

# Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone

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**ABSTRACT:** Phosphatase (P-ase) activity was determined together with other extracellular enzyme activities, bacterial abundance and production rates during the 2 SW Monsoon process studies of the German JGOFS Arabian Sea Program. Water samples were collected along the cruise tracks from the equator to the upwelling region at the shelf edge off Oman. Depth profiles of P-ase activity were strikingly different from those of the other enzymes. While values of aminopeptidase and  $\beta$ -glucosidase generally decreased below the euphotic zone, P-ase increased by factors of 1 to 7. The relation between peptidase- and P-ase activity was from 4 to 21 at the surface and from 3 to 5 at 800 m depth. Because P-ase production (dissolved and cell-bound) in deep waters is mainly dependent on bacteria, P-ase activities per bacterial cell were calculated: these were, on average, 37 times higher at 800 m than at the surface. We also observed a positive correlation of P-ase activity with phosphate concentrations in the depth profiles below the euphotic zone, while this relationship was much more variable in the mixed surface layer. These observations suggest that C-limited bacteria in the deep strata did not primarily focus on the phosphate generated by their P-ase activity but on the organic C compounds which were simultaneously produced and which could probably not be taken up prior to the hydrolytic detachment of phosphate. It is hypothesised that a considerable part of the measured P-ase activity was dissolved (though it might have originated from bacteria). These enzymes may be important for the slow, but steady regeneration of phosphate and organic C in mesopelagic waters.

**KEY WORDS:** Ectoenzymes · Phosphatase · Aminopeptidase · Glucosidase · Bacteria · Indian Ocean · Arabian Sea · Mesopelagic zone · Deep sea

## INTRODUCTION

In offshore, oceanic waters far below the euphotic zone bacteria are hampered by an insufficient supply of easily degradable organic compounds. Sedimenting and suspended particles at greater depths are generally depleted of their easily degradable constituents, something which is particularly pertinent for dissolved organic matter in the surrounding water. It can thus be hypothesised that bacteria activate their enzymatic potential to mitigate C limitation.

In practice, however, only a few field studies have been made on extracellular enzyme activities in deep waters or sediments (e.g. Rosso & Azam 1987, Hoppe

et al. 1993, Boetius & Lochte 1994, Boetius 1995, Poremba & Hoppe 1995, Talbot & Bianchi 1997, Koike & Nagata 1998). This is partly due to the inadequate sensitivity of methods for the assessment of enzyme activities in the deep open ocean and also likely due to the inadequate recognition of the importance of enzymatic decomposition processes in deep waters.

The available information on bacterial enzyme activities in deep oceanic regions indicates that (1) these activities correlate with decreasing bacterial abundance and growth although exceptions do occasionally occur for specific enzymes (Hoppe et al. 1993, Koike & Nagata 1998) and (2) the relative importance and dominance within a set of hydrolytic enzymes changes with increasing water depth and especially in the sediments (Kim 1985, Boetius & Lochte 1996, Koike & Nagata 1998).

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The western Indian Ocean during SW Monsoon and the deep upwelling zones at the shelf edge off the Arabian coast are characterised by high primary production and sedimentation rates (Haake et al. 1993). Therefore it was expected that the sensitivity of the available methods would be sufficient to obtain reliable measurements of bacterial extracellular enzyme activities even in deep water.

Phosphatase (P-ase) activities in deep waters have been measured even less than other hydrolytic activities. This may be due to the fact that phosphate is generally not the growth limiting factor in the euphotic zone of the sea (Rivkin & Anderson 1997). Some major exceptions to this are found in the Mediterranean Sea (Krom et al. 1991, Thingstad & Rassoulzadegan 1995), the Sargasso Sea and the Gulf Stream (Cotner et al. 1997, Rivkin & Anderson 1997). In addition, phosphate is highly abundant in deep oceanic water. However, extracellular enzymatic P regeneration is necessarily coupled with the regeneration of organic C. Thus P-ase is bi-functional and interacts with the C cycle (Chróst 1990, Siuda & Güde 1994, Thingstad & Rassoulzadegan 1995), which may be important for C-limited bacteria in the deep sea.

In this paper we present some unexpected observations of P-ase activity in the mesopelagic and bathypelagic zones of the Indian Ocean, complemented by observations of other hydrolytic enzymes and general microbiological and phytoplanktological variables.

## MATERIALS AND METHODS

The presented results are from 2 JGOFS cruises in the Indian Ocean (Fig. 1): (1) Meteor cruise 32/5 took place from 14 June to 12 July 1996 during the SW

Monsoon. Stations were located on a transect from the equator to the shelf edge off Oman, crossing the region of the Findlater jet axis. The Findlater jet over the northern Arabian Sea is the strongest trophospheric jet known. Its axis extends generally from Cape Guardafui, Somalia (12° N, 52.2° E), to the Gulf of Cambay, India (20.8° N, 71° E). The jet causes deep water mixing and wind-driven offshore upwelling in the region north-west of its axis (Smith & Bottero 1977) and downwelling on its eastern side. (2) Sonne cruise 120 was conducted from 22 June to 24 July 1997 during the SW Monsoon. Stations were arranged along the trajectory of a drift buoy in a cold water upwelling filament off the coast of Oman and an offshore transect (Stns 26 to 34, Fig. 1) which was not affected by upwelling according to temperature characteristics.

