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Free and Particle-associated Extracellular Enzyme Activity and Bacterial Production in the Lower Elbe Estuary, Germany

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A high abundance of particles and aggregates is a characteristic of estuaries and may represent important loci of increased bioproductivity and microbial activity. From a survey performed in the Elbe estuary, particle concentration (using a particle counter), bacterial biomass and abundance (AODC method), bacterial production rates (³H thymidine method) and the microbial extracellular enzyme activity of 11 enzymes (MUF and MCA method) were determined with particular respect to their relationship to particles and aggregates (size-fractionated). Although higher particle abundance was found compared to other sections of the river Elbe, neither relative nor absolute bacterial activity associated with particles and aggregates was enhanced. The activity of free dissolved microbial enzymes was found to dominate the breakdown of organic material. The productivity of free dispersed bacteria $\geq 0.2 \dots < 2 \mu\text{m}$ (2...69%) and bacteria attached to small particles $\geq 2 \dots < 10 \mu\text{m}$ (27...93%) considerably surpassed that of bacteria associated with particles $> 10 \mu\text{m}$ (0...40%). This indicates that the larger particles and aggregates do not always contribute substantially to the overall microbial activity in rivers and estuaries.

Freie und partikelassoziierte extrazelluläre Enzymaktivität und bakterielle Produktion im unteren Elbeästuar, Deutschland

Hohe Abundanzen von Partikeln und Aggregaten sind für Ästuarare charakteristisch, und ihre Textur und Oberflächen können wichtige Orte für eine gesteigerte Bioproductivität und mikrobielle Aktivität repräsentieren. Von einer im Elbeästuar durchgeführten Studie wurden Partikelanzahl und -größenverteilung (Partikelzähler), Abundanz und Biomasse von Bakterien (AODC-Methode), bakterielle Produktionsraten (³H Thymidin-Methode) und mikrobielle extrazelluläre Enzymaktivitäten (MUF- und MCA-Methode) mit spezieller Hinsicht auf ihre Beziehung zu Partikeln und Aggregaten (Größenfraktionierung) untersucht. Obwohl im Vergleich zu anderen Flussabschnitten der Elbe eine höhere Partikelabundanz vorgefunden wurde, konnte keine relativ oder – mit Ausnahme der meisten Peptidasen, Galactosidasen und der Phosphatase – absolut erhöhte bakterielle Aktivität, assoziiert mit Partikeln und Aggregaten, bestimmt werden. Für den Abbau des organischen Materials war hauptsächlich die Aktivität der freien gelösten mikrobiellen Enzyme verantwortlich. Die Produktivität der freien Bakterien ($\geq 0.2 \dots < 2 \mu\text{m}$, 2...69%) und der an kleine Partikel angehefteten Bakterien ($\geq 2 \dots < 10 \mu\text{m}$, 27...93%) übertraf deutlich die bakterielle Produktion an Partikeln $> 10 \mu\text{m}$ (0...40%). Damit konnte gezeigt werden, dass Partikel und Aggregate nicht zu allen Zeiten in Fließgewässern und Ästuaren substantiell zur mikrobiellen Gesamtaktivität beitragen.

Keywords: Bacterial Abundance, Bacterial Biomass, Microbial Self-purification Capacity, Microbial Activity, Suspended Particulate Matter, Aggregate

Schlagwörter: Bakterienabundanz, Bakterienbiomasse, Mikrobielles Selbstreinigungsvermögen, Mikrobielle Aktivität, Schwebstoff, Aggregat

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1 Introduction

The complex dynamics and variability of physical and chemical factors influencing microbial processes are one of the main features of estuaries. The load of nutrients in rivers coupled with the energy from tidal action intensifies the stirring-up and cycling of nutrients from shallow sediments, making estuaries one of the most productive environments on earth [1]. Moreover, abundant particles and aggregates are a prominent characteristic of estuaries, where their formation (besides their ubiquitous occurrence within running waters) is partly fostered by processes and functions such as particle concentration and surface properties, shear forces and sedimentation, as well as precipitation, polymerization, and the adsorption of dissolved organic and inorganic matter (reviewed by Zimmermann-Timm [2]).

These particles and aggregates consist of minerals, detritus and organisms and enfold bacteria, protozoans and metazoans (Zimmermann [3] and literature cited therein), and may represent important loci of increased bioproductivity and microbial activity [4]. The significance of fluxes of material and energy associated with particles or aggregates is that they are transported in more or less the same manner as their carriers. For example, the microbial self-purification capacity attributed to particles has the same sedimentation probability as the particles themselves. Accordingly, this share of self-purification capacity ought to sediment out under low turbulence conditions.

Few papers have been published on microbial extracellular enzyme activities, which represent the first step of microbiological self-cleaning in the water column of rivers and estuaries [5, 6]. Some authors have dealt with free dissolved and particle-associated microbial extracellular enzyme activities in rivers, for instance for various rivers in south-eastern Australia [7], the Elbe estuary [8], and the Elbe and other rivers ([9] and literature cited therein).

Compared to the adjacent coastal seas, the productivity of heterotrophic bacterioplankton in estuaries is generally high. These intensive growth rates of bacteria are accounted for by the relatively high organic carbon and nutrient concentrations caused by river inputs [10]. The coupling of bacterial activity and productivity to particles or aggregates versus the production rates of free living bacteria in estuaries is an important factor in understanding energy fluxes and the microbial conversion of organic material. In contrast to the marine environment ("marine snow") and lentic freshwater systems ("lake snow"), only few papers deal with particle-related microbial activities within rivers and estuarine waters ([11] and literature cited therein).

