig. 5. The number of cells in colonies of different sizes increases proportional to the diffusion limited fluxes at shear rates of 1.0 s^{-1} ($R^2 = 0.998$; $p < 0.001$) because both parameters increase with $r_0^{1.5}$ in this size range. A shear rate of 0.1 s^{-1} also results in a linear relationship between cell numbers and the diffusion limited flux (data not shown). These similar size dependencies indicate, that the number of cells in *Phaeocystis* colonies is determined by or adapted to the diffusion limitation, i.e., the physical–chemical micro-

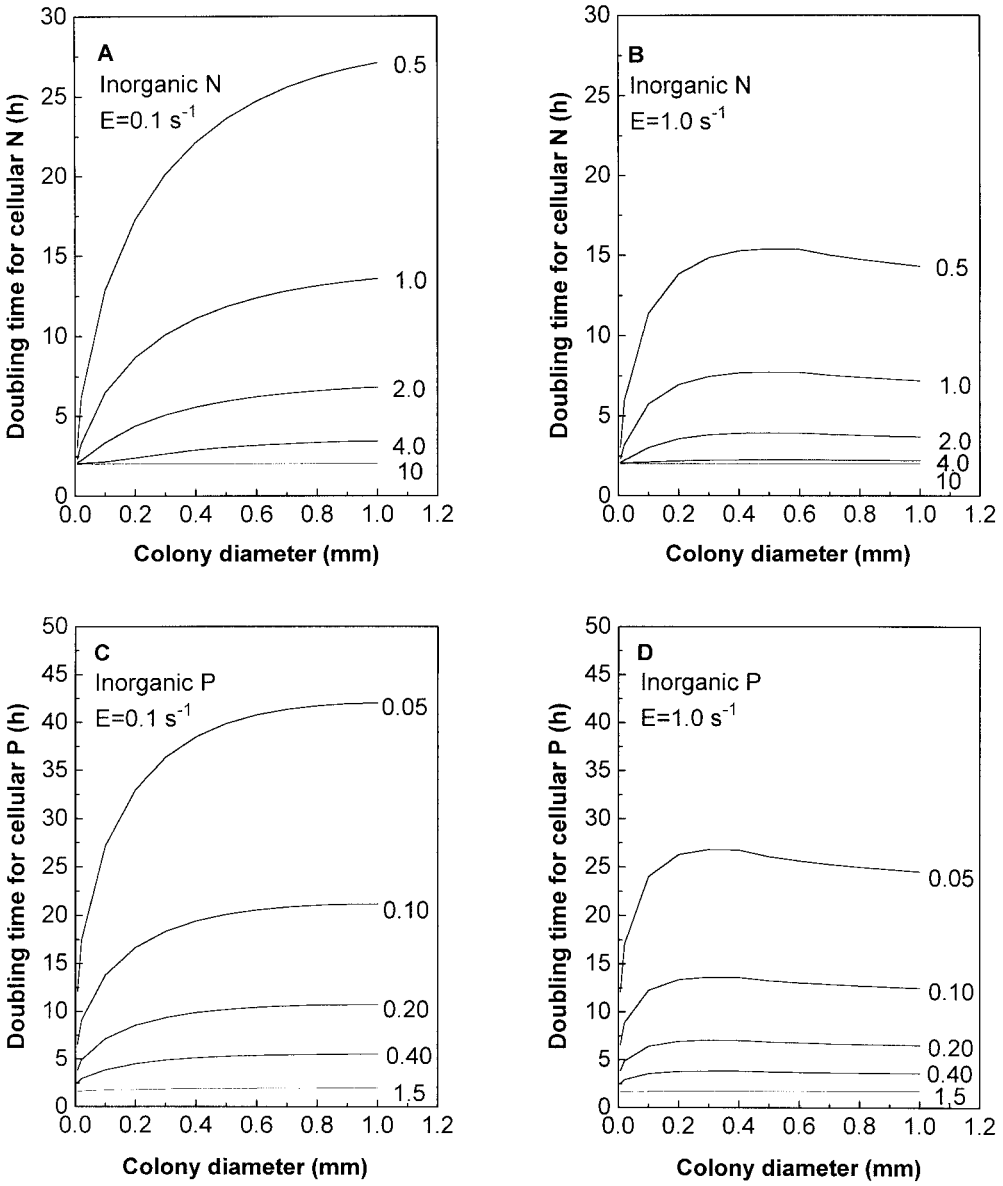

Fig. 3. Time required for doubling of cellular N and P through the uptake of these nutrients across the DBL of free-living cells and colonies at shear rates of 0.1 and 1.0 s^{-1}. Numbers on curves indicate the bulk water concentrations (μM).

environment, to meet the reduction in cellular nutrient supply as the size of the colony increases during growth.

Discussion

The classical work on fluid motion and nutrient uptake in aquatic plants was done by Munk and Riley (1952), and diffusion limitation of nutrient and CO_2 uptake have been analyzed for single phytoplankton cells and macroalgae (Pasciak and Gavis 1974; MacFarlane and Raven 1985; Riebesell et al. 1993). The Sherwood