Microbial communities and respiration on aggregates in the Elbe Estuary, Germany

Helle Ploug[1],*, Heike Zimmermann-Timm[2],**, Bernd Schweitzer[3]

[1]**Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany**
[2]**Institute for Hydrobiology and Fisheries, University of Hamburg, Zeiseweg 9, 22765 Hamburg, Germany**
[3]**Institute for Limnology, University of Konstanz, Postbox 5560, 78434 Konstanz, Germany**

ABSTRACT: Estuaries are characterized by a very high abundance of small aggregates enriched with bacteria, protozoa and sometimes metazoa compared to their abundance in the surrounding water. In this study, the microbial community and respiration rates on estuarine aggregates were analyzed by the combination of microscopy (bacteria, protozoa and metazoa enumeration), *in situ* hybridization and microsensor techniques. Aggregates were isolated and kept in darkness to prevent scavenging or production of new organic carbon. Bacterial abundance and respiration rates were stable during the first 2 d after collection. Bacterial numbers ranged from 0.84×10^6 to 23.7×10^6 cells aggregate $(agg)^{-1}$. Protozoa were dominated by nanoflagellates, which varied between 24 and 1497 cells agg^{-1}. Total protozoan biovolume accounted on average for 4 to 13% of the total estimated microbial biomass on aggregates, and the total grazing rate on attached bacteria was apparently low. Size-specific respiration rates were described by $R = 5.86 \times d^{1.82}$, where R is measured in ng C agg^{-1} h^{-1} and the diameter (d) is measured in mm. The average respiration rate was 0.150 µg C agg^{-1} d^{-1}, and the estimated average bacterial respiration rate was 22 fg C cell^{-1} d^{-1}. Total respiration and cell-specific respiration decreased to 0.039 µg C agg^{-1} d^{-1} and 2.6 fg C cell^{-1} d^{-1}, respectively, after 6 d. The bacterial community was dominated by members of β-proteobacteria and the *Cytophaga-Flavobacterium* cluster, which together accounted for 54% of all 4',6-diamidino-2-phenylindole (DAPI) stained bacteria at the end of the study. The respiration rate on aggregates larger than 400 µm in the water column of the Elbe Estuary was 11.8 µg C l^{-1} h^{-1} as determined from the size-specific respiration rates, the aggregate abundance and size distribution. It accounted for 84 to 94% of the estimated total respiration in the upper water column. The critical ambient O_2 concentration, at which the center of the aggregate interior turns anoxic, was found at ~16 µM.

KEY WORDS: Estuarine aggregates · Oxygen microelectrodes · Respiration · Protozoa · Bacteria · *In situ* hybridization

INTRODUCTION

A high occurrence of aggregates is a conspicuous feature of the estuarine environment (Kausch 1990, Eisma 1993). Such aggregates are composed of phytoplankton, fecal pellets, loricae, macrophytes, mineral particles and detrital particles (Alldredge & Silver 1988, Velmirov 1991). In estuaries, aggregates may sink out of the water column or be carried downstream toward the sea as dead or living particulate organic matter (POM) (Eisma 1993, Berger et al. 1996, Zimmermann 1997). Suspended particulate material in flowing waters is, thus, an important energetic link between upstream and downstream communities (Naiman 1983), as well as between the pelagic and benthic environment in estuaries (Eisma 1993). The colonization of estuarine aggregates has been investigated in several studies (Laybourn-Parry et al. 1992, Rogerson & Laybourn-Parry 1992a,b, Crump & Baross 1996, Zimmer-

*E-mail: hploug@mpi-bremen.de
**Present address: Institute for Ecology, Department for Limnology, Friedrich-Schiller-University of Jena, Winzerlaerstraße 10, 07743 Jena, Germany

mann & Kausch 1996, Zimmermann-Timm et al. 1998). Estuarine aggregates contain relatively more benthic organisms and material than marine and lake snow (Zimmermann-Timm et al. 1998, Wörner et al. 2000) and the aggregate size range is, in most cases, smaller than those in the marine environment and in lakes (Alldredge & Silver 1988, Zimmermann 1997, Grossart & Simon 1998). Particle-attached bacteria have been shown to account for 90% of total bacterial production and to correlate with particulate organic carbon (POC) and turbidity in the Columbia River Estuary. By assuming a bacterial net growth efficiency of 0.10, the respiratory turnover of POC was estimated to be 8 d (Crump et al. 1998).

