

Anoxic aggregates — an ephemeral phenomenon in the pelagic environment?

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ABSTRACT: Radial microscale distributions of oxygen and pH were studied in ca 1.5 mm large laboratory-made aggregates composed of phytoplankton detritus and fecal pellets. Microsensor measurements were done at spatial increments down to 0.05 mm in a vertical flow system in which the individual aggregates stabilized their position in the water phase according to the upward flow velocity. The aggregates were surrounded by a diffusive boundary layer with steep gradients of oxygen and pH. They were highly heterotrophic communities both under natural light conditions and in darkness. pH was lowered from 8.2 in the surrounding water to 7.4 in the center of an anoxic aggregate. Sulfide was not detectable by use of sulfide microelectrodes in anoxic aggregates, and methanogenic bacteria could not be detected after PCR (polymerase chain reaction) amplification using archaeobacterial-specific primers. The oxygen respiration rate decreased exponentially over time with a $T_{1/2}$ of 2.3 d. Theoretical calculations of the volumetric oxygen respiration rate needed to deplete oxygen inside aggregates was compared to the density of organic matter in natural marine aggregates. These calculations showed that carbon limitation of heterotrophic processes would limit anoxic conditions to occurring only over a few hours, depending on the size of the aggregates. Therefore slow-growing obligate anaerobic microorganisms such as sulfate reducing bacteria and methanogenic bacteria may be limited by the relatively short persistence of anoxia in marine aggregates.

KEY WORDS: Microelectrodes · Molecular techniques · Diffusive boundary layers · Modeling

INTRODUCTION

A significant portion of suspended matter in the sea and in lakes exists in the form of rapidly sinking aggregates comprised of phytoplankton and other microorganisms, of organic detritus, zooplankton fecal pellets and inorganic particles (Alldredge & Silver 1988). The degradation of phytoplankton detritus aggregates is characterized by a microbial succession involving heterotrophic bacteria and bacterivorous protozoa (Biddanda 1988, Biddanda & Pomeroy 1988). Although aggregates can reach sizes up to 75 mm in maximum length (Alldredge & Gotschalk 1988), it is not evident from direct measurements by microelectrodes if anoxia develops during the concurrent decay and sedimentation processes of marine snow comprised of diatoms (Alldredge & Cohen 1987). Anoxia has, however, been measured in a several millimeter large crustacean fecal pellet with an intact peritrophic membrane (All-

dredge & Cohen 1987). Anoxic microenvironments harboring strictly anaerobic bacteria have been suggested as a source for the observed methane production in oxygenated waters (Lamontagne et al. 1973, Sieburth 1993). Low redox potentials measured by the use of tetrazolium salts and sulfide measured spectrophotometrically have suggested that sulfate reduction occurs in marine snow (Shanks & Reeder 1993). Isolation of strictly anaerobic bacteria from fresh zooplankton fecal pellets and large aggregates with numerous large fecal pellets from sediment traps (Bianchi et al. 1992) and measurements of methane fluxes associated with sediment trap material (Karl & Tilbrook 1994) have shown that anoxic microenvironments may be present in zooplankton guts and/or sinking zooplankton fecal pellets (Karl & Tilbrook 1994, Tilbrook & Karl 1995).

Here we present direct measurements of anoxic conditions in freely sinking aggregates composed of phytoplankton detritus and fecal pellets surrounded by fully oxygenated seawater. The volumetric oxygen res-

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Table 1. List of symbols and their definitions

C (nmol cm ⁻³)	Concentration
C_r (nmol cm ⁻³)	Concentration at the radial distance, r
C_0 (nmol cm ⁻³)	Concentration at the surface, r_0
C_∞ (nmol cm ⁻³)	Bulk water concentration
D (cm ² s ⁻¹)	Molecular diffusion coefficient
$D_{agg.}$ (cm ² s ⁻¹)	Effective diffusion coefficient in an aggregate
D_w (cm ² s ⁻¹)	Molecular diffusion coefficient in seawater
$\delta_{eff.}$ (cm)	Effective diffusive boundary layer thickness
E (s ⁻¹)	Shear rate
ϵ (cm ² s ⁻³)	Energy dissipation rate
k_c (cm s ⁻¹)	Mass transport coefficient
K_m (μ M)	Half-saturation constant
J (nmol cm ⁻² s ⁻¹)	Diffusive flux
ν (cm ² s ⁻¹)	Kinematic viscosity
R (nmol cm ⁻³ s ⁻¹)	Volumetric respiration rate
r_0 (cm)	Surface radius
r_c (cm)	Radius at which the O ₂ concentration is zero
r (cm)	Radius
Sh (dimensionless)	Sherwood number
U (cm s ⁻¹)	Sinking velocity
Q (nmol s ⁻¹)	Area-integrated diffusive flux