This paper describes the study of free microbial activities and those related to particles and aggregates in the Elbe estuary.

The aim of this research is to investigate the horizontal distribution and range of microbial secondary productivity and the activity of 11 different extracellular enzymes along with the impact of particles and aggregates on the pelagic microbial processes within an estuarine system.

2 Materials and methods

Samples were collected during two RV Tromper Wiek cruises on 14 and 15 October 1997 in the tidally influenced stretch of the lower Elbe from the funnel-shaped river mouth (stream distance km 707) upstream to the Port of Hamburg (km 620) (Fig. 1). The mean river discharge is about $700 \text{ m}^3 \text{ s}^{-1}$ and the current velocity is some $1\text{--}2 \text{ m s}^{-1}$ [12].

On the first day, stations were sampled at stream distance km 707, km 695, and km 690. On the second day, stations at km 707 and km 690 were sampled again, and additional samples from a transect upstream with stations at km 680, km 665, km 645, km 635, and km 620 were collected.

Particle abundance and distribution were determined using a particle counter (Partmaster L made by AUCOTEAM GmbH, Berlin) covering a particle size range of $2\text{--}200 \mu\text{m}$.

Bacterial abundance and biomass of free bacteria were determined using the AODC method [13]. The samples (2 mL) were filtered ($< -24 \text{ kPa}$) onto Irgalan Black stained polycarbonate Nuclepore filters ($0.2 \mu\text{m}$ pore size), and 1 mL acridine orange (0.01% final conc.) was added. After a staining time of 5 min the filters were vacuum-filtered, dried and immediately counted using a Zeiss Axioplan epifluorescence microscope (365 nm excitation filter, 395 nm beam splitter, 397 nm emission filter, Zeiss). A minimum of 250 bacterial cells were counted and the length (L) and width (W) of 50 bacterial cells per sample were measured using a New Porton Grid (Graticules, Ltd.). The cell volume (V) was calculated as follows: cell volume = $\pi/4 \cdot W^2 \cdot (L - W/3)$ (with W : width in μm and L : length in μm). Bacterial biomass was determined using the non-linear equation derived from Simon and Azam [14]:

$$\text{cell carbon/fg} = (\text{cell volume}/\mu\text{m}^3)^{0.59} \cdot 88.6 \cdot 1.04878.$$

The extracellular activity of 11 enzymes was determined as a measure of extracellular breakdown of dissolved and particulate organic macromolecules in accordance with Hoppe [15]. Phosphatase, aminopeptidase, α - and β -glucosidase, α - and β -galactosidase, lipase and chitinase activity were measured by using the following analogue substrates: 4-methylumbelliferyl phosphate, *l*-leucine 7-amido-4-methylcoumarin, *l*-arginine 7-amido-4-methylcoumarin, *l*-tyrosine 7-amido-4-methylcoumarin, glycine 7-amido-4-methylcoumarin, 4-methylumbelliferyl- α -D-glucoside, 4-methylumbelliferyl- β -D-glucoside.

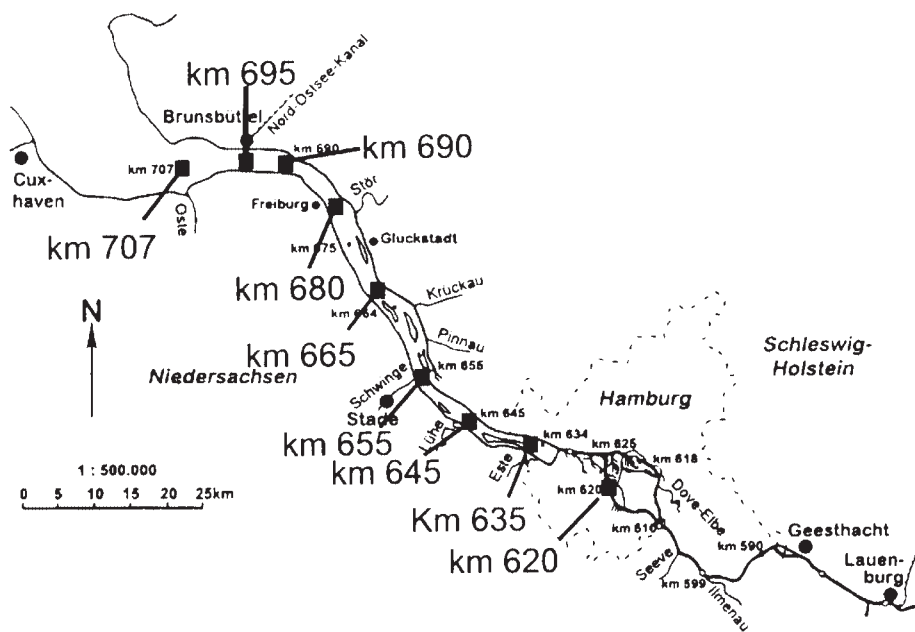


Fig. 1: Map of the study site and the location of the stations in the Elbe estuary, Germany.

Karte vom Untersuchungsgebiet und den Stationen im Elbeästuar, Deutschland.

side, 4-methylumbelliferyl- α -D-galactoside, 4-methylumbelliferyl- β -D-galactoside, 4-methylumbelliferyl stearate, and 4-methylumbelliferyl-*N*-acetyl- β -D-glucosaminide (Sigma Chemical Company).