The abundance of aggregates larger than 400 µm is often in the order of several hundreds of aggregates per liter in the water column of the Elbe Estuary. Aggregate-attached bacteria are up to 4×10^3 more concentrated on the aggregates than are free-living bacteria in the surrounding water and they constitute up to 80% of total bacteria (Zimmermann 1997, Zimmermann & Kausch 1996). Respiration on aggregates may cause gradients of oxygen within and around the flocs and even anoxic conditions as earlier demonstrated in large fecal pellets (Alldredge & Cohen 1987). However, the critical ambient O_2 concentration at which the aggregate interior becomes anoxic and the respiratory turnover of organic matter in suspended aggregates have not yet been quantified in the Elbe Estuary.

By the use of recently developed techniques we made the first direct measurements of the O_2-uptake rates on 400 to 3000 µm sized field-sampled estuarine aggregates while they were suspended as in the natural environment (Ploug & Jørgensen 1999). The microbial community and respiration rates were analyzed by the combination of microscopy (bacteria, protozoa and metazoa enumeration), in situ hybridization and microsensor techniques. Single aggregates were kept separately for up to 1 wk in darkness in order to study the dynamics of the attached microbial community and respiration rates under controlled conditions without scavenging or production of new organic carbon. The critical ambient O_2 concentration at which the aggregate interior turns anoxic was determined, and the role of these relatively small aggregates in the carbon turnover was estimated from the size-specific respiration rates, the aggregate abundance and the size distribution in the Elbe.

MATERIALS AND METHODS

Study site and sampling. The Elbe Estuary is a coastal-plain estuary, classified as 'partially mixed' (Day 1981) and characterized by salinity gradients and a large amount of suspended particulate matter (Kausch 1990). It extends about 140 km between the funnel-shaped river mouth at Brunsbüttel and Cuxhaven, where it flows into the North Sea, and a weir at Geesthacht, 45 km upstream from Hamburg, which forms the upper tidal boundary. The mean river discharge is about 700 m^3 s^{-1}, and the current velocity reaches 1 to 2 m s^{-1}. Although the pollution of the Elbe River has been reduced since 1990, it is still one of the most polluted European rivers and carries high loads of sewage and wastes (Adams et al. 1996). Samples were taken in October 1997 (station: 634 km downstream of the German-Czech border; Zimmermann 1997) in Hamburg-Blankenese, Germany.

Water samples were taken in the morning from a depth of 1 m through a horizontal tube (Hydro-Bios). The water temperature was 18.3°C and the oxygen concentration was 6.5 mg l^{-1} (69% of air saturation) The conductivity was fairly constant at 1118 µS cm^{-1}. The pH was 7.9. Aggregates remained in glass bottles in a cooling box for 2 h until they arrived at the laboratory. Aggregates were then isolated individually in vials with 0.5 ml surrounding water and stored in darkness at in situ temperature under still conditions in order to prevent scavenging and production of new organic matter. Anoxic conditions in the sedimented aggregates were not induced by this procedure as demonstrated in larger aggregates (Ploug & Jørgensen 1999) because the aggregates were small and the oxygen consumption was below the critical limit for development of anoxia during contact with the solid surface of the vial. The microbial community was enumerated and respiration on aggregates was measured within a few hours after sampling in the Elbe, and on Days 2 and 6 after the aggregates had been collected in the field.

Enumeration of aggregates. Individual macroscopic aggregates were collected with a Pasteur pipette from the water sample and counted under a dissection microscope. The sizes of the aggregates were measured directly using a calibrated ocular micrometer.

Microscopic characterization of the aggregates. Aggregates were observed under a phase contrast microscope (Olympus BH-2) at a magnification from 100× to 400× to characterize their content of phytoplankton, macrophytes, fecal pellets and detritus (Zimmermann-Timm et al. 1998).