number has been used to asses advantages of large versus small cell size relative to nutrient uptake in phytoplankton communities (Kiørboe 1993). Conclusions from such studies are consistent with the fact that small (<10 μm) motile phytoplankton predominate in stagnant and nutrient-poor waters whereas large phytoplankton predominate in turbulent, nutrient-rich waters.

In which respect colony formation is an advantage in *Phaeocystis* is currently discussed. *Phaeocystis* blooms often follow after diatom spring blooms, when nutrients are partly depleted (Riegman et al. 1990; van Boekel et al. 1992). The nutrient status is believed to be a major factor for colony formation (Rousseau et al. 1994). According to the present study, the cell-specific nutrient fluxes in colonies are consistently lower than those of single free-living cells at low nutrient concentrations due to the presence of relatively thick DBLs surrounding the colonies even in turbulent waters. Diffusion limitation was reflected by an apparently higher

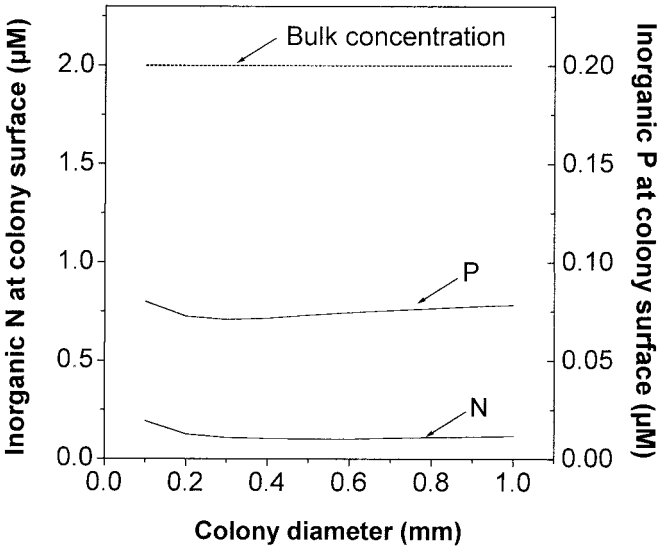

Fig. 4. The concentration of inorganic N and orthophosphate at the colony surface at a shear rate of 1.0 s^{-1}, when the bulk water concentrations of inorganic N and orthophosphate is 2.0 and 0.2 μM, respectively.