10 μ L of each analogue substrate was added to 200 μ L samples (8 parallels, 1000 μ mol L⁻¹ final conc.) and incubated in micro-plates for 2 h in the dark at in situ temperatures. The saturation of a substrate concentration of 1000 μ mol L⁻¹ was tested by kinetic experiments (data not shown). Fluorescence reading was done using a micro-plate fluorescence reader (Labsystems, Ascent) at $\lambda = 364$ nm (excitation) and $\lambda = 445$ nm (emission). To calculate the maximum hydrolysis velocity, internal standard additions were used. The standard deviations of the extracellular enzyme activities varied between 0.7% and 17.2%, with double-digit percentage deviations occurring just 3 times.

To divide the specific extracellular enzyme activities into ectoenzyme and free dissolved enzyme activities, size-fractionated "pre"-filtration (<math>-24 kPa) was performed at stations km 695, km 680, km 665, km 655, and km 645 using 0.2 μ m, 2 μ m and 10 μ m Nuclepore polycarbonate filters. The enzyme activities were determined as described above. The <math><0.2 μ m fraction defines free dissolved enzymes, the size class of ≥ 0.2 ...<math>2 μ m represents ectoenzymes mainly attached to free living bacteria, the ≥ 2 ...<math>10 μ m range is a composition of ectoenzymes attached to free living bacteria, to particle-bound bacteria and originally free dissolved enzymes now adsorbed onto particle surfaces, while the >math>10 μ m size fraction mainly represents ectoenzymes attached to particle-bound bacteria or originally free dissolved enzymes adsorbed to particles.

Bacterial production was calculated from [³H] thymidine incorporation rates in accordance with [16] with some modifications. In detail, [³H] thymidine (spec. activity = 2.74 TBq mmol⁻¹) was added to 5 mL samples (triplicates + 1 killed blank) to give a final concentration of 20 nmol L⁻¹. Treated samples were incubated (starting at the in situ temperature) for 1 h in a thermo-container in the dark to minimize temperature effects. Incubation was terminated by adding 200 μ L 37% formaldehyde. Afterwards, the bacteria were concentrated on polycarbonate filters (Poretics, 25 mm diameter). The size fractionation of bacterial production was determined at all stations using Poretics polycarbonate filters with pore sizes of 0.2 μ m, 2.0 μ m, and 10.0 μ m. During filtration the filters were rinsed ten times with 1 mL ice-cold 5% TCA solution (but not ethanol) and then placed in polyethylene vials (Packard) with 5 mL Lumagel scintillation cocktail.

The samples were radioassayed in a scintillation counter (Packard Instruments 1600) after 24 h. Thymidine uptake was converted into bacterial carbon production using a factor of $1.1 \cdot 10^{18}$ cells produced per mole of incorporated thymidine [17] and 20 fg C per cell [18].

3 Results

Particle number concentrations ranged from $111 \cdot 10^6$ to $715 \cdot 10^6$ L⁻¹ (Fig. 2). From km 707 upstream to km 655, a more or less steady increase in particle abundance and subsequently a decrease to a minimum within the Port of Hamburg is observable. The number of particles determined at stations which were sampled again on the second day of investigation

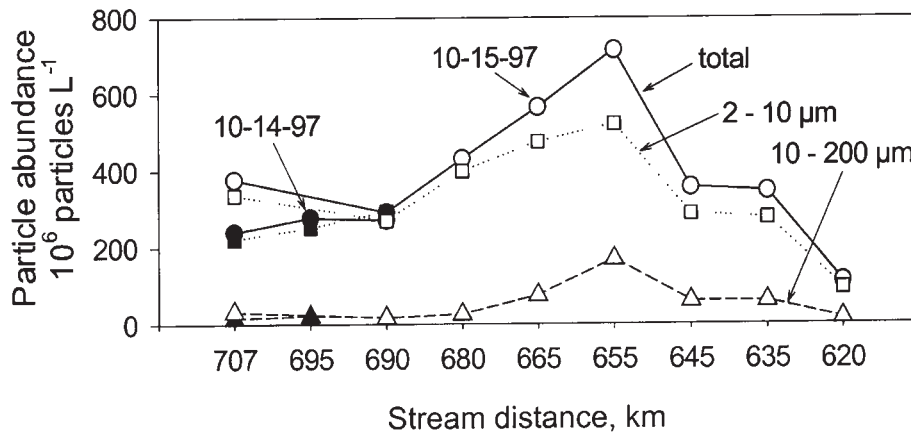


Fig. 2: Distribution of total particle abundance (circles), 2...10 μm (squares), and 10...200 μm particle size fraction (triangles) on 14 (filled symbols) and 15 October 1997 (open symbols) along the Elbe estuary.

Verteilung der Gesamtpartikelzahl (Kreise), 2...10 μm (Quadrate) und 10...200 μm (Dreiecke), Partikelgrößenfraktion vom 14. (gefüllte Symbole) und 15. Oktober 1997 (offene Symbole) entlang des Elbeästuars.

