

Bacterial production and growth efficiencies: Direct measurements on riverine aggregates

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Abstract

Heterotrophic bacteria transform organic matter by respiration and production of new biomass. Because there are only a limited number of studies on the respiration of bacteria attached to particulate organic matter, their role in the carbon cycle of aquatic systems is not well known. In this study, we combine radiotracer with microsensor techniques to measure bacterial production and respiration rates on the same aggregate and to directly determine the growth efficiency of the microbial community attached to aggregates. Aggregates of defined age were formed after incubation of water samples of the river Weser, Northern Germany, in roller tanks and their bacterial community was analyzed by in situ hybridization. The growth efficiency was 0.45 ± 0.04 (SE) on 1–3-d-old aggregates, and it was independent of the growth rate (μ). There was no correlation between respiration and the particulate organic carbon (POC) or particulate organic nitrogen (PON) content of the same aggregate. Bacterial growth efficiencies on aggregates decreased after 5 d of incubation, as bacterial production decreased and respiration increased. On 7- and 14-d-old aggregates, the growth efficiency was 0.23 ± 0.06 and 0.04 ± 0.01 , respectively, and proportional to μ . The bacterial production was thus apparently substrate limited. Respiration was then correlated with both POC and PON content of the same aggregates. The changes in bacterial production and respiration occurred with concurrent changes in the bacterial community. The percentage of members of the α - and β -subclass of *Proteobacteria* decreased from 13% and 33.7% to 2.6% and 9.0%, respectively, whereas those of the γ -subclass of filamentous *Proteobacteria* and *Cytophaga* increased from 31.9% to 50.4% and from 8.5% to 24.9%, respectively, during the 14 d of incubation. These results demonstrate that bacterial production and respiration on aggregates are dependent on the bacterial community and the substrate composition of aggregate. High growth efficiencies of aggregate-associated bacteria, especially during the first days of colonization, suggest that aggregates are spots of high bacterial growth where a rapid and efficient transfer of organic matter into bacterial biomass takes place.

Macroscopic organic aggregates larger than 0.5 mm (marine snow) and 0.3 mm in diameter (lake snow) are known to be preferential sites of microbial colonization in aquatic systems (Shanks and Trent 1980; Caron et al. 1982; Alldredge and Silver 1988; Grossart and Simon 1998a). However, the carbon demand of attached bacteria both in marine and limnetic systems, estimated by their production rates, is often very small, leading to unrealistically long turnover times of the aggregate-associated organic carbon of months

to years (Karl et al. 1988; Simon et al. 1990; Smith et al. 1992; Grossart 1995). Since the actual carbon turnover by the bacterial community on aggregates further depends on their respiration rates (Del Giorgio and Cole 1998) and their ectoenzymatic hydrolysis (Hoppe et al. 1988; Smith et al. 1992), it is assumed that bacterial production measurements alone are insufficient to calculate the carbon turnover by attached bacteria.

Particle dissolution via bacterial hydrolysis of the organic carbon and particulate combined amino acids (PCAA) on aggregates has been characterized as a fast and efficient decomposition process during sinking (Smith et al. 1992; Grossart and Simon 1998a). Moreover, enzymatic hydrolysis and hydrolysate uptake by bacteria seem to be only loosely coupled on aggregates, as indicated by a net release of amino acids into the surrounding water and the relatively low bacterial production rates as compared with high enzymatic hydrolysis (Smith et al. 1992; Grossart and Simon 1998a,b). Thus, a large fraction of the particulate organic matter (POM) in aquatic systems serves to supply free-living bacteria with dissolved organic matter and nutrients (Cho and Azam 1988; Smith et al. 1992; Grossart and Simon 1998b). This notion is true only when one assumes that measured rates of bacterial production are valid and not substantially underestimated because of experimental errors.

Bacterial production rates on aggregates may have been

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Acknowledgments

Microelectrodes were constructed by Gaby Eickert, Anja Eggers, and Vera Hübner at the Max Planck Institute for Marine Microbiology in Bremen. Thanks are due to Thomas Richter for software development to determine the surface area of ellipsoids and Petra Merkl for POC and PON analysis. Statistical analysis were conducted by Kirsten Pohlmann and is gratefully acknowledged. Our study was supported by the Max Planck Society (Germany), by the University of Oldenburg (Germany), and by the Carlsberg Foundation (J. 970213/20-886 to HP). We thank Bo Barker Jørgensen and Meinhard Simon for numerous discussions and many helpful comments, and two anonymous reviewers for commenting on the manuscript.

underestimated by pooling several aggregates in one sample with a few milliliters of sterile, filtered surrounding water in order to limit the variability among individual aggregates and thus to obtain more representative measurements (Simon et al. 1990; Grossart and Simon 1993, 1998a). Pooling aggregates significantly influences the diffusion field during incubation and alters the measured bacterial production rates, as compared with more natural conditions (Ploug and Grossart 1999). By using a vertical flow system in which aggregates are stabilized in suspension by an upward directed flow, it has been shown that diffusion of oxygen from the surrounding water into sinking aggregates is greatly facilitated (Ploug and Jørgensen 1999). Since the flow past the sinking aggregates influences growth conditions of attached bacteria, the low rates of bacterial production measured by traditional methods are partly the result of the incubation method used (Ploug and Grossart 1999).

Turnover times of carbon on macroscopic organic aggregates calculated on the basis of release of dissolved organic matter (Smith et al. 1992; Grossart and Simon 1998a) and respiration (Ploug et al. 1999) are much shorter than previously estimated by bacterial production. High respiration but low bacterial production rates on aggregates suggest a very low growth yield of attached bacteria. However, the actual growth efficiency of attached bacteria is still unknown, since respiration and bacterial production have not been studied simultaneously on the same aggregate.