Chemical characterization. Aggregates were characterized histochemically with conventional staining procedures: Alcian blue 8GX in 3% acetic acid to characterize acid mucopolysaccharides (Pearse 1968), Sudan black B for lipids (Jensen 1962), periodic acid Schiff for hexoses (Pearse 1968) and sulfated carbohydrates by using the Alcian blue 8GX, pH 0.5 (Parker &

Diboll 1966). POC was determined with a LECO Carbon Determinator E12. The C:N ratio was determined by CHN analysis with a Heraeus CHN-O-Rapid instrument.

Protozoan numbers. Ciliates were counted on single aggregates after O_2 measurements. Living organisms were observed under a phase contrast microscope (Olympus BH-2) at magnifications from 100× to 400×. If possible they were identified to the species level; otherwise, it was done to genus level. Qualitatively and quantitatively examined aggregates were fixed with formaldehyde (4%). Flagellates were counted on single fixed aggregates after 4',6-diamidino-2-phenylindole (DAPI) staining by epifluorescence microscopy (Porter & Feig 1980). To remove attached organisms from single aggregates, they were treated with ultrasound in 2 mM Na-pyrophosphate (Velji & Albright 1986) prior to filtration.

Grazing rates of bacterivorous protozoa. Grazing rates of the aggregate-attached bacterial population by flagellates was estimated as described by Artolozaga et al. (2002). The grazing rate by heterotrophic flagellates is described by log (grazing rate) = 0.833 × log (bacterial abundance) – 6.701, where the grazing rate is measured as bacteria per protist h^{-1} and the bacterial abundance is measured as bacterial cells ml^{-1} aggregate. The grazing rate by ciliates was assumed to be ~27 bacteria per protist h^{-1} as measured by Artolozaga et al. (2002). The total grazing rate of bacteria on aggregates was calculated from the number of bacteria per protist h^{-1} multiplied by the number of protists per aggregate to equal the number of bacteria per aggregate h^{-1}.

Bacterial enumeration and *in situ* hybridization. After O_2 measurements, as well as qualitative and quantitative analysis of ciliates and flagellates, bacteria were counted on single aggregates after DAPI staining as described for flagellates above. For *in situ* hybridization, 20 µl aliquots of sonicated aggregates were pipetted onto gelatin-coated Teflon microslides and dried at 46°C. The samples were then fixed in 40 µl of fresh paraformaldehyde (4%) for 4 h at 4°C. EUB338, which is specific for bacteria and targets the 16S rRNA, and ARCH915, which is specific for *Archaea* and targets the 23S rRNA, were used (Amann et al. 1995). Members of the α-, β-, and γ-subclass of proteobacteria were examined by *in situ* hybridization with 23S rRNA-targeted fluorescent oligonucleotide probes (ALF1B, BET 42a, GAM42a), and members of the *Cytophaga-Flavobacterium* (CFB) cluster were detected by CF319a according to Manz et al. (1992). Exact sequences of oligonucleotide probes to detect *Archaea*, *Bacteria*, α-, β- and γ-proteobacteria, and the CFB cluster are given by Amann et al. (1995). Bacteria belonging to the different phylogenetic groups were counted in at least 10 fields. The standard deviation of the mean value was <10%.

Respiration measurements. Oxygen gradients were measured by a slender Clark-type O_2 microelectrode (Revsbech 1989) in the diffusive boundary layer (DBL) surrounding individual aggregates, which were kept in suspension by an upward-directed flow opposing sinking velocity (Ploug & Jørgensen 1999). The oxygen microelectrode was attached to a micromanipulator, and it had a 4 µm wide sensing tip, a 90% response time of 1.0 s and a stirring sensitivity of <0.3%. It was calibrated in air-saturated and N_2-flushed water. Its signal was measured by a picoamperemeter connected to a strip chart recorder. The position of the aggregate surface was determined visually by advancing the electrode toward the aggregates until it touched the upper surface as observed under a dissecting microscope. The oxygen gradient was measured with a spatial resolution of 50 µm in the DBL at steady state in darkness. Dimensions of the aggregate were measured under the dissection microscope with a calibrated ocular micrometer. The flow system was filled with 10 l of surface water filtered through microfiber glass filters (GF/C, Whatman). Oxygen was measured at *in situ* temperature (18°C) and at air saturation in the ambient water.