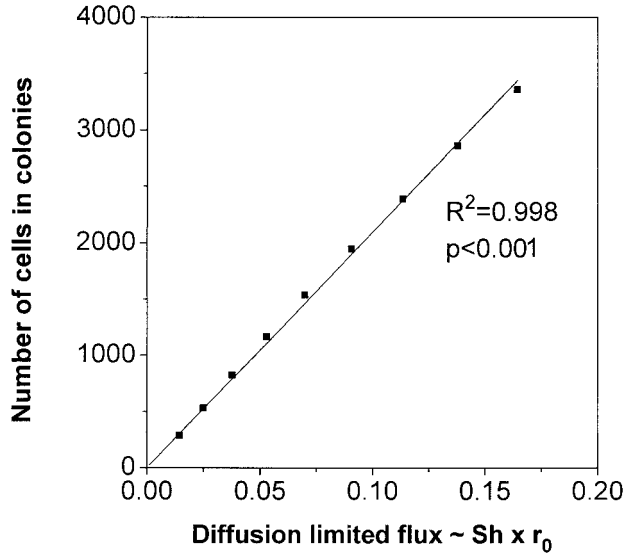

Fig. 5. Cells per colony of different sizes versus diffusion limited fluxes at a shear rate of 1.0 s^{-1} (*see text*).

half-saturation constant for nutrient uptake, K_M, for colonial cells compared with that for single cells (Fig. 2), which was earlier observed in phosphate uptake studies (Veldhuis et al. 1991). The physiological K_M used in the model is lower and correlates to the concentration of the nutrient at the colony surface, which has not been directly measured in nutrient uptake studies. Plankton algae have been shown to increase their uptake capacity (V_{max}) under nutrient limitation (Zevenboom et al. 1982). The V_{max} used in our model has been measured under N and P limitation, respectively (Riegmann et al. 1990; Veldhuis et al. 1991). At high nutrient concentrations, they thus represent transient uptake rates during short-term incubations rather than steady uptake rates, and the short doubling times of cellular quota for nutrients at high nutrient concentrations are therefore probably overestimated.

A second response of phytoplankton to nutrient limitation is the reduction of the cellular quota for that nutrient. Phosphorus quota can be reduced ca. fivefold under P limitation (Rhee 1973; Nalewajko and Lean 1980). With respect to the diffusive transport toward and the cellular uptake of phosphorus by the colony cells, the time needed to double phosphorus content would be lower when cellular phosphorus quota are reduced. However, reduction of cellular nutrient quota always coincides with reduction of the specific growth rate, indicating that this will only play a role when growth rate is already reduced because of nutrient limitation. Quantitatively, cell quota reduction will decrease the negative effect of diffusion limitation of nutrient uptake on the growth response of colonial *Phaeocystis* cells. However, it does not affect the qualitative conclusions, i.e., that colony cells will always grow slower than free-living cells. For a sound quantitative analysis, the relation of cellular P content with growth rate is indispensable.

Maximum specific growth rates of 0.8–1.0 d^{-1} in *Phaeocystis* cultures have been found at initial phosphate concentrations of 1–4 μM in batch cultures (Veldhuis and Admiraal 1987). The growth rate of colony cells was reduced by 50% at a phosphate concentration of 0.8 μM. In contrast, the growth rate of single cells was only reduced by 11% at a phosphate concentration of 0.8 μM (Veldhuis and Admiraal 1987). In the sea, *Phaeocystis* forms blooms in the colony phase at orthophosphate concentrations of <0.5 μM (Veldhuis et al. 1986). The orthophosphate fluxes in a turbulent environment as calculated here could cover P demands for one doubling in cellular biomass in <6 h for colonies with a diameter up to 1 mm at a shear rate of 0.1 s^{-1} even at a bulk water concentration of 0.40 μM (Fig. 3). At a bulk water concentration of 0.10 μM and a shear rate of 0.1 s^{-1}, the diffusive supply was not sufficient to support growth rates of one doubling per day in colonies with a cellular C : P ratio of 106, assuming a strictly light dependent P uptake in a light : dark cycle of 12 : 12 h. At bulk water concentrations of orthophosphate below 0.15 μM, alkaline phosphatase activities increased up to 1.4×10^{-20} mol $cell^{-1}$ s^{-1} in *Phaeocystis* cultures. Concurrently, the fraction of colonial cells decreased (Veldhuis and Admiraal 1987). Thus, the bulk water concentration of orthophosphate, which limits growth rates due to DBLs, is close to the concentrations where alkaline phosphatase activity is increased in response to phosphate limitation.