The combination of radiotracer with microsensor techniques allowed us for the first time to analyze bacterial production rates and respiration rates on the same aggregate and thus to determine directly the growth efficiency of the microbial community attached to aggregates. We incubated natural water samples in rolling tanks to form aggregates of well-defined age and composition to study changes in bacterial growth efficiency in relation to changes in bacterial community during aging of the aggregates. Furthermore, community respiration, bacterial production, and enumeration and identification of attached bacteria were analyzed in relation to the particulate organic carbon (POC) and particulate organic nitrogen (PON) content of the aggregates, which in turn allowed us to directly calculate POC turnover times.

Materials and methods

Sampling and aggregate formation—Water samples were collected in a Van Dorn bottle from the surface of the river Weser at Sta. Etelsen, ca. 20 km southeast of Bremen, Germany. The river Weser originates by the confluence of the rivers Werra and Fulda and has a total length of 432 km. The annual mean drain at the sampling station was $\sim 250 \text{ m}^3 \text{ s}^{-1}$ in 1997, and the salinity was $<1.0\text{‰}$. About 1 h after sampling, the samples were incubated in 1.4-liter Plexiglas cylinders rotating at 2.5 rpm at in situ temperature (22°C) and in darkness (Shanks and Edmondson 1989). Aggregates $\geq 1 \text{ mm}$ in diameter formed within 2 h of incubation. In total, we conducted three experiments in which we measured respiration as well as bacterial production on single aggregates at a defined time after aggregate formation (Table 1).

Table 1. Approaches for respiration and bacterial production measurements.

Experiment	Date of start	Measurements (day after aggregate formation)
1	9 Jul 1998	5 and 14
2	15 Jul 1998	1, 5, and 7
3	20 Jul 1998	1, 3, and 5

Respiration measurements—Oxygen gradients were directly measured in the diffusive boundary layer (DBL) of individual aggregates that were kept in suspension above a net by an upward-directed flow that opposed their sinking velocities (Ploug and Jørgensen 1999). The position of the aggregate surface was determined visually by advancing a slender Clark-type microelectrode (Revsbech 1989) toward the aggregate until it touched the upper surface, as observed under a dissecting microscope. The oxygen gradient was measured with a spatial resolution of $50\text{-}\mu\text{m}$ step intervals in the DBL at steady state in darkness. Using a dissecting microscope with a calibrated ocular micrometer, the dimensions of every aggregate were directly measured in the flow system. The flow system was filled with 10 liters of surface water filtered through microfiber glass filters (GF/C, Whatman) to avoid smaller particles being collected in the net of the flow system. Previous measurements showed that filtering water did not influence the respiration rates on the aggregates. The oxygen measurements were done at in situ temperature (22°C). The oxygen microelectrode had a $4\text{-}\mu\text{m}$ -wide sensing tip, a 90% response time of 0.2 s, and a stirring sensitivity of $<0.3\%$. It was calibrated in air-saturated and N_2 -flushed water. The electrode signal was measured by a picoamperemeter connected to a strip chart recorder. Immediately after oxygen measurements, aggregates were collected for bacterial production measurements (*see below*).

Calculation of respiration rates—The respiration rates of the whole aggregate community in the dark determine 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. The respiration rates in the aggregates were accordingly calculated from the measured oxygen fluxes through the DBL (Ploug et al. 1997). However, the aggregate surface area was calculated for ellipsoids as described by Ploug et al. (1999). Respiration rates were converted to carbon equivalents by using a conversion factor of 1.2 mol O_2 to 1 mol carbon. The diffusion coefficient for oxygen in water at salinities $<1\text{‰}$ and at 22°C is $2.24 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Broecker and Peng 1974).

Bacterial production rates—Bacterial production rates were measured directly after respiration had been determined on the same aggregate using the microcentrifugation method (Smith and Azam 1992). Incorporation of [^3H] thymidine (^3H -TdR; Fuhrman and Azam 1980) and [^{14}C] leucine (^{14}C -Leu; Kirchman et al. 1985; Simon and Azam 1989) into the ice-cold trichloroacetic acid precipitate was measured with

a dual-label approach (Chin-Leo and Kirchman 1988). ^3H -TdR (75 Ci mmol $^{-1}$) and ^{14}C -Leu (312 mCi mmol $^{-1}$, both from Amersham) were added to samples and to a formalin-killed control and incubated at in situ temperature for 1 h in the dark. Single aggregates were incubated in test tubes filled with 5 ml of sterile, filtered water and kept in suspension by rotating the test tube vertically at 2.5 rpm on a roller table. Final concentrations of the radiotracers were 120 nM, which saturated the uptake systems of both tracers (Ploug and Grossart 1999). Bacterial cell multiplication was calculated from ^3H -TdR incubation, assuming an isotope dilution factor of four and a conversion factor of 2×10^{18} cells mol $^{-1}$ ^3H -TdR (Simon 1990). Bacterial carbon production (BCP) by attached bacteria was calculated from ^{14}C -Leu incorporation assuming an intracellular isotope dilution factor of two (Simon and Azam 1989). The minimum bacterial growth efficiency (BGE) was determined from [BCP/(BCP + community respiration)] measured on the same aggregate.

Bacterial enumeration and in situ hybridization—Bacteria on aggregates were counted by use of epifluorescence microscopy after 4',6-diamidino-2-phenylindole (DAPI staining) (Porter and Feig 1980). To remove attached bacteria, single aggregates were treated with ultrasonication in 2 mM Na-pyrophosphate (Velji and Albright 1986) prior to filtration onto 0.2- μm Nuclepore membranes.