Water samples were taken from the 10 l Niskin bottles of a CTD rosette sampler (Multi Water Sampler, Hydrobios). Samples for microbial activity measurements were immediately processed on board the ship.

Total bacterial counts were obtained by epifluorescence-microscopical counting of acridine-orange-stained bacteria on black polycarbonate filters (Poretics) with a pore size of 0.2 µm (Hobbie et al. 1977), as standardised by the 'JGOFS core measurement protocols' (SCOR 1994). After filtration, the bacteria were stained and mounted in immersion oil on board. They were kept deep frozen at -20°C until they were microscopically analysed in the home laboratory.

Extracellular enzyme activities were measured by using fluorogenic substrate analogues (Hoppe 1983, 1993). The measured extracellular enzymes (ectoenzymes according to the definition by Chróst 1991) were P-ase, leu-aminopeptidase and β-glucosidase using the substrate analogues MUF-P (4-methylumbelliferone-phosphate), Leu-MCA (L-leucine-4-methylcoumarinyl-

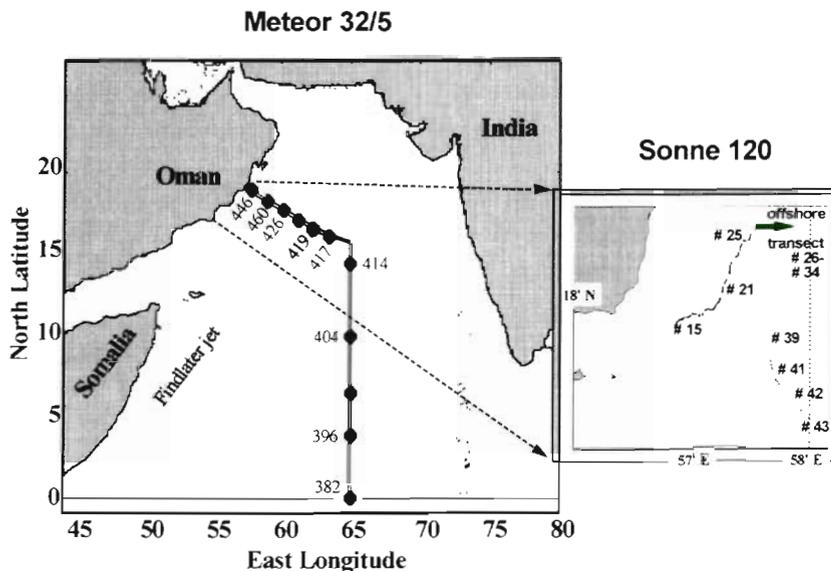


Fig. 1 Cruise tracks of RV 'Meteor' (cruise 32/5) and RV 'Sonne' (cruise 120) during the German JGOFS Indian Ocean SW Monsoon expeditions. Selected sampling stations are marked for orientation. The region of the Findlater jet was crossed at Stn 419 during the Meteor cruise. Note the stations of the separate offshore transect not affected by inshore upwelling during the Sonne cruise 120



Findlater jet was clearly reflected by elevated values (Stn 426, north-west of the jet axis). At depth, P-ase activity increased strongly in the region next to the equator and remained constant or increased slightly towards the shelf edge. Values at 800 m varied between 2 and 5  $\text{nM h}^{-1}$ . Aminopeptidase activities at depth in comparison to surface values remained constant or increased slightly in the oligotrophic region (Stns 382, 396, 404 and 414, between the equator and the Findlater jet). Towards the shelf edge activities decreased strongly at depth. Note, however, that the values at 800 m were always between 10 and 15  $\text{nM h}^{-1}$ , independent of the surface value and thus less variable than the corresponding values for P-ase.  $\beta$ -glucosidase activity always decreased at depth to very low values.

Total bacterial counts decreased regularly with depth in the profiles along the transect (Fig. 3). The same held true for the patterns of bacterial secondary production. Phosphate increased strongly immediately below the euphotic zone and correlated in most cases significantly with community P-ase activity (Fig. 2). Relating total bacterial counts to community P-ase activity results in cell-specific enzyme activity. This

is potentially a maximum value because measured enzyme activities may not be entirely attributed to bacteria but could also originate from other sources and from dissolved enzymes (see 'Discussion'). Cell-specific P-ase activities increased nearly linearly with depth, reaching values at 800 m which were on average 37 times ( $n = 9$ ) greater than the corresponding values from bacteria at the surface. The cell-specific activities of aminopeptidase also occasionally increased, although less than those of P-ase.

Generally, in the inshore upwelling region inorganic phosphate concentrations and P-ase activity were much higher than in the oligotrophic offshore region. Surprisingly, this was not as pronounced in the chlorophyll *a* content (compare Fig. 4a and b), which is likely due to effects of grazing on phytoplankton (Zeller 1998). In the offshore system (Fig. 4a, unfortunately no deeper samples were taken) concentrations of inorganic phosphate and P-ase activity were highly correlated ( $p \leq 0.05$ ). Chlorophyll *a* correlated occasionally with P-ase activity in the euphotic zone (about 80 m). Increasing values of P-ase activity below the euphotic zone apparently originated from sources other than phytoplankton.