Calculation of respiration rates. The respiration rate of the whole aggregate community in the dark determines the oxygen gradient within the DBL at the aggregate-water interface because the net oxygen exchange between the aggregate community and the surrounding water occurs through the DBL, when aggregates are impermeable to flow. The respiration rates in the aggregates were, thus, calculated from the measured oxygen gradient through the DBL (Ploug et al. 1997). The aggregate surface area was calculated for ellipsoids as described by Maas (1994). The equivalent spherical radius was calculated as $r = (a \times b \times c)^{1/3}$, where a, b and c are the half-axes in the 3 dimensions. Respiration rates were converted to carbon equivalents using a respiratory quotient of 1 mol O_2 to 1 mol CO_2.

RESULTS

Microbial dynamics and respiration over time

Aggregates in the size range of 400 to 3000 µm were studied during a time series experiment. They contained very few phytoplankton but some *Actinocyclus normannii*, some macrophytes and copepod fecal pellets, and high amounts of detritus. Their content of acid mucopolysaccharides, lipids and sulfated polysaccharides was high.
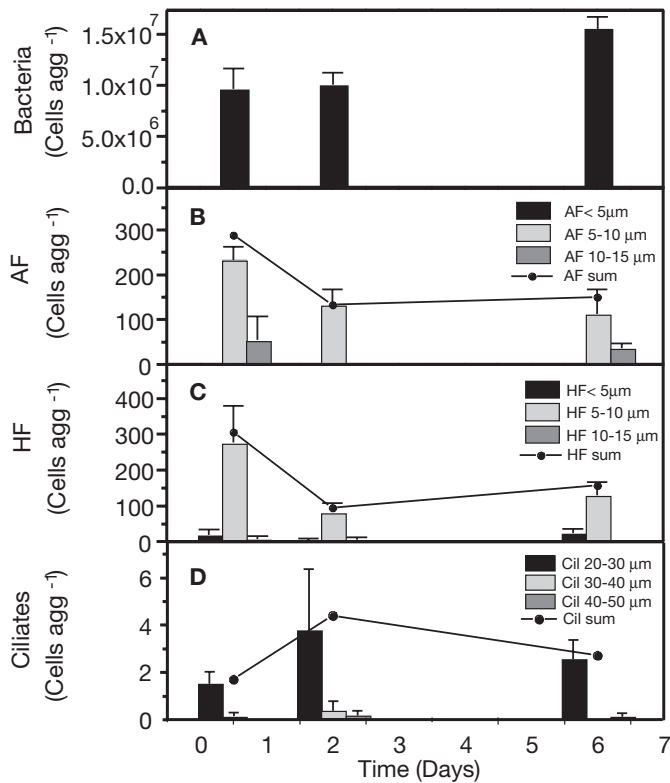
Fig. 1. (A) 4′,6-diamidino-2-phenylindole (DAPI)-specific counts of bacteria, (B) autotrophic flagellates (AF), (C) heterotrophic nanoflagellates (HF) and (D) ciliates per aggregate (agg) during the time series experiment. The line shows whole average abundance of the organism group, and the 3 size classes are shown as bars: <5 μm, 5 to 10 μm and 10 to 15 μm for flagellates and 20 to 30 μm, 30 to 40 μm and 40 to 50 μm for ciliates, which distinguish 3 sizes in all 3 protozoan diagrams. Bars represent the average value with the standard error (n = 10 to 14)

DAPI-specific bacterial numbers on single aggregates varied between 0.84×10^6 and 23.69×10^6 cells aggregate (agg)$^{-1}$, and the average value increased with increasing age of the aggregates after being sampled in the Elbe (Fig. 1). The composition of the protozoan community is summarized in Table 1. On individual aggregates, heterotrophic nanoflagellates varied between 24 and 1497 cells agg^{-1}, whereas autotrophic nanoflagellates varied between 24 and 794 cells agg^{-1}. The average number of flagellates decreased during the first 2 d followed by an increase on Day 6, whereas the average number of ciliates in the 20 to 30 μm size class increased during the first 2 d followed by a decrease on Day 6 (Fig. 1). Ciliates in the 20 to 30 μm size class were more abundant than larger ciliates. Grazing rates of bacterivorous protozoa were estimated from bacterial and protozoan abundance on aggregates (Artolozaga et al. 2002). The rate ranged between 27 and 179 bacteria per protist h^{-1}, and
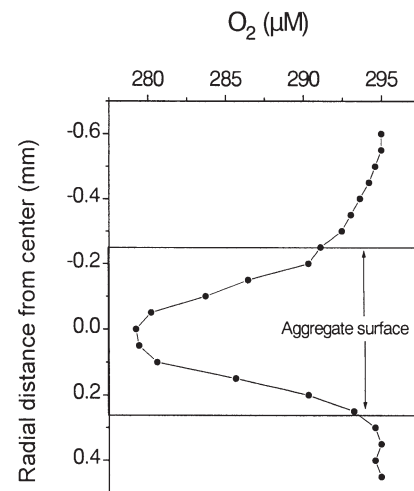