Twenty-microliter aliquots of sonicated aggregates were pipetted onto gelatin-coated Teflon microslides (P. Marienfeld KG) and dried at 46°C. The samples were then fixed in 40 μl of fresh paraformaldehyde (4%) for 4 h at 4°C. Percentages of *Archaea* and *Bacteria*, members of the α -, β -, and γ -subclass of *Proteobacteria*, of the *Cytophaga*-flexibacterium bacteroides cluster (*Cytophaga*) and of sulfate-reducing bacteria were examined by in situ hybridization with 16S rRNA targeted fluorescent oligonucleotide probes according to Weiss et al. (1996). Exact sequences of oligonucleotide probes to detect *Archaea*, *Bacteria*, α -, β -, and γ -*Proteobacteria*, as well as *Cytophaga* are given by Amann et al. (1995), whereas those of sulfate-reducing bacteria are given by Amann et al. (1990; SRB 385) and Rabus et al. (1996; SRB dB).

Carbon, hydrogen, nitrogen (CHN) analysis—Particulate organic carbon (POC) and nitrogen (PON) content of single aggregates used for respiration measurements were determined in a carbon, nitrogen, sulfur (CNS) analyzer (Perkin Elmer). The aggregates were transferred to preweighed tin cups (Perkin Elmer), dried at 110°C for 1 h, and weighed on a microbalance with a closed weighing chamber for accurate dry weight determination. All samples were fumed overnight with HCl (20% final concentration) to remove the inorganic carbon. The amount of organic carbon or nitrogen per aggregate was calculated from the dry weight, and the percentage of carbon or nitrogen was measured by CNS analysis.

Statistics—To test for significance of our results, we applied multiple analysis of variance because the experimental design included multiple factors. Calculations were done by using the program JMP (Version 3.1.5, SAS Institute Inc.).

Results

Aggregate composition and bacterial colonization—The aggregates contained very few phytoplankton but high amounts of detritus and resuspended sediment material. Bacterial numbers on aggregates varied between 1.0×10^6 and 1.5×10^6 and did not change significantly during the incubation time. However, in situ hybridization with 16 rRNA-targeted fluorescent oligonucleotide probes revealed significant changes in bacterial community structure during incubation, especially after 5 d (Fig. 1). The percentage of members of the α - and β -subclasses of *Proteobacteria* decreased from 13% to 2.6% and from 33.7% to 9.0%, respectively, whereas those of the γ -subclass of filamentous *Proteobacteria* and *Cytophaga* increased from 31.9% to 50.4% and from 8.5% to 24.9%, respectively, over time. The very low percentages of *Archaea* and sulfate-reducing bacteria at each sampling indicate high resuspension of sediments and demonstrate their low contribution to the bacterial community on the always oxic aggregates.

Bacterial production and respiration on aggregates—The growth efficiency (GE) was 0.45 ± 0.04 (SE) during the first 3 d after aggregate formation. Concurrent with the changes in bacterial community, GE decreased significantly during the fifth day of incubation (Fig. 2). Bacterial production (BP) and community respiration (R) ranged between 1.63 and 23 ng C agg $^{-1}$ h $^{-1}$ and 2.33 and 52 ng C agg $^{-1}$ h $^{-1}$, respectively, on 1–5-d-old aggregates with high GE (Fig. 3A,B). Both parameters were dependent on aggregate size. On 5–14-d-old aggregates with low GE, the bacterial production was up to two orders of magnitude lower relative to the younger aggregates with high GE. The BP ranged between 0.14 and 5.98 ng C agg $^{-1}$ h $^{-1}$ and was independent on aggregate size (Fig. 3C,D). In contrast, the respiration rates remained dependent on aggregate size and were up to twofold higher on 5–14-d-old aggregates as compared with those on 1–5-d-old aggregates of similar sizes. The decrease in GE was thus due to a decrease in bacterial production and an increase in community respiration as the aggregates aged.

Although the GE was variable in individual aggregates, BP and R were correlated in 1–5-d-old aggregates with high GE: $R = 1.57 \times \text{BP}^{0.86}$; $r^2 = 0.58$ ($P < 0.05$; Fig. 4A). The average GE was 0.45 ± 0.04 (SE). The GE was independent of growth rates (μ) as measured by ^3H -TdR, indicating relatively uniform growth conditions of the bacteria and indicating that they were not substrate limited (Fig. 4B). On 7- and 14-d-old aggregates, the respiration was independent and much higher than the BP (Fig. 4C,E). The GE, however, was then linearly dependent on μ , which indicates that the bacterial production was substrate limited (Fig. 4D,F). Interestingly, bacteria showed higher growth rates but lower growth efficiencies on day 14 compared with day 7, which coincided with the high relative increase of filamentous γ -*Proteobacteria* (Fig. 1).

Respiration and POC/PON content of aggregates—To examine factors controlling the bacterial activities, we measured respiration on the same aggregates as PON and POC content. POC and PON comprised 6.0% and 0.66%, respec-