piration rates and carbon turnover times in anoxic aggregates are analyzed with respect to the carbon content in naturally occurring aggregates of marine snow and fecal pellets.

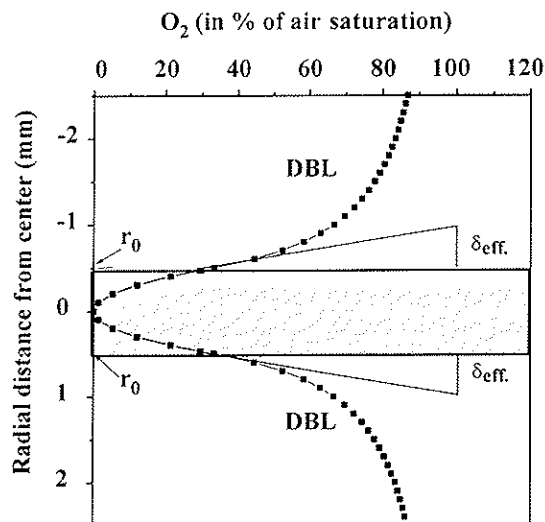


Fig. 1. A hypothetical (see text), radial oxygen distribution in the diffusive boundary layer (DBL) and within a 1 mm spherical aggregate (shaded area), which is anoxic exactly at the center under stagnant conditions. The effective DBL thickness, $\delta_{eff.}$ is shown

MATERIAL AND METHODS

Theoretical calculations. Diffusive boundary layers:

When mass transfer to a sphere proceeds only by molecular diffusion, e.g. in a diffusive boundary layer (DBL) at the sphere-water interface, 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, r_0 , C_1 is the concentration at the radial distance r_1 , and $r_0 < r < r_1$.

The radial flux of oxygen is expressed by Fick's first law of diffusion:

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

where J is the flux of oxygen per surface area, D_w is the molecular diffusion coefficient of oxygen in seawater. A list of symbols is shown in Table 1. The area-integrated flux per unit time, Q_t , through the DBL is described by (Crank 1975):

$$Q_t = 4\pi D_w \frac{r_0 r_1}{r_1 - r_0} (C_1 - C_0) \quad (3)$$

A hypothetical example of the radial oxygen distribution inside and around a 1 mm aggregate which is anoxic at the very center under stagnant conditions (r_1 tends to ∞) is shown in Fig. 1. Regarding the DBL as an array of concentric shells, the surface area of each shell increases with r^2 . As the flux per surface area is proportional to the radial gradient through each concentric shell, the radial gradient therefore decreases with r^2 without a decrease in the (total) area-integrated flux. The DBL thickness can thus be determined from the curvature of the oxygen profile as the largest radial distance which yields the same total flux in the water phase as the total flux at the sphere surface, r_0 .

In mass transfer theory, DBLs have been replaced by the mass transfer coefficient, k_c , which equals the ratio between the diffusion coefficient, D_w , and the effective (or hypothetical) DBL thickness, $\delta_{eff.}$. The $\delta_{eff.}$ is defined by extrapolating the radial oxygen gradient at the sphere-water interface, r_0 , to the bulk water phase concentration. Thus,

$$J = -D_w \frac{C_\infty - C_0}{\delta_{eff.}} = -k_c (C_\infty - C_0) \quad (4)$$

where k_c is the mass transport coefficient, which equals $D_w/\delta_{eff.}$. It should be noticed that this extrapolation yields the same flux as Eq. (2). The mass transport coefficient increases with increasing flow, due to a decrease in the DBL thickness.