Fig. 2. Oxygen distribution in an aggregate suspended by an upward-directed flow velocity opposing sinking velocity

approximately 2.3 to 6.8% of the bacterial population was grazed per day.

Aggregates were never anoxic despite the high abundance of attached bacteria. An example of the average oxygen gradient in aggregates is shown in Fig. 2. The shortest axis of the aggregate was 0.45 mm and its longest axis was 1.0 mm. The maximum oxygen concentration difference between the surrounding water and the aggregate interior was 16 μM O_2. The sinking velocities were on average 1.5 mm s^{-1}, and flow in the vicinity of aggregates was, therefore, important for the exchange of solutes at the aggregate-water interface and the steady-state concentrations of oxygen inside the aggregates (Ploug 2001).

Average aggregate size and respiration rates are shown in Fig. 3. The average aggregate diameter was

Table 1. Protozoa associated with estuarine aggregates during this experiment

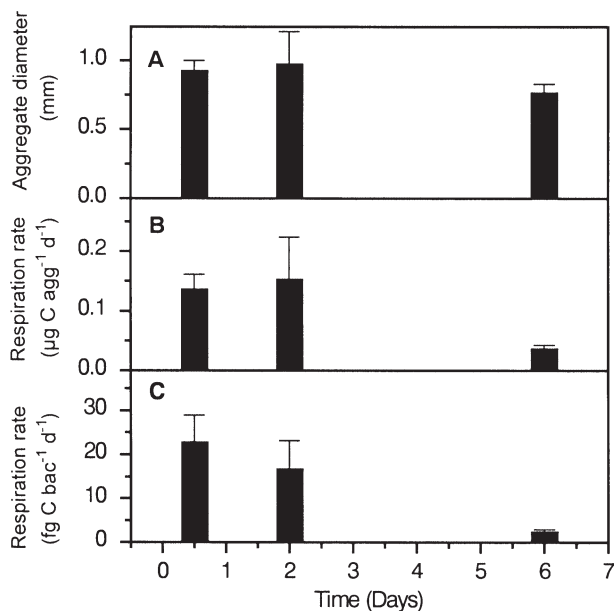| Group of organisms	Species
Flagellates	*Antophysa vegetans*
	Bodo saltans
	Bodo sp.
	Cryptomonas sp. (25 μm)
	Goniomons sp.
	Katablepharis sp.
	Ryhnchomonas nasuta
	Choanoflagellata
Ciliophora	*Lacrymaria olor*
	Mesodinium sp.
	Strombidium sp.
Rhizopoda, Amoebida	Species in the 30 μm size class
Actinopoda, Heliozoa	Species in the 30 μm size class

Fig. 3. (A) Aggregate size, (B) respiration rate agg^{-1} and (C) average bacteria-specific respiration rates. Bacteria were counted in the same aggregate after respiration measurements. Bars represent the average value measured in individual aggregates with the standard error (n = 10 to 14)

Fig. 5. Aggregate abundance (agg l^{-1}) and aggregate size (µm) in the Elbe Estuary near Hamburg-Blankenese (Elbe 634 km downstream from the German-Czech border)

slightly lower on Day 6 (0.77 mm) than on the day the aggregates had been sampled in the field (0.91 mm) and on Day 2 (0.95 mm). The average respiration rate agg^{-1} was 0.134 and 0.154 µg C agg^{-1} d^{-1} on the day of sampling and Day 2, respectively, but significantly lower on Day 6, when it was 0.038 µg C agg^{-1} d^{-1}. Respiration by protozoa was ignored due to their apparently low grazing rates on bacteria. Hence, the bacterial respiration rate decreased nearly 10-fold throughout the experiment from 22 fg C bacteria^{-1} d^{-1} on the day of sampling to 2.6 fg C bacteria^{-1} d^{-1} on

Day 6, when bacterial growth presumably was substrate limited. The C:N (w:w) ratio was 7.0 at the end of the experiment, and the average POC content was then 0.37 ± 0.07 µg C agg^{-1}.