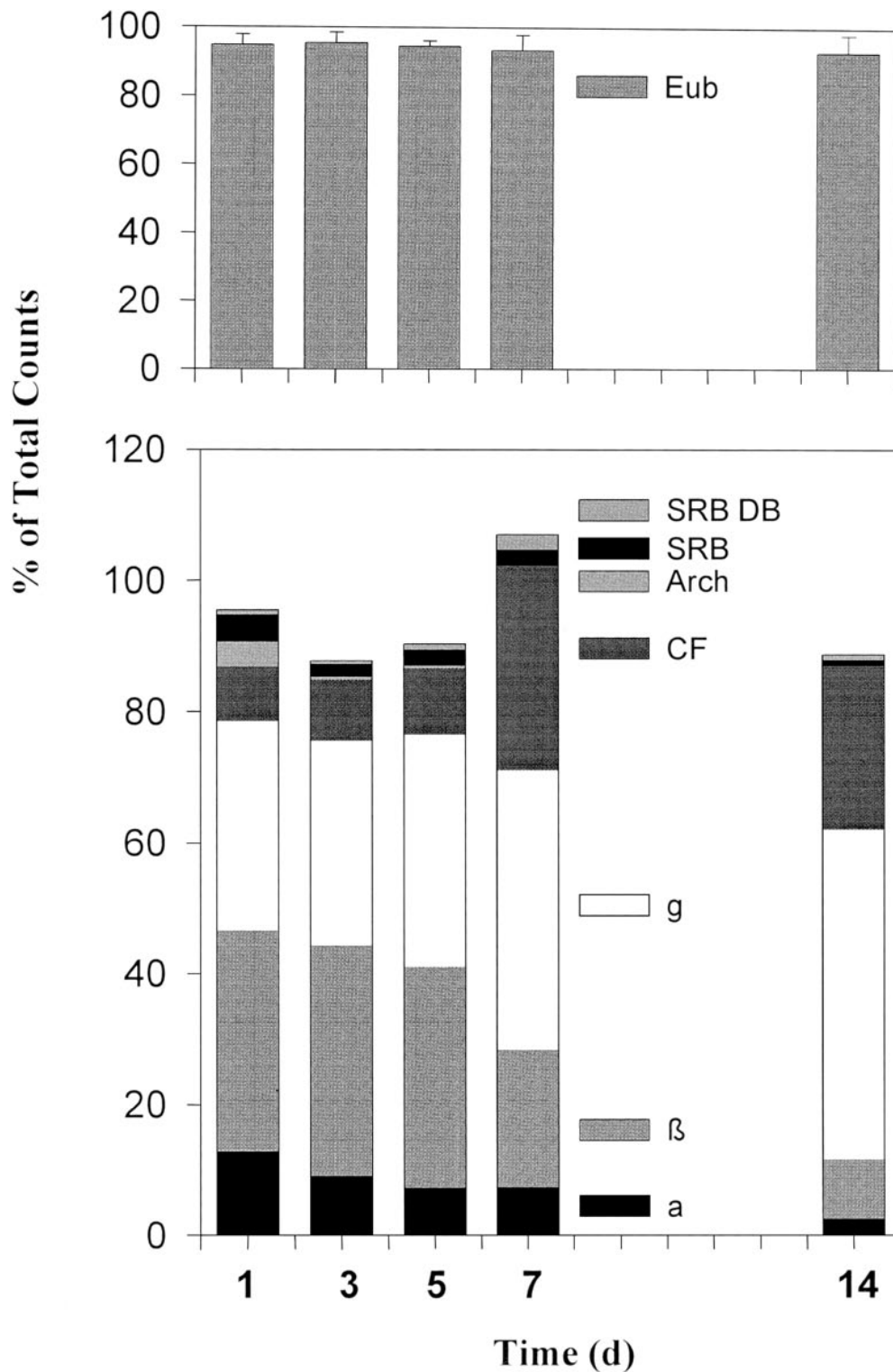


Fig. 1. Bacterial community structure on laboratory-made aggregates of different age. Error bars give the standard deviation of three independent measurements for bacteria (Eub). Standard deviations are for α -, β -, and γ -subclasses of *Proteobacteria* (α , β , and γ , respectively). Cytophaga (CF), Archaea (Arch), and sulfate-reducing bacteria (SRB and SRB DB) are not shown but were usually less than 10%.

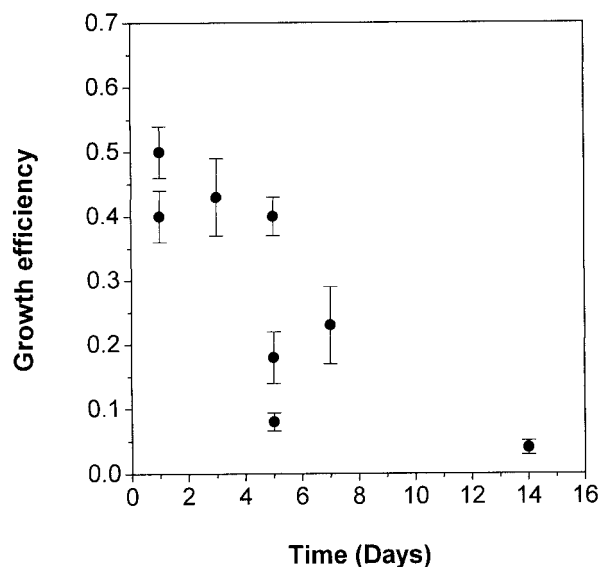


Fig. 2. The growth efficiencies (GE) as calculated from bacterial production and respiration measured on the same aggregate. The error bar gives the standard error of 5 to 20 independent measurements.

tively, of aggregate dry weight. The C:N molar ratio was 13.3 ± 8.68 (SD) on 1–5-d-old aggregates and 11.3 ± 3.14 on 7–14-d-old aggregates. However, POC and PON content were not significantly dependent on incubation time (F ratio = 0.2172, Prob > F = 0.8968, df = 2; and F ratio = 0.2943, Prob > 0.7486, df = 2, respectively). Respiration was only weakly correlated to POC and PON content on 1–5-d-old aggregates (Fig. 5A,B) when bacterial production and respiration were correlated (Fig. 4A), and the GE was independent on the growth rate (Fig. 4B). However, on 7- and 14-d-old aggregates, respiration rates were linear and were dependent on PON and POC content (Fig. 5C,D) when BP presumably was substrate limited, since the growth efficiencies were low and linearly dependent on the growth rate (Fig. 4D,F). The interceptions with the y-axes were $12 \mu\text{g C agg}^{-1}$ and $0.93 \mu\text{g N agg}^{-1}$, suggesting that the aggregates mostly consisted of refractory organic matter, which is also supported by the high C:N ratio. The high amount of refractory organic matter was also reflected in high POC turnover time due to respiration (20–280 d), which decreased with aggregate size (Fig. 6). The average POC turnover time due to respiration was 97 ± 62 d.