The Sherwood number, Sh , describes the increase in mass transfer due to flow (Sherwood et al. 1975). In a stagnant fluid:

$$Sh = \frac{k_c r_0}{D_w} = 1 \quad (5)$$

where r_0 is the radius of the sphere. However, as k_c equals the ratio of the diffusion coefficient and the effective DBL thickness, δ_{eff} (Sherwood et al. 1975):

$$Sh = \frac{r_0}{\delta_{\text{eff}}} = 1 \quad (6)$$

Whereas the real DBL is infinite in a stagnant fluid, a hypothetical DBL, δ_{eff} , which yields an equivalent flux of mass transfer to a sphere, thus extends to a distance outside the sphere which is equal to the radius of the sphere, as shown in Fig. 1. This hypothetical boundary layer decreases inversely proportional to Sh for sinking spheres or in a turbulent environment.

Concentration gradients within a sphere: The flux of oxygen and, thus, the radial gradient of oxygen decreases towards the center of an aggregate, as oxygen is consumed within the aggregate. At steady state, the oxygen concentration inside a sphere with zero order oxygen consumption and spherical diffusion is described by (Jørgensen 1977):

$$C_r = -\frac{R}{6D_{\text{agg}}}(r_0^2 - r^2) + C_0 \quad (7)$$

where D_{agg} is the effective diffusion coefficient of oxygen in the aggregate and R is the volumetric respiration rate.

The concentration at the surface, C_0 , is dependent on the volumetric oxygen respiration rate in the aggregate, as the area-integrated flux of oxygen through the DBL at steady state is equal to the total oxygen being consumed within the aggregate. Assuming an evenly distributed oxygen consumption and that anoxia does not occur, the volumetric oxygen respiration rate is, therefore, described by:

$$R = \frac{4\pi r_0^2 D_w (C_\infty - C_0)}{\frac{4}{3}\pi r_0^3 \delta_{\text{eff}}} = \frac{3D_w}{r_0 \delta_{\text{eff}}}(C_\infty - C_0) \quad (8)$$

where $4\pi r_0^2$ and $\frac{4}{3}\pi r_0^3$ are the surface area and volume, respectively, of a sphere.

C_0 decreases linearly with an increase in the oxygen uptake, R :

$$C_0 = -R \frac{r_0 \delta_{\text{eff}}}{3D_w} + C_\infty \quad (9)$$

By substitution of Eq. (9) into Eq. (7), the concentration at the radial distance, r , inside a sphere, which is surrounded by a DBL, is described by:

$$C_r = -\frac{R}{3} \left(\frac{r_0^2 - r^2}{2D_{\text{agg}}} + \frac{r_0 \delta_{\text{eff}}}{D_w} \right) + C_\infty \quad (10)$$

where the concentration exactly at the center is

$$C_{r=0} = -\frac{R}{3} \left(\frac{r_0^2}{2D_{\text{agg}}} + \frac{r_0 \delta_{\text{eff}}}{D_w} \right) + C_\infty \quad (11)$$

which shows that the oxygen concentration at the center decreases proportionally to the volumetric respiration rate, to the product of the radius of the sphere and its DBL thickness, and to the square of the radius of the sphere, whereas it decreases inversely proportionally to the diffusion coefficients in the surrounding water and inside the aggregate.

Anoxic aggregates: When an anoxic central part is present in a sphere and thus all the oxygen is consumed close to the surface of the sphere, R can be described by:

$$R = \frac{4\pi r_0^2}{\frac{4}{3}\pi(r_0^3 - r_c^3)} \frac{D_w (C_\infty - C_0)}{\delta_{\text{eff}}} \quad (12)$$

where r_c is the radial distance from the center at which the concentration of oxygen is zero, and

$$C_0 = -R \frac{\delta_{\text{eff}}(r_0 - r_c^3/r_0^2)}{3D_w} + C_\infty \quad (13)$$