Bacteria and *Archaea* accounted for 83 and 2%, respectively, of the total DAPI counts on Day 6. The α-subclass of proteobacteria accounted for 8.7 to 10.1% of the DAPI counts, while β- and γ-proteobacteria accounted for 26 to 35.4% and 5.8 to 7.8%, respectively, of the DAPI counts. Members of the CFB reached values between 22.4 and 26.0% of total DAPI counts. The average distribution of phylogenetic groups in percentage of DAPI counts is shown in Fig. 4.

Respiration on aggregates in the Elbe Estuary

Aggregates collected in the upper meter of the Elbe were enumerated in size classes between 5 and 1500 µm. Nanoparticles with a maximum abundance of 5 µm large particles dominated the size spectrum (Fig. 5). Aggregates with sizes ranging between 400 and 1500 µm reached a total concentration of 1300 agg l^{-1} of which 500 agg l^{-1} belonged to the 1500 µm size class. The microbial biomass and respiration rates measured on the same aggregates in the 500 to 3000 µm size range are shown as a function of aggregate size in Fig. 5. The bacterial and protozoan biomass were calculated from their size and abundance on single aggregates assuming a carbon content of 100 fg C (µm^3 biovolume)$^{-1}$ and an average specific biovolume of 0.15 µm^3 bacteria^{-1} for attached bacteria (Alldredge & Gotschalk 1990). Protozoa contributed only 4 to 13% to the total microbial biomass (data not shown). The microbial biomass on aggregates was highly variable and showed a relatively poor correla-
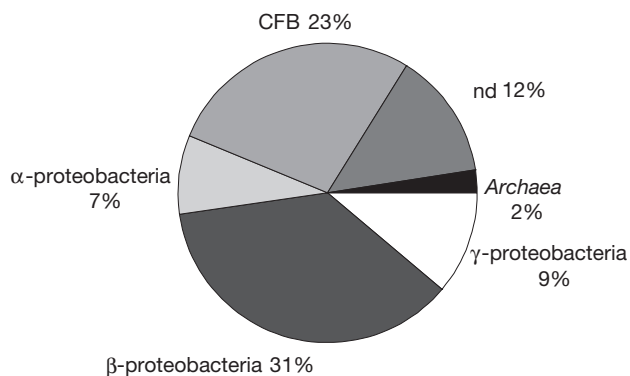

Fig. 4. Distribution of detected bacteria as a percentage of DAPI-stained bacteria in different clusters (α-, β-, γ-proteobacteria, *Cytophaga-Flavobacterium* cluster [CFB], *Archaea*) at the end of the experiment. nd: not detected
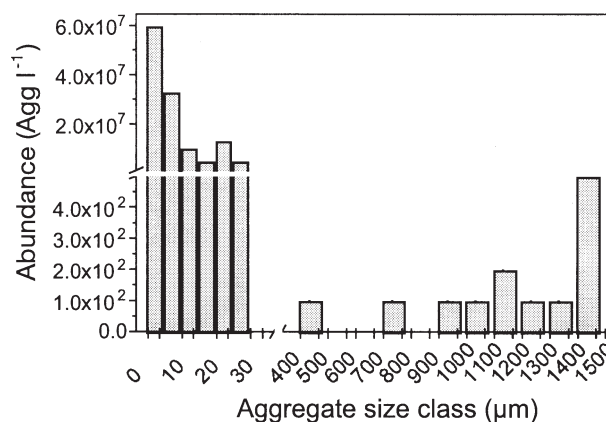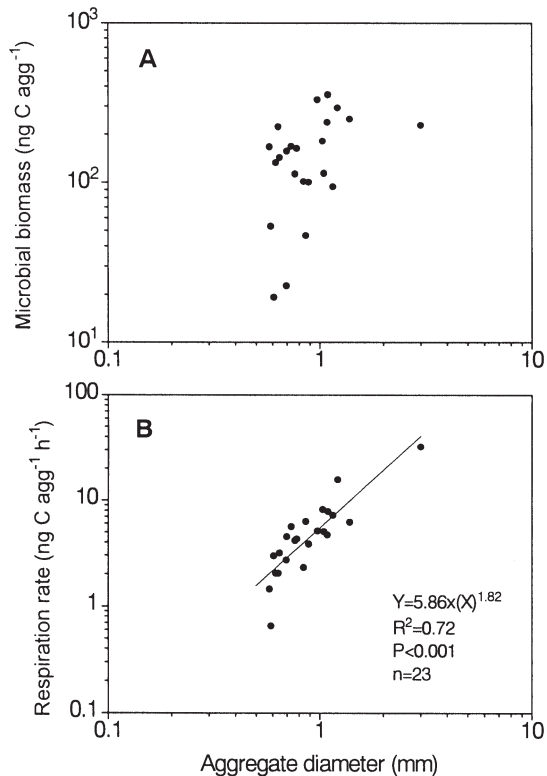
Fig. 6. (A) Attached microbial biovolume as a function of aggregate size. (B) Respiration rate as a function of aggregate size. The microbial community was enumerated after respiration measurements on the same aggregates