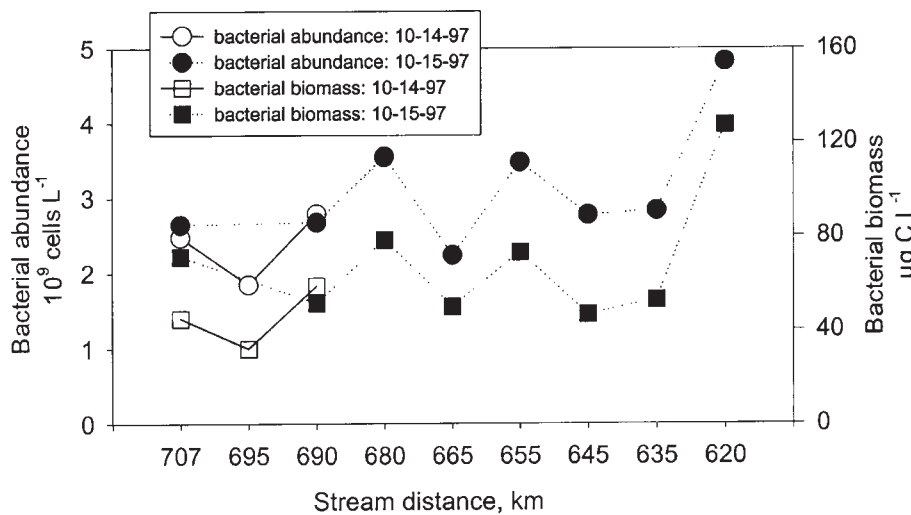


Fig. 3: Distribution of bacterial abundance (acridine orange direct counts; circles) and bacterial biomass (squares) on 14 (solid line) and 15 October 1997 (dashed line) along the Elbe estuary.

Verteilung der Bakterienzahl (Kreise) und Bakterienbiomasse (Quadrate) vom 14. (durchgezogene Linie) und 15. Oktober 1997 (gestrichelte Linie) entlang des Elbeästuars.

showed no pronounced difference from the first day. Particles in the 2...10 μm size fraction dominated the particle pool (73...93%). Only at the station with the highest particle concentration (km 655) was the particle fraction of >10...200 μm also quantitatively important (27%).

The bacterial abundance and biomass gradually increased upstream from $1.8 \cdot 10^9$ cells L⁻¹ and $32 \mu\text{g C L}^{-1}$ in the Elbe estuary (km 707) to $4.8 \cdot 10^9$ cells L⁻¹ and $127 \mu\text{g C L}^{-1}$, respectively, in the Port of Hamburg (km 620) (Fig. 3). No pronounced differences in bacterial numbers were found at km 690 and km 707 between the two sampling days.

The extracellular enzyme hydrolysis velocities ranged from undetectable to $181 \mu\text{g C L}^{-1} \text{h}^{-1}$ (Fig. 4). The lowest enzyme activities ($0...29 \mu\text{g C L}^{-1} \text{h}^{-1}$) were determined for aminopeptidase (leucine), α -, β -D-glucosidase, chitinase and α -D-galactosidase, followed by aminopeptidase (glycine), phosphatase, aminopeptidase (tyrosine), and aminopeptidase (arginine) ($2.5...54 \mu\text{g C L}^{-1} \text{h}^{-1}$). The highest degrada-

tion rates ($9...153 \mu\text{g C L}^{-1} \text{h}^{-1}$) were measured for β -D-galactosidase and lipase. All enzyme activities were lowest in the mouth region of the river Elbe and increased upstream with maxima at km 655 (phosphatase, aminopeptidase (arginine), α -, β -D-glucosidase, α -D-galactosidase and lipase) and the Port of Hamburg area. The day-to-day variability of the enzyme activities was relatively low for aminopeptidases (leucine and tyrosine) and for α -D-galactosidase, but high for aminopeptidases (arginine and glycine) and lipase.

Hydrolysis rates of 9 of the 11 enzymes tested showed the highest activities (mean 57%) in the size class $<0.2 \mu\text{m}$, suggesting that the degradation of these corresponding organic compounds could mainly be attributed to free dissolved enzymes (Fig. 5). Only leucine and tyrosine aminopeptidases and β -D-glucosidase achieved the highest activity rates in the $\geq 10 \mu\text{m}$ fraction, implying that the main hydrolysis locality of leucine, tyrosine, and β -D-glucose-rich macromolecules was strongly associated with particles covered indirectly (ectoenzymes from attached bacteria) and directly with adsorbed