Assuming zero order oxygen consumption, i.e. ignoring the small volume of the aggregate which follows first order kinetics (see 'Discussion'), C_r is described by:

$$C_r = -\frac{R}{3} \left(\frac{r_0^2 - r^2}{2D_{\text{agg}}} + \frac{\delta_{\text{eff}} r_0}{D_w} - \frac{\delta_{\text{eff}} r_c^3 / r_0^2}{D_w} \right) + C_\infty \quad (14)$$

where the respiration rate needed to create anoxia at the radial distance r_c within a sphere is described by:

$$R = 3C_\infty \left(\frac{r_0^2 - r_c^2}{2D_{\text{agg}}} + \frac{\delta_{\text{eff}} r_0}{D_w} - \frac{\delta_{\text{eff}} r_c^3 / r_0^2}{D_w} \right)^{-1} \quad (15)$$

Aggregates. Aggregates of detritus from a zooplankton culture, *Acartia tonsa*, which had been grown on *Rhodomonas baltica*, were made in roller tanks (Shanks & Edmonson 1989). The detritus included phytoplankton debris, fecal pellets and carcasses. The detritus was suspended in artificial seawater (hw Meer-salz) of 30‰ with a pH of 8.2 in the roller tanks at 23°C. Eight aggregates were examined per day by the use of microelectrodes over a time period of 5 d.

Microelectrode measurements. Oxygen distributions were measured by a Clark-type oxygen microelectrode with a guard cathode (Revsbech 1989) mounted in a micromanipulator and calibrated in air-saturated and N₂-flushed seawater. The electrode current was measured by a picoammeter and read on a strip chart recorder. The tip diameter was 6 μm, the stirring sensitivity was <1%, and the 90% response time was 0.2 s. Sulfide was measured by a ~20 μm wide Ag/Ag₂S microelectrode, as described in Revsbech & Jørgensen (1986), connected to a mV-meter. The lower detection limit of sulfide was ~1 μM at pH 7.4 (Kühl & Jørgensen 1992). The pH microelectrodes were constructed ac-

diffusion coefficient, ϵ is the energy dissipation rate and ν is the kinematic viscosity of sea water; $\sqrt{\frac{\epsilon}{\nu}}$ is the turbulent shear rate, E (s^{-1}). Pe is the Peclet number. The equation is valid for $Pe \gg 1$. In the surface waters of the ocean the shear rate normally ranges from 0.1 to 1.0 s^{-1} (MacKenzie & Leggett 1991). In the presence of turbulence with a shear rate of 1.0 s^{-1} , Sh ranges from 2.77 at neutral buoyancy to 4.59 for a 1 mm sinking sphere (170 $m d^{-1}$) (cf. Eq. 14). These Sherwood numbers correspond to a $\delta_{eff.}$ of 0.18 to 0.11 mm (cf. Eq. 6), which is close to the measured $\delta_{eff.}$ of 0.17 mm.

The measured profiles in the aggregates of the present study could be well described by the theoretical distributions of oxygen in a sphere assuming a diffusion coefficient of O_2 inside the aggregates similar to that in seawater and an evenly distributed respiration of zero order (Fig. 4). The molecular diffusion coefficient of O_2 in mucus produced by the colony forming microalgae *Phaeocystis* sp. has recently been experimentally shown to be similar to that in seawater (H. Ploug, W. Stolte, E. H. G. Epping & B. B. Jørgensen unpubl.). A diffusion coefficient of O_2 inside marine aggregates similar to that in seawater may therefore be expected as well. A lower diffusion coefficient in the aggregates would imply steeper gradients of oxygen inside the aggregates. As the spatial resolution of oxygen measurements by microelectrodes is the same as the tip diameter, e.g. 6 μm , and the 90% response time was 0.2 s, anoxic 'microzones' with dimensions <100 μm , e.g. formed due to a locally lower diffusion coefficient in marine aggregates, would in principle be detectable with the microelectrode technique unless surrounded by a protecting wall. We did not detect such microzones in the present study.

The calculations of the oxygen consumption rates were done assuming zero order kinetics. The half-saturation constant, K_m , for oxygen respiration is approximately 1 μM in heterotrophic microorganisms (Focht & Verstraete 1977). An anoxic center in an aggregate being immersed in oxygenated water implies very steep oxygen gradients within the DBL and inside the aggregate. The fraction of the aggregate (Fig. 2) in which respiration presumably was oxygen limited was very small, i.e. the anoxic zone of the aggregate comprised ca 8% of the total aggregate volume. The zone in which O_2 was present at concentrations <10 μM (10-fold K_m) and, thus, presumably followed first order kinetics was found at the radial distance $0.225 < r < 0.350$ mm, which comprised <9% of the total aggregate volume, assuming spherical geometry. The error in the calculated respiration rates introduced by assuming zero order kinetics was accordingly <9%.