tion with aggregate size (Fig. 6A). The community respiration rate ranged between 0.64 and 32 ng C agg^{-1} h^{-1}, and it was significantly related to aggregate size ($R^2 = 0.72$, p < 0.001; Fig. 6B). The size-specific respiration rate was described by $R = 5.86 \times d^{1.82}$, where R is the respiration rate in ng C agg^{-1} h^{-1} and the diameter (d) is measured in mm. The total respiration on aggregates larger than 400 µm was 11.8 µg C l^{-1} h^{-1} as determined from the aggregate abundance and size distribution, and the size-specific respiration rates.

DISCUSSION

Microbial colonization and respiration on aggregates

Previous studies have shown that up to 80% of all bacteria, amoebas and ciliates occurred on aggregates in the Elbe Estuary, 50 to 75% of total rotifers were dwelling on aggregates, and only <10% of all flagellates occurred on aggregates (Zimmermann & Kausch 1996, Zimmermann 1997, Zimmermann-Timm et al. 1998). The aggregate-attached species observed in the present study and other studies are more common in

benthic habitats than in the surrounding water (Artolozaga et al. 1997, Zimmermann-Timm et al. 1998, Wörner et al. 2000). Grazing rates on attached bacteria appeared low. Choanoflagellata are filtrators and may, therefore, preferentially graze on free-living bacteria while being attached to particles.

Low protozoan grazing rates on aggregate-attached bacteria have also been observed in other studies (Jürgens & Güde 1994, Artolozaga et al. 2002). Considering the physical structure of aggregates in which bacteria are embedded in mucus, bacterial attachment to particles has been hypothesized to be a spatial refuge to decrease grazing rates by protozoa (Jürgens & Güde 1994). The cell-specific grazing rates by protozoa estimated from bacterial densities (Artolozaga et al. 2002) were 2-fold higher than those estimated on larger diatom aggregates colonized by natural populations of bacteria and protozoa. Total grazing rate on the bacterial population of those aggregates, however, was higher because the number of protozoa relative to that of bacteria were higher (Ploug & Grossart 2000).

The estimated biomass of bacteria and protozoa on aggregates of the present study showed a relatively poor correlation to aggregate size as compared to the respiration rate. The biovolume and carbon content may vary largely between different groups of microorganisms and protozoa. Earlier studies have shown that bacterial and flagellate abundance on aggregates is better correlated to aggregate diameter than to surface area (Zimmermann 1997). Respiration was measured before bacterial numbers and protozoa were determined on the same aggregates in the present study. The respiration rate was not proportional to bacterial number, protozoan biovolume or biomass of the total attached microbial community of the same aggregates (data not shown). Therefore, it seems that some of the bacteria on aggregates were dormant. Respiration on other riverine aggregates has been shown to correlate with bacterial production rather than with bacterial number measured on the same aggregate (Grossart & Ploug 2000).