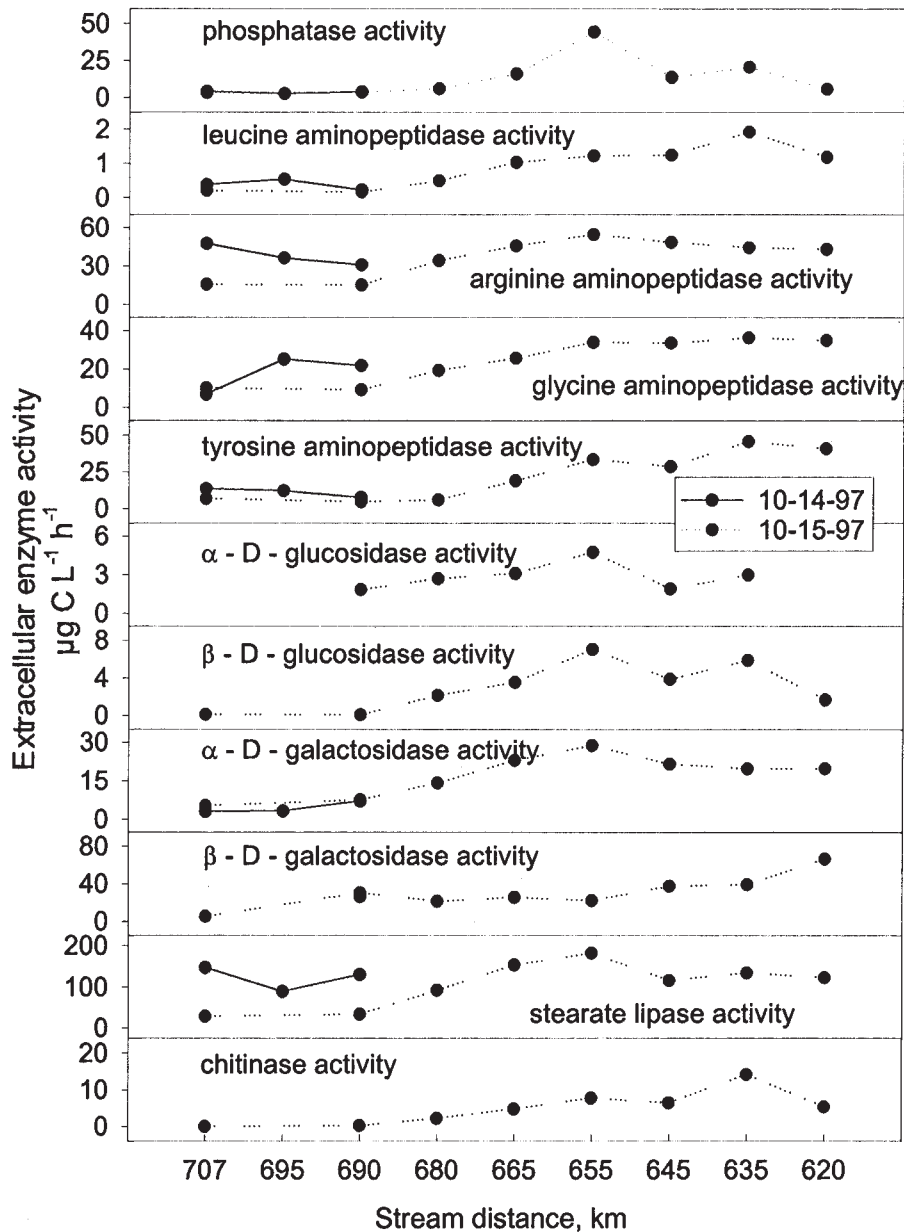


Fig. 4: Distribution of extracellular enzyme activity on 14 (solid line) and 15 October 1997 (dashed line) along the Elbe estuary.

Verteilung der extrazellulären Enzymaktivität (Phosphatase, Leucin-, Arginin-, Glycin- und Tyrosin-Aminopeptidase, α -D-Glucosidase, β -D-Glucosidase, α -D-Galactosidase, β -D-Galactosidase, Stearat-Lipase, Chitinase) vom 14. (durchgezogene Linie) und 15. Oktober 1997 (gestrichelte Linie) entlang des Elbeästuars.

originally free dissolved enzymes. The relative portion of the enzyme activities in the $\geq 0.2 \dots < 2 \mu\text{m}$ range amounted to just 3...12%. Therefore it can be assumed that ectoenzymes of the bulk of free living bacteria only played a minor role in the degradation of organic compounds.

The [^3H] thymidine bacterial production rates ranged from 0.6...4.6 $\mu\text{g C L}^{-1} \text{h}^{-1}$ with low rates at the seawards stations and higher values in the sector km 655...620 (Fig. 6). Again, only slight differences were observed in the mouth region (km 707 and km 690) between the two investigation days. The size-fractionated bacterial production rates related to large

free living bacteria and bacteria associated with smaller particles ($< 10 \mu\text{m}$) clearly dominated the overall bacterial productivity (Fig. 7). The share of production rates which could be attributed almost exclusively to free bacteria cells ($\geq 0.2 \dots < 2 \mu\text{m}$) generally ranged between 2% and 37%, but between 45% and 69% at km 695 and km 620. The bacterial production of the $\geq 10 \mu\text{m}$ size fraction, which mainly represents bacteria attached to larger particles and aggregates, was only detectable in the upstream area (41%) at km 680 and in the range of 4...12% at the subsequent stations towards the Port of Hamburg.

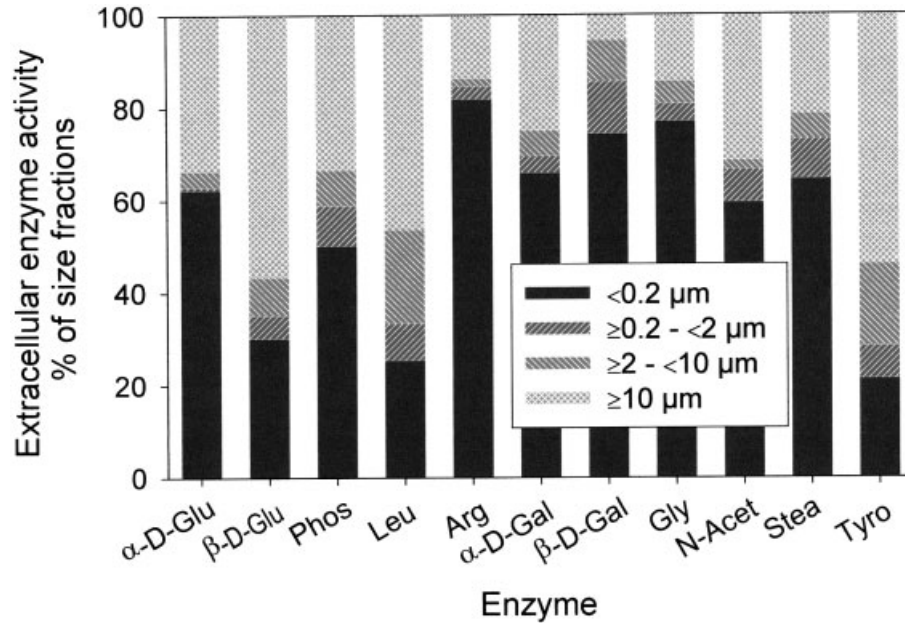


Fig. 5: Extracellular enzyme activity in different particle size fractions, averaged from the stations km 695, km 680, km 665, km 655, and km 645 of the Elbe estuary. Abbreviations: α -D-Glu: α -D-glucosidase, β -D-Glu: β -D-glucosidase, Phos: phosphatase, Leu: leucine aminopeptidase, Arg: arginine aminopeptidase, α -D-Gal: α -D-galactosidase, β -D-Gal: β -D-galactosidase, Gly: glycine aminopeptidase, N-Acet: chitinase, Stea: stearate lipase, Tyro: tyrosine aminopeptidase.