The mass transfer resistance in the DBL surrounding the aggregates with a mean size of 1 mm lowered the oxygen respiration required to create anoxic condi-

tions by 33%. The lower limit for the oxygen respiration rate needed to create anoxia at the center of 1 mm large aggregates was found at 28 $nmol O_2 mm^{-3} h^{-1}$ (Fig. 5). Anoxia was only detected in the largest aggregates, and a 0.4 mm wide zone with anoxic conditions was measured early in the degradation process of a 1.4 mm aggregate with a volumetric oxygen respiration rate of 22.1 $nmol O_2 mm^{-3} h^{-1}$ (Fig. 2). With a respiratory quotient of 1, carbon must be degraded at the same rate, and such anoxic microenvironments may therefore rapidly become carbon limited.

Hansen & Bech (1996) found that fecal pellets of the copepod *Acartia tonsa* are mainly colonized by bacterioplankton from the surrounding waters, and the bacterial numbers associated with fecal pellets were stable after 150 h (Hansen et al. 1996). The exponential decay of oxygen respiration rate in the aggregates of the present study (Fig. 3) with a $T_{1/2}$ of 2.3 d indicates that the oxygen respiration rates in these aggregates were carbon limited and not limited by e.g. sub-optimal colonization by microorganisms. Studies of the bacterial colonization and carbon degradation of fecal pellets from *A. tonsa* cultures have also shown an immediate exponential decay of fecal pellets with $T_{1/2}$ ranging between 0.09 and 17.2 d at 18°C (Lee & Fisher 1992a, Hansen et al. 1996). The observed half-time of respiration in the present study was, thus, within the same range as observed for decomposition of zooplankton debris at similar temperature. $T_{1/2}$ for phyto- and zooplankton debris is up to 100-fold longer at 2 to 4°C, which may be due to lower bacterial respiration and growth rates (Lee & Fisher 1992, 1993). Anoxic aggregates harboring methanogenic bacteria may therefore not be expected during algal blooms at low temperature. A previous study on the microbial population structure of marine phytodetrital macroaggregates comprised of diatoms and *Phaeocystis* colonies, being typical during algal blooms, showed that archaeal DNA and RNA were not associated with such aggregates (DeLong et al. 1993). Microelectrode studies in *Phaeocystis* colonies have also shown 50-fold lower respiration rates as compared to the present study, whereby such colonies very unlikely become anoxic (Ploug et al. unpubl.).

DBLs and anoxia have been measured in a several millimeter large crustacean fecal pellet attached to a particle of marine snow (Aldredge & Cohen 1987). Copepod fecal pellets are, however, significantly smaller with a long axis ranging from 88 to 168 μm , while the short axis is approximately 40 μm (Hansen et al. 1996). Depending on the DBL thickness, anoxic conditions in a 'spherical' fecal pellet with a diameter of 70 μm would require an oxygen respiration rate of 3.2 to 9.8 $\times 10^3 nmol O_2 mm^{-3} h^{-1}$, assuming a diffusion coefficient of O_2 similar to that in seawater. The carbon

content of fecal pellets produced by copepods is approximately $0.060 \text{ pg C } \mu\text{m}^{-3}$, equal to $5.0 \text{ } \mu\text{mol C mm}^{-3}$ (Gonzales & Smetacek 1994). Anoxic conditions occurring only at the very center would accordingly predict a carbon turnover time of 0.06 d assuming spherical geometry, maximal DBL thickness, and a diffusion coefficient similar to that in seawater, and require a 100% remineralization to CO_2 . With an observed $T_{1/2}$ of 0.09 to 17.2 d (Lee & Fisher 1992a, Hansen et al. 1996) for fecal pellets which also release DOC, such small fecal pellets would not be anoxic. Anoxic conditions in such fecal pellets with a volumetric oxygen respiration rate of $22.1 \text{ nmol O}_2 \text{ mm}^{-3} \text{ h}^{-1}$, i.e. the highest measured in the present study, would require a diffusion coefficient 100-fold less than that of seawater or alternatively that pO_2 of the surrounding water is reduced down to 1% of air saturation.