Discussion

To our knowledge, this study is the first in which the relationships between bacterial production, respiration, and POC and PON content have been directly determined on single suspended macroscopic organic aggregates. The simultaneous measurement of respiration and bacterial production on a single aggregate has numerous advantages compared with more classic methods. For example, the high sensitivity of the methods used allowed measurement on single aggregates without any pooling or filtration artifacts. In addition, short incubation times and the simulation of the

natural physical environment ensured more realistic conditions for reliable determination of bacterial production and respiration. Leucine incorporation rates measured in the present study were much higher than those previously measured on natural marine and limnetic aggregates with similar bacterial numbers. This we partly attribute to the fact that the aggregates were kept in suspension during the incubation (Ploug and Grossart 1999).

The combination of the tracer and microsensors techniques for determination of bacterial production and respiration, respectively, enabled us to directly estimate the growth efficiency of attached bacteria on individual aggregates. Our estimates of growth efficiencies represent minimum estimates of the bacterial growth efficiency, as the growth efficiencies were calculated from the BP and the community respiration. Flagellates grazing on the bacteria can account for 30% of the community respiration if the bacteria are grazed as fast as they grow and if the flagellates have a growth efficiency of 50% (Ploug and Grossart 1999). Increased abundance of grazing-resistant filamentous bacteria in planktonic bacterial communities and shifts in community structure are observed during increased grazing pressure by protozoans (Jürgens et al. 1999). The very high respiration rates but low bacterial production rates on 14-d-old aggregates, which were strongly dominated by filamentous γ -*Proteobacteria*, may be due to preferential grazing of smaller forms as well as high cell-specific respiration rates by the large bacteria and respiration due to protozoa.

Mean growth efficiencies of bacteria on 1–3-d-old aggregates of the present study were much higher than the estimated 9–17% for aggregate-associated bacteria during the breakdown of a diatom bloom (Smith et al. 1995), and the 17% measured for bacteria associated with lacustrine seston in Lake Kinneret (Parparov et al. 1998). However, Smith et al. (1995) did not measure respiration directly, whereas Parparov et al. (1998) measured respiration directly by the standard dark bottle method. Because of methodological limitations, Parparov et al. (1998) pooled high numbers of seston particles and aggregates and measured community respiration, including the algal dark respiration and respiration by protozoa. Thus, their calculated growth efficiencies are also minimum estimates.

Aggregates in the present study were relatively uniform, small in size, and low in phytoplankton content, in comparison with most marine and lake snow (Alldredge and Silver 1988; Herndl 1992; Smith et al. 1992; Alldredge 1998; Grossart and Simon 1998a). However, bacterial numbers were similar to those published for most marine and limnetic aggregates, and the respiration rates were similar to those measured on marine snow of similar sizes (Ploug et al. 1999). On the other hand, bacterial numbers on marine as well as limnetic aggregates were higher when formed in rolling tanks (Grossart and Simon unpubl. data) and may be due to the formation of more compact aggregates in roller tanks. This is indicated by the higher PON and POC content of aggregates of the present study compared with those of marine snow (Alldredge 1998). Although laboratory-made aggregates are compact, they provide a useful model to examine changes in activities and community structure of attached bacteria over time.