Aggregates are fractal, and their porosity, therefore, increases with increasing aggregate size (Logan & Wilkinson 1990, Chen & Eisma 1995). Respiration rates in the present study showed a similar dependency on aggregate size to those on other riverine aggregates and diatom aggregates formed in roller tanks. In those aggregates and in marine snow, respiration was proportional to the aggregate content of POC and particulate organic nitrogen (PON) rather than to apparent aggregate volume including pore water content. Bacterial production and respiration on aggregates may, therefore, be linked to POC and PON content through enzymatic hydrolysis of the POM within the aggregates (Ploug et al. 1999, Grossart & Ploug 2000, Ploug & Grossart 2000).

Combined measurements of bacterial production and respiration in riverine aggregates have shown that the bacterial net growth efficiency ranges between 0.05 and 0.60. In 7 d old aggregates, it was proportional to the growth rate, which indicates substrate limitation of bacterial growth (Grossart & Ploug 2000). The very high bacterial abundance on Elbe aggregates, and decrease in cell-specific respiration rates over time suggest that bacterial growth also became substrate limited during our experiment. The C:N (w:w) ratio was 7, and the organic content of aggregates was low at the end of the experiment. The bacterial community was dominated by members of the β-proteobacteria and the CFB cluster at the end of the study similar to the bacterial communities found in old riverine aggregates and lake snow (Grossart & Ploug 2000, Schweitzer et al. 2001).

Oxygen deficiency and carbon turnover in estuaries

Oxygen deficiency often occurs in the water column of estuaries, e.g. the Schelde Estuary (Boderie et al. 1993), the Loire (Thouvenin 1992), the Chanjiang Estuary (Tian et al. 1993), Chesapeake Bay (Officer et al. 1984) and the Elbe Estuary (Kerner et al. 1995). The O_2 uptake in aggregates becomes transport limited when the oxygen demand is higher than the potential diffusive O_2 flux to the aggregate, and the O_2 concentration at the surface and within aggregates is close to the half-saturation constant for oxygen uptake, the Michaelis-Menter constant k_m. Heterotrophic bacteria, ciliates and amoebas have a high affinity for O_2 and their k_m for oxygen respiration is 0.1 to 3 µM O_2 (Focht & Verstraete 1977, Fenchel & Finlay 1995). The critical ambient O_2 concentration at which the center of the aggregate interior becomes anoxic can, therefore, be estimated from the maximum difference in oxygen concentration between the aggregate interior and the surrounding water, which was ~16 µM. It was significantly lower than the ambient O_2 concentration (~90 µM) at which glycoaldehyde from anaerobic microbial degradation processes have been demonstrated in the water column of the Elbe during late spring and summer (Kerner et al. 1995). Suspended aggregates and the sediment are likely the major sites where anaerobic processes occur at low ambient O_2 concentrations in the water column in the Elbe Estuary (Kerner & Edelkraut 1995). Aggregates of the present study contained glycoaldehyde. It is, however, not possible to know whether the aggregates previously had been in an anoxic transition state (Ploug et al. 1997) because the chemical composition of aggregates also is dependent on sedimentation and contact with anoxic conditions in the sediment and resuspension processes

(Kerner & Edelkraut 1995, Kerner et al. 1995).

Respiration on Elbe aggregates may vary many-fold depending on their abundance, the quality of organic matter, season, tide and human activities (Zimmermann 1997). Estuarine aggregates presumably undergo several sedimentation and re-suspension events. Total respiration on aggregates larger than 400 µm was 11.8 µg C l^{-1} h^{-1} equal to 0.98 mmol O_2 m^{-2} h^{-1} in the upper meter of the water column. We did not measure the respiration in the surrounding water. Bacterial production on aggregates, however, accounted for 66 to 88% of total bacterial production (free-living and attached bacteria) in the upper meter of the water column, of which the bacterial production of free-living bacteria ranged between 0.1 to 0.4 µg C l^{-1} h^{-1} (Schweitzer & Simon unpubl.). Assuming a bacterial net growth efficiency of 0.16 for free-living bacteria (Roland & Cole 1999), their respiration would account for 4 to 14% of total respiration in the upper meter of the water column. Hence, respiration on these relatively small but highly abundant aggregates is highly significant for total oxygen consumption and aerobic carbon turnover in the pelagic environment of the Elbe.

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