The diffusion distance of oxygen and the accumulation of organic matter are both highly increased when fecal pellets are trapped in marine snow. Thereby anoxic conditions are more likely to occur as the oxygen respiration rate necessary to create anoxic conditions decreases 100-fold as the radius increases 10-fold (Fig. 6). The calculated turnover times of carbon required to generate an anoxic center in marine aggregates with sizes ranging from $100 \text{ } \mu\text{m}$ to 1 cm were in the order of 1.5 to 34 h (Fig. 7). As the degradation process becomes carbon limited, the oxygen consumption rate will decrease as the carbon is consumed and remineralized or released as DOC. Because anoxia will prevail only until the respiration becomes carbon limited, the calculated time range represents a maximum estimate of the turnover time of anoxic aggregates. Advective transport of oxygen inside large porous aggregates will enhance the availability of oxygen and, thus, decrease the carbon turnover time of large anoxic aggregates as here calculated, assuming molecular diffusion as the only mass transfer process. The sinking velocity and the porosity of the aggregates increase with size (Alldredge & Gotschalk 1988), and advective transport oxygen may therefore be more important for mass transfer in several mm to cm large aggregates (Logan & Alldredge 1989). Carbon turnover times in marine snow have earlier been estimated from bacterial production rates (Simon et al. 1990, Smith et al. 1992). These studies showed carbon turnover times ranging from 20 to 1429 d. These degradation rates were, thus, 10- to 1000-fold lower than the respiratory rates necessary to create anoxic conditions. Intense hydrolysis of particulate amino acids by bacterial ectoenzymes have shown turnover times of 0.2 to 2.1 d for such pools with very little of the hydrolysate being taken up by the attached bacteria in marine aggregates, whereby

the nutritional value of marine aggregates rapidly decreases (Smith et al. 1992).

In situ measurements of methane fluxes from sediment trap material have suggested that methanogenic bacteria are associated with sinking particulate matter (Karl & Tilbrook 1994). The exact source of methane accumulating in the traps could, however, not be determined (Tilbrook & Karl 1995). Nitrification and methanogenesis have earlier been measured in sediment trap material composed of detritus and fecal pellets in the size range of 43 to $150 \text{ } \mu\text{m}$ (Bianchi et al. 1992). Methanogenic activity associated with sediment trap material including such fecal pellets was, however, only detectable after long incubation times of 30 to 40 d under anaerobic conditions (Bianchi et al. 1992, Marty 1993). By the use of archaeal-specific primers we did not detect any methanogenic bacteria in the anoxic aggregates. The ephemeral anoxic state and the very dynamic change of the oxygen microenvironment in the aggregates suggest that such microenvironments are too unstable for the relatively slow growth of obligatory anaerobic bacteria such as methanogenic and sulfate-reducing bacteria, and it may explain why neither archaea nor sulfide was detected inside the aggregates. Only bacteria and other microorganisms that can grow over a wide range of oxygen concentration and pH may efficiently exploit such a microenvironment rich in carbon and nutrients.

As shown in the present study, stable anoxic conditions require a high and continuous carbon supply to support the oxygen consumption rates. The methane and sulfide production observed in oxygenated waters may be due to zooplankton grazing as previously suggested by De Angelis & Lee (1994) and it is most likely produced in meso- and/or macrozooplankton guts. A continuous passage and degradation of organic matter and/or a strong diffusive barrier for O_2 is required to provide stable anoxic conditions, where methanogenic bacteria can grow.

Acknowledgements. Detritus from *Acartia tonsa* cultures was kindly provided by Thomas Kjørboe. We thank Eric H. G. Epping for developing the computer analysis software. Cindy Lee is thanked for discussions. Microelectrodes were constructed by Anja Eggert and Gaby Eickert at the Max Planck Institute. This work was supported by the Danish Natural Science Research Council (J: 11-0557-1 to H.P.), the Danish Research Academy (J: V930148 to H.P.), and by the Max Planck Gesellschaft (Germany).

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