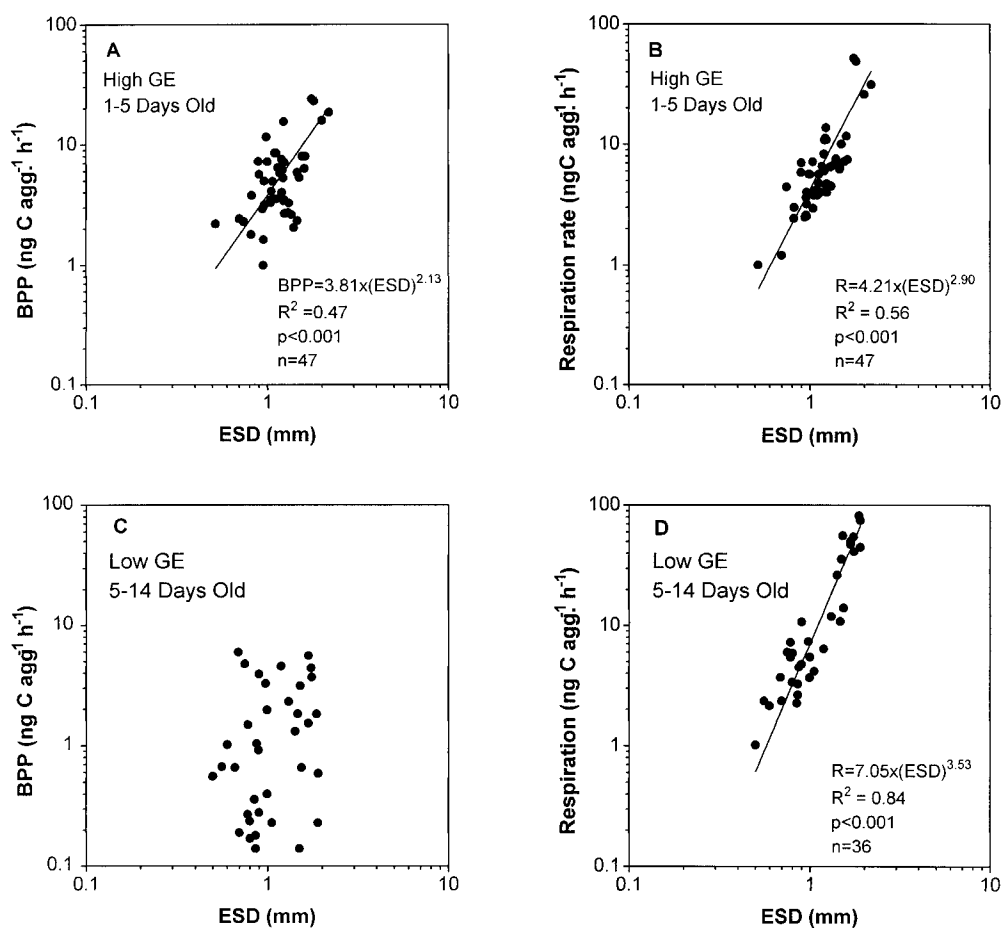


Fig. 3. Bacterial production and respiration as a function of equivalent spherical diameter (ESD) of the aggregate.

Bacterial growth efficiencies have been shown to vary between 8–39% for free-living bacteria when degrading high molecular DOC and 16–66% when degrading low molecular DOC (Amon and Benner 1996). The broad range of growth efficiencies is similar to those of the present study and may reflect the multitude of substrates utilized by aggregate-associated bacteria. In a recent review of bacterial growth efficiencies in natural aquatic systems, Del Giorgio and Cole (1998) give two models describing the positive relationships between bacterial respiration (BR) and production (BP) of free-living bacteria:

$$\begin{aligned} BR &= 3.70 \times BP^{0.41}, & r^2 &= 0.46 & \text{and} \\ BR &= 3.42 \times BP^{0.61}, & r^2 &= 0.46. \end{aligned} \quad (1)$$

According to these models, respiration is high relatively to bacterial production rates when these are low, but increases slowly relatively to increasing bacterial production. Bacterial growth efficiencies therefore increase with increasing bacterial production. However, the given equations do not hold true for aggregates in the present study that show a wide range of growth efficiencies. On 1–5-d-old aggregates, where the growth efficiencies were high, respiration and bacterial production were correlated as $R = 1.57 \times BP^{0.86}$ (r^2

$= 0.58$), which imply generally higher growth efficiencies also at low BP, and that BR increases faster in proportion to increasing BP than it does for free-living bacteria (cf. Eq. 1). Accordingly, R and BP shows a tight coupling for bacteria attached to newly formed aggregates. On 5–14-d-old aggregates, the respiration increased and was apparently independent of BP, which decreased. However, GE was then linear dependent on growth rate. There are two possible reasons: newly formed aggregates comprise a relatively uniform substrate quality for attached bacteria, which is processed quickly and efficiently by the attached bacteria compared with the variety of substrates available for free-living bacteria in different aquatic systems; and the physiological state of free-living bacteria in natural assemblages is highly variable, and only a minor fraction of bacteria is actively growing in less productive systems, whereas on aggregates, most of the bacteria can be actively growing (Allredge et al. 1986; Smith et al. 1995; Grossart and Simon 1998a).

Average bacterial growth rates on 1–3-d-old aggregates in the present study are in the same range as reported for free-living bacteria growing on organic carbon freshly excreted by phytoplankton (Del Giorgio and Cole 1998). However, aggregates in the present study were mostly composed of detritus. The high bacterial growth efficiencies during early