Größenfraktionen der extrazellulären Enzymaktivität, zusammengefasst von den Stationen km 695, km 680, km 665, km 655 und km 645 des Elbeästuars. Abkürzungen: α -D-Glu: α -D-Glucosidase, β -D-Glu: β -D-Glucosidase, Phos: Phosphatase, Leu: Leucin-Aminopeptidase, Arg: Arginin-Aminopeptidase, α -D-Gal: α -D-Galactosidase, β -D-Gal: β -D-Galactosidase, Gly: Glycin-Aminopeptidase, N-Acet: Chitinase, Stea: Stearat-Lipase, Tyro: Tyrosin-Aminopeptidase.

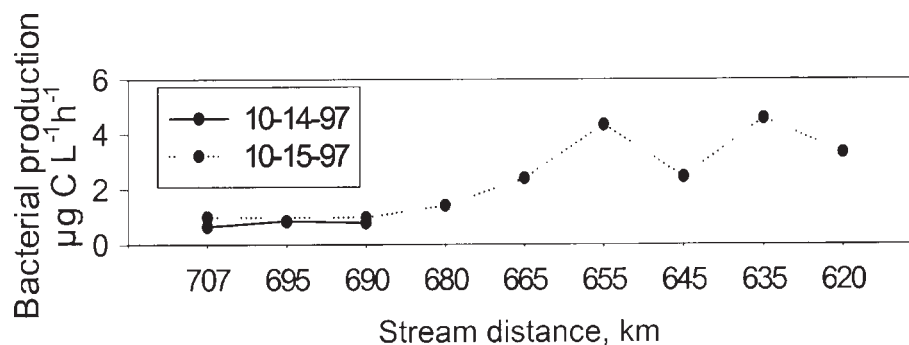


Fig. 6: Distribution of bacterial production rates on 14 (solid line) and 15 October 1997 (dashed line) along the Elbe estuary.

Verteilung der Bakterienproduktionsraten vom 14. (durchgezogene Linie) und 15. Oktober 1997 (gestrichelte Linie) entlang des Elbeästuars.

4 Discussion

Estuaries are strongly influenced by tides and variation in salinity and flows of material and energy. Thus the conversion of substances within estuarine environments is more complex

than that of streams, rivers, lakes, and the sea. In this context suspended particulate matter, especially organic particles, appears to be an important focus for the biological conversion of substances [19], which may sometimes dominate microbial activity compared to the surrounding water [20]. Here we

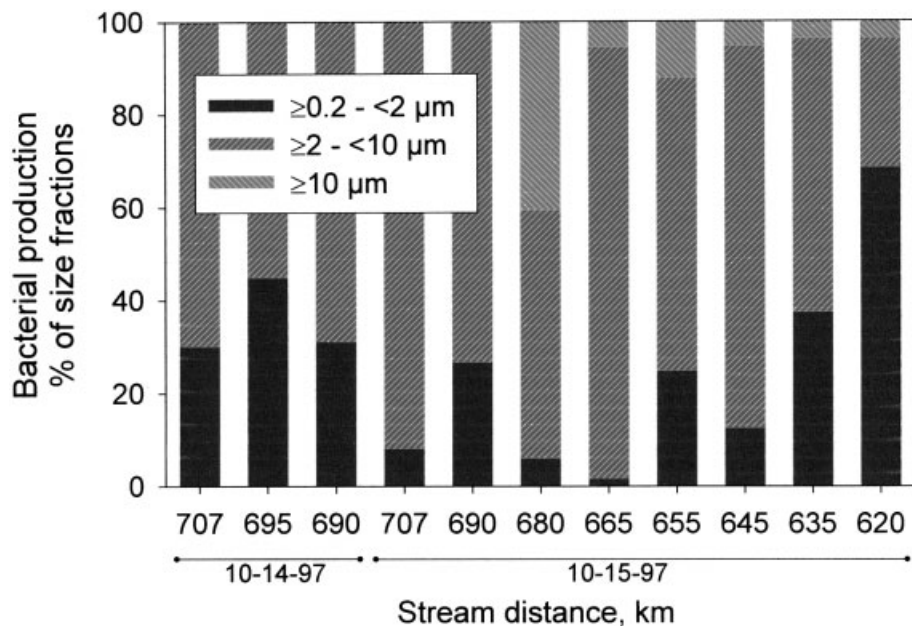


Fig. 7: Bacterial production in different particle size fractions on 14 and 15 October 1997 along the Elbe estuary.

Größenfraktionen der Bakterienproduktionsraten vom 14. und 15. Oktober 1997 entlang des Elbeästuars.

present results concerning the contribution of particles and aggregates to the initial step of microbial self-purification capability (extracellular enzyme activity) and the productivity of bacterioplankton within an estuarine system.