Continued nutrient uptake of both inorganic N and P at night may partly compensate for diffusion limited nutrient uptake. Continued uptake of inorganic nitrogen and protein synthesis at the expense of mucus as a carbon source during dark have been demonstrated in *Phaeocystis* colonies isolated from temperate areas (Lancelot et al. 1986). The protein synthesis in dark was dependent, however, on the ''previous light history,'' i.e., the N uptake and protein synthesis in dark increased with increasing duration and intensity of the preceding illumination. Such a utilization of newly incorporated carbon in the mucus matrix was not observed in Antarctic clones of *Phaeocystis* (Matrai et al. 1995). Veldhuis et al.

(1991) measured a 70% decrease in orthophosphate uptake rate from light to dark for free-living single cells, whereas the P uptake rate for colonies remained unchanged during light and dark. A dark uptake of orthophosphate by colonial cells proceeding at the same rate as in light would imply that growth at one doubling per day would be balanced according to the Redfield ratio of 106 C : P for all colony sizes, even with a bulk water concentration of orthophosphate of only 0.1 μM at a low shear rate of 0.1 s^{-1}.

Adsorption and accumulation of phosphate may occur in the mucus matrix when the daily cellular P uptake is not limited by the maximum potential P fluxes. Adsorbed phosphate in *Phaeocystis* colonies has been observed to gradually increase during growth and reach a concentration 3,000-fold higher than that of the surrounding water, which concurrently decreased to 0.3 μM. This reservoir diminished 2 d after the bulk water concentration had decreased to 0.3 μM accompanied by a decrease in colony cell numbers and an increase in alkaline phosphatase activities (Veldhuis et al. 1991). Such an absorbed reservoir is thus probably of little quantitative importance for cellular growth.

The *Phaeocystis* cells are located 15–20 μm below the surface of the mucus matrix, and the cells per surface area decrease significantly with increasing colony size (Rousseau et al. 1994). Under P limitation, growth may be restricted to the periphery because cells closer to the center would be severely diffusion limited by nutrients and deprived of orthophosphate being scavenged by adsorption in the mucus and nutrient uptake by peripheral cells. Additionally, the distribution of the cells over the colony surface might be controlled by the diffusion-limited flux of the limiting nutrient (Fig. 5). Enhanced mucilage formation by colonial cells, induced by nutrient limitation (Myklestad and Haug 1972), would cause a relative increase of colony size compared with the colony cell number and would result in a lower cell density in the colonial matrix. Supporting evidence shows that colonial cells have found to excrete more of their fixed carbon (13–64%) than single cells (only 3%) (Guillard and Hellebust 1971). Although this does not necessarily have to be caused by diffusion limited flux of nutrients, cell number in natural *Phaeocystis* colonies, which is the balanced result of cell division and mucus excretion, correlates noticeably with diffusive flux calculations (Fig. 5).

The organization of cells near the colony surface probably also reduces self-shading within the colonies., e.g., a strong self-shading has been measured in colonies of cyanobacteria (Prufert-Bebout et al. 1993). *Phaeocystis* colonies can be neutrally buoyant or sink at only low velocities (van Boekel et al. 1992; Ploug et al. in press). The mucus may thus reduce the excess density of the colonies, i.e., their sinking velocities, to reduce export of the colonies from the euphotic zone.

Flagellated free-living cells, however, would always have their N and P demand covered in a shorter time compared with colonial cells at low nutrient concentrations, and they do not sink or experience strong self-shading due to their small size. Copepod grazing on *Phaeocystis* single cells and colonies have been shown to be equally low compared with that on diatoms (Verity and Smayda 1989). Other studies have indicated that the grazing pressure by protozoans on *Phaeocystis* single cells is high because of their size class (Admiraal and Venekamp 1986; Weisse et al. 1994). *Phaeocystis* colonies are only colonized by bacteria and protozoans in the stationary phase of blooms when net growth has ceased (Davidson and Marchant 1987; Lancelot 1995). A reduction in grazing pressure may compensate for reduction in nutrient supply during colony formation and thus may be of major significance for the colony formation during *Phaeocystis* blooms.

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