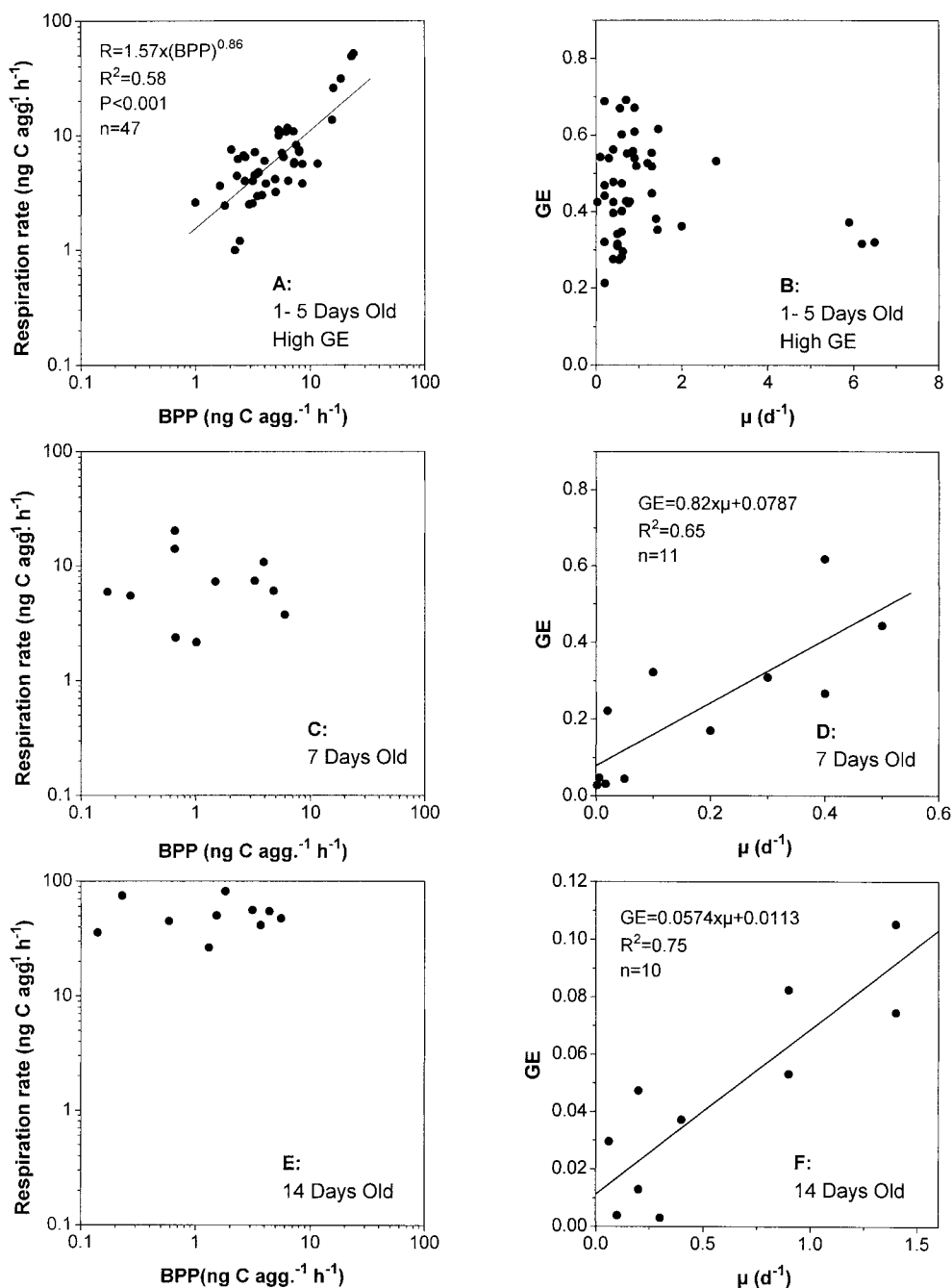


Fig. 4. Bacterial production (BPP) versus respiration in aggregates of different ages (left panel). Growth rate (μ) versus growth efficiency (GE) are shown in the right panel. Note the different scales in the right panel.

colonization on freshly formed aggregates are in good agreement with the high growth efficiencies found in estuaries with high autochthonous production and high amounts of particulate organic matter. On the other hand, increasing fractions of hard-to-degrade substrates such as lignin and humic substances of terrestrial origin may explain that bacterial growth efficiencies on aggregates constantly decreased over time in the present study.

The relatively high turnover times of organic carbon due to the respiration may be due to the high content but rela-

tively poor quality of the POC of these aggregates compared with natural marine snow of the same size (Allredge 1998). Macroscopic organic aggregates, e.g., marine and lake snow, are often composed of numerous organic compounds that lead to a very broad range of POC turnover times calculated either by respiration, bacterial production, or potential ectoenzyme activities (Allredge and Youngbluth 1985; Allredge et al. 1986; Smith et al. 1992; Grossart and Simon 1998a; Parparov et al. 1998; Ploug et al. 1999). In contrast, turnover times of particulate combined amino acids, such as

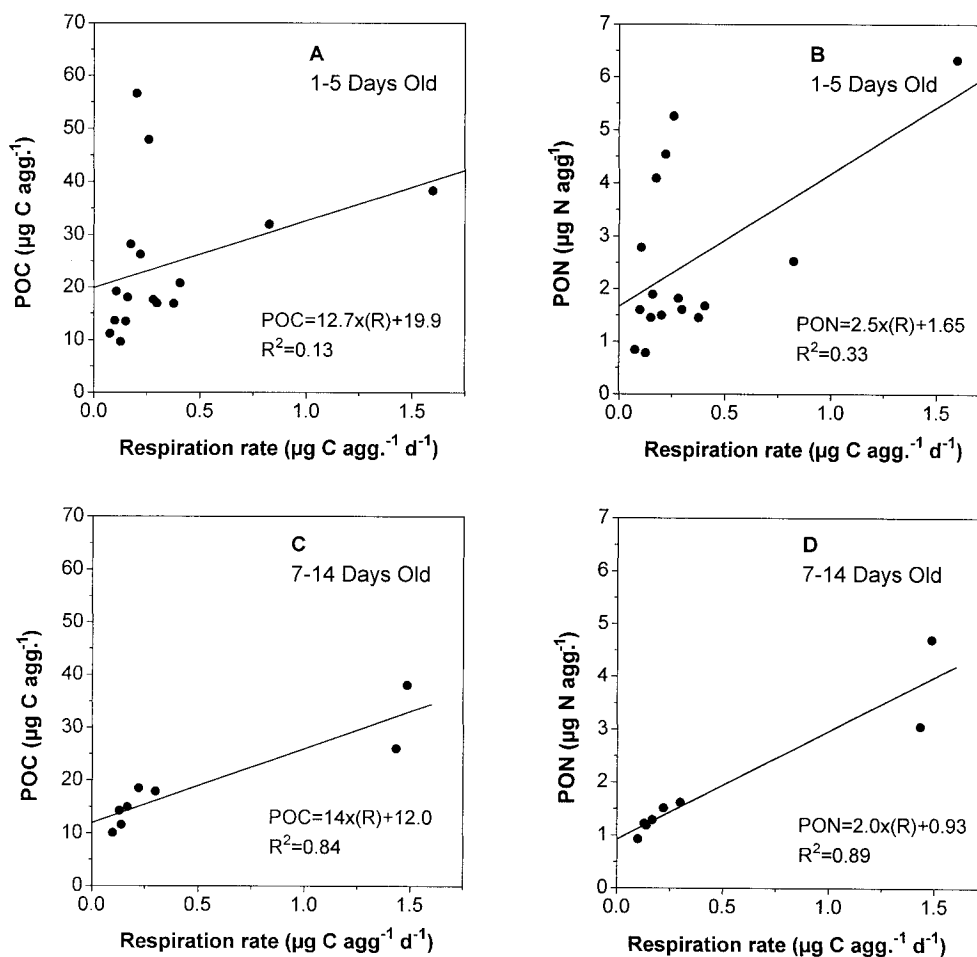


Fig. 5. Respiration versus POC and PON content measured on the same aggregates.