With $(111...715) \cdot 10^6$ particles L^{-1} compared to $(20...80) \cdot 10^6$ particles L^{-1} determined for the tidally unaffected reach of the river Elbe at Magdeburg (Baborowski, pers. com.), concentrations about one order of magnitude higher were determined for the estuarine stretch of the river Elbe. In all cases the 2...10 μm particle size fraction dominates the overall particle pool. With respect to larger particles, higher concentrations (up to a factor of 4) were also determined for the Elbe estuary (on the basis of $>20 \mu m$ values) compared to values determined in the river Elbe at Magdeburg (Baborowski, pers. com.) as well as in the Elbe and Saale [21].

Due to the low detection limit of the particle counter used of 2 μm , particles in the pico range (0.2...2 μm ; mainly bacterioplankton) were not included which otherwise, based on the microscopical results of bacterial abundance, would have enhanced this size fraction by a factor of about 3.5. Owing to the particle counter's specific detection range, direct comparison with published particle abundance ascertained with other equipment is difficult. Nevertheless, these results are helpful in indicating the particle transport capacity and the overall particle concentration.

Estimates of bacterial abundance $((1.8...4.8) \cdot 10^9$ cells L^{-1}) reported here were relatively low compared to previous investigations in the lower Elbe, where bacterial cell numbers ranged between $0.6 \cdot 10^9$ cells L^{-1} and $27 \cdot 10^9$ cells L^{-1} [3, 8, 22, 23]. Corresponding to bacterial abundance, low bacterial

biomass ($32...127 \mu g C L^{-1}$) was also determined compared to previous studies [24, 25] or was within the same range [26]. This might be due to seasonal conditions at the time of investigation (e.g., low temperatures and the low entrainment of organic matter from terrestrial sources).

A decrease in bacterial standing stocks downstream was also observed by Zimmermann [3]. This is very probably due to the increasing dilution of waters highly loaded with anthropogenic sources by seawater, which has a low concentration of easily degradable DOC. Another regulating factor of bacterial abundance and biomass might be the downstream increase in salinity, possibly functioning as a selective factor for freshwater-adapted bacteria.

Concerning extracellular enzyme activity, only two investigations have been published for the Elbe estuary, and these were limited to the determination of proteolytic activity in turnover ($\% h^{-1}$) and relative proteolytic activity units [6, 8]. Compared to the investigations carried out in the spring [9] and autumn in the tidally unaffected part of the river Elbe as well as for the river Havel (Karrasch et al., in prep.), the results of the enzyme activities from the Elbe estuary exhibit in some cases sharp differences.

In most cases the maximum hydrolysis rates of α - and β -D-glucosidase and chitinase of the tidally unaffected part of the river Elbe as well as for the river Havel were comparable to the rates determined in this investigation, and are also consistent with estimates reported for other rivers [27, 28]. With the exception of aminopeptidase (leucine) activity, which was unexpectedly low, all other peptidases (arginine, glycine, and tyrosine) showed higher values for the Elbe estuary than for

the unaffected part of the river Elbe. Moreover, compared to the results from [9] and (Karrasch et al., in prep.) unusually high hydrolysis rates were determined for α - and β -D-galactosidase and phosphatase activity. Discussion of the high lipase (stearate) activity is not possible because these measurements represent the first publication of this enzyme activity. The difference of the extracellular enzyme activities compared to the tidally unaffected part of the river [9] reflects the particular abiotic and biotic conditions within the estuarine region.

With the exception of α - and β -D-glucosidase and chitinase activity, all the other enzymes tested were detected at every station. Due to the energetic outlay for the cell metabolism, extracellular enzyme activities are more or less only measurable when the actual enzyme substrate is abundant. These results indicate a corresponding qualitative and quantitative organic load of particulate and dissolved organic compounds along the study area. The mostly steady increase in the extracellular enzyme activities from the mouth region upstream to km 655 and within the port area could be induced by a rising concentration of organic material (OM). Besides high human activity introducing a strong organic load in mainly the upper Elbe region, processes within the river itself, such as the breakdown of phytoplankton below the Port of Hamburg region mainly attributable to a salt wedge [29, 30], could be responsible for elevated OM. Whether and to what extent in this region in particular the activity of extracellular enzymes was enhanced by enzymes stemming from industry and sewage works [5] cannot be assessed.

As demonstrated in this study and by other publications [3, 12], the Elbe estuary is characterized by large numbers of particles and aggregates which might also serve as important loci for extracellular enzyme activities. However, it was shown that dissolved free enzymes were predominantly (22...82%, mean 57%) responsible for extracellular enzyme activities within the water column. Ectoenzymes coupled to free bacteria and smaller particles played a lesser role. Large particles and aggregates as well as ectoenzymes only became significant in the hydrolysis of leucine- and tyrosine-containing proteins – which was also observed by [8] – and for the degradation of polymeric β -D-glucose (cellulose).

From an ecological viewpoint, the coupling and uncoupling of extracellular enzyme activity to particles or aggregates is very important. A more particle-related degradation of organic material is subject to a higher sedimentation probability, leading to a reduced self-purification capability under less turbulent conditions while dissolved free enzymes remain more or less unaffected by the hydrographical situation, representing as it were the dissolved behavior of the first step of biological self-purification capacity. A different size fraction pattern of the extracellular enzyme activity (exoenzyme $<0.2 \mu\text{m}$ vs. ectoenzymes $\geq 0.2 \mu\text{m}$) was determined in a tidally unaffected

stretch of the river Elbe during a spring flood [9] and also during autumn for an investigation of the Saale and Elbe (Karrasch et al., in prep.). In both cases the activity of the ectoenzymes clearly dominated the breakdown of OM.

With regard to the season, the bacterial [^3H] thymidine production rates estimated here tallied well with the microbial productivity level determined for the Elbe and other estuaries [10, 11, 31, 32], for other sections of the river Elbe as well as for other rivers such as the Saale and Neckar (Karrasch et al., in prep.). This might point to the fact that a pronounced increase in overall bacterial activity/productivity cannot be conclusively demonstrated under particular estuarine conditions. Towards the mouth of the river Elbe, the microbial production rates steadily decreased, which could be primarily explained by the shift in the environmental conditions (e.g., dilution of organic load, change of osmotic conditions) from a freshwater through brackish water to a marine system.

The pattern of bacterial productivity in different size classes is quite different from that of the tidally unaffected reach of the river Elbe ([9], Karrasch et al., in prep.). For the Elbe estuary, the contributions of free dispersed bacteria ($\geq 0.2...<2 \mu\text{m}$; mean 27%) and the $\geq 2...<10 \mu\text{m}$ fraction (67%) clearly indicate that bacterial productivity related to larger particles ($>10 \mu\text{m}$) played no role whatsoever (stations at km 707, km 695, km 690) or at best merely a minor one in the degradation and cycling of OM. In contrast to the Elbe estuary, the percentage of the size fraction comprising primarily bacteria associated with particles or aggregates ($>10 \mu\text{m}$) possesses the same importance (around 40%) as the size class dominated mainly by free dispersed bacteria ($\geq 0.2...<2 \mu\text{m}$). With respect to microbiological capabilities (extracellular enzyme activity, bacterial production), it can be ascertained that in the upper river Elbe these particles and aggregates had a higher impact on the overall bacterial capacity within the system. The extent to which the elevated contribution of this size fraction can be explained by for instance a longer development and colonization time for the aggregates during river passage whereas in estuarine waters a relatively high share of aggregates could be newly generated by various physical, chemical, and biological particle/aggregate-forming processes, which are often intensified in estuary regions ([2] and literature cited therein).

All in all, the results concerning the relation of microbial activity to particles or aggregates are contradictory. Crump et al. [11] found that on average 90% of bacterial carbon production in estuarine water was attributable to particle-attached bacteria ($>3 \mu\text{m}$). But in agreement with our results, Plummer et al. [32] and Painchaud and Therriault [33] found particle-related production rates to be less important. The relative contribution of free living vs. particle-attached bacteria is generally variable depending on the abundance and growth rates within the two microbial groups. Griffith et al. [34] and Crump

and Baross [35] found the importance of these groups to alternate seasonally for the upper Chesapeake Bay and the Columbia River, respectively.

It should be mentioned that all the results have to be considered with some caution with respect to the size fractions. Due to the necessary "pre"-filtration procedure for the estimation of extracellular enzyme activities, the <0.2 µm fraction and not the whole system was incubated, so that bacteria which might have produced additional extracellular enzymes during incubation were excluded. Regarding the "pre"-filtration of the extracellular enzyme and ectoenzyme activities through 2 and 10 µm filters, only parts of the whole system were included during incubation. It cannot be ruled out that with the pressure difference applied (<–24 kPa) some cell- or particle-associated enzymes may have been stripped during filtration, causing the activity of the free dissolved enzyme activity to be overestimated. With <–24 kPa we applied a slightly higher pressure difference than usually considered (<20 kPa) as a gentle suction pressure (e. g., [36]).

When estimating bacterial production rates, sources of error include the possible need for other conversion factors to calculate microbial productivity, because bacteria attached to particles are generally thought to be larger and more active per cell [37, 38], or the supply of [³H] thymidine inside particles may be limited. Another potential uncertainty stems from the "post"-filtration of the bacterial production rates, which could include cells which may have become detached from particles during the filtration procedure [39].

For the parameters investigated on both days (14 and 15 October), generally only slight differences between days were found. Although estuaries represent very heterogeneous environments under comparable meteorological circumstances and short-time scales (day to day) within the tide cycles, the physicochemical conditions may remain more or less constant.

Although estuaries have features in common (e.g., the interface between saline and freshwater, salt gradients), many features differ, accounting for their individuality. Taking into account the temporal and spatial scales of various abiotic and biotic conditions (e.g., geographical location, morphology of the estuary, discharge, tidal impact, residence time of water, different loads of particulate matter, biota, inorganic and organic nutrients), the fact that study findings are sometimes contradictory comes as no surprise. The impact of particle- and aggregate-associated microbial activities could be raised temporally and locally during episodes of intense sediment resuspension, e.g., intensified by storm events, high tides, and wakes of vessels. In this case these suspension-deposition cycles export bacteria with a largely benthic origin attached to particles or aggregates into the water column. From this it can be assumed that the free bacteria of the water col-

umn and the bacteria attached mainly to the larger particles and aggregates form two distinct and possibly uncoupled populations as hypothesized by Painchaud and Theriault [33], and that the latter are largely controlled by sediment dynamics.

Judging by the overall scale of biological benefits, it may be that particles and aggregates in running waters and estuaries do not always play an important role in the conversion of substances and the ecological flow of energy and matter. Nevertheless they still represent loci which, by virtue of their abilities to for instance accumulate vigorous substances on their surfaces and to establish local specific environmental conditions, facilitate transformations of substances which could otherwise only be realized within the water column to a limited extent – if at all.

Acknowledgements

We would like to thank Martina Baborowski for assisting us with the AUCOTEAM GmbH particle counter and the crew of the RV Tromper Wiek for their obliging help during the sampling cruises.

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[Received: 28 August 2002; accepted: 10 June 2003]