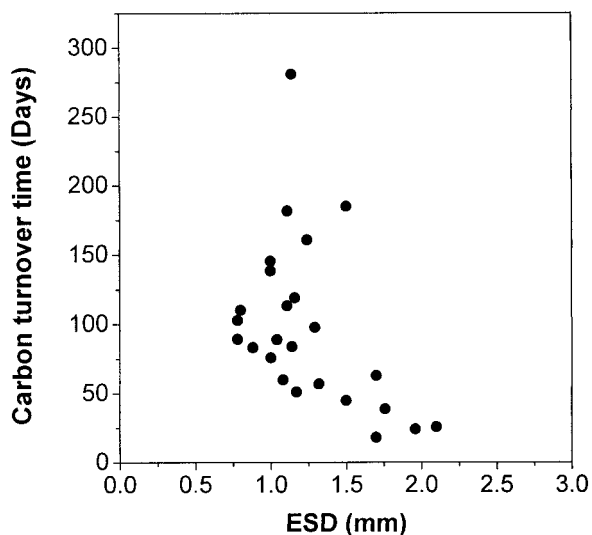


Fig. 6. Carbon turnover time calculated from the POC and respiration rates measured on the same aggregate.

proteins on aggregates, were significantly shorter (Smith et al. 1992; Grossart and Simon 1993, 1998a) and suggest that the rate and efficiency of bacterial degradation strongly depend on the quality of the organic matter present. High growth efficiencies in combination with high bacterial production on 1–3-d-old aggregates demonstrate that at least the less refractory fraction of the POC was rapidly and efficiently used by attached bacteria. Because of similar size dependency of POC content and respiration on marine snow aggregates (Ploug et al. 1999), respiration is correlated to POC, indicating a tight coupling between the amount of POC and respiration on aggregates. Further, direct studies of respiration and POC and PON measured on the same aggregate have shown that respiration rate is proportional to aggregate POC and PON when ectoenzymatic activities by the aggregate attached bacteria are high (Ploug and Grossart unpubl. data).

On 5–14-d-old aggregates, GE was proportional to the growth rate, and the respiration rate was linear dependent on the POC and PON content of the aggregates, which indicates substrate limitation in these aggregates. Total bacterial numbers remained more or less constant and did not reflect any changes in bacterial activities, but the community structure changed significantly, i.e., percentages of filamentous *γ-Proteobacteria* and *Cytophaga* increased, especially on day 7,

and percentages of α - as well as β -*Proteobacteria* decreased. Similar communities were also found on marine and limnetic aggregate studies (Schweitzer 1998; Ploug et al. 1999). *Cytophaga* are known to hydrolyze a variety of refractory compounds such as chitin and other polysaccharides, implying a high potential to degrade highly polymeric substrates by aggregate-associated bacteria (DeLong et al. 1993; Rath et al. 1998). The cost of producing exoenzymes may also lead to low growth efficiencies of the attached bacteria. Besides from grazing, changes in community structure and GE may thus be related to changes in substrate quality over time.

On feces of pelagic tunicates, bacterial populations develop rapidly during the first 24 h, and respiratory rate increases with the increase in bacteria (Pomeroy et al. 1984). Our findings of higher bacterial production and growth efficiencies on fresh aggregates compared with older ones are also in good agreement with recent studies, which have shown high bacterial production and low enzymatic activities on newly formed aggregates but decreased bacterial production and highly increased hydrolysis on aggregates >3 d old (Unanue et al. 1998). Increasing ectoenzyme activities such as β -glucosidase during the incubation of various types of aggregates have been measured frequently (Smith et al. 1995; Grossart and Simon 1998a; Unanue et al. 1998), and growth efficiencies and β -glucosidase activities of free-living bacteria in numerous aquatic systems have been shown to be negatively correlated (Del Giorgio and Cole 1998). Thus, high respiration rates in combination with high ectoenzymatic activities found on artificial as well as on natural marine snow (Smith et al. 1995; Ploug et al. 1999) may indicate lower substrate quality and thus lower bacterial growth efficiencies.

Since marine as well as limnetic aggregates are composed of more or less refractory POC compounds, an interesting question arises: does the presence of less refractory POC compounds, e.g., rich in organic N and P, facilitate the microbial degradation of the more refractory POC compounds? For example, DOC from extracellular release of phytoplankton leads to an accelerated loss of riverine DOC (Cifuentes and Eldridge 1998), implying a synergism between autochthonous inputs and the reactivity of allochthonous sources (Zweifel et al. 1995). Aggregates in the same size range as those of the present study occur in high numbers (100 agg L⁻¹) in the Elbe Estuary of northern Germany (Zimmermann and Kausch 1996). Bacterial numbers on aggregates of the present study were lower, but the respiration rates were comparable to those on natural riverine aggregates in the Elbe Estuary (Zimmermann and Ploug unpubl. data). When assuming 100 agg L⁻¹ and that the average BP and respiration rates measured in the present study are representative for riverine aggregates, 6.29 ng C agg⁻¹ h⁻¹ × 24 h d⁻¹ × 100 agg L⁻¹ = 15 μ g C L⁻¹ d⁻¹ is channeled through BP, and 8.59 ng C agg⁻¹ h⁻¹ × 24 h d⁻¹ × 100 agg L⁻¹ = 21 μ g C L⁻¹ d⁻¹ is respired in <5-d-old riverine aggregates. Whereas BP decreased, the respiration rates increased during the 2-week study, leading to even higher respiratory losses of organic matter. Aggregates in riverine systems are thus loci of highly efficient microbial degradation of organic matter with profound implications for overall flux of organic C and nutrients.

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Received: 19 April 1999

Accepted: 13 October 1999

Amended: 27 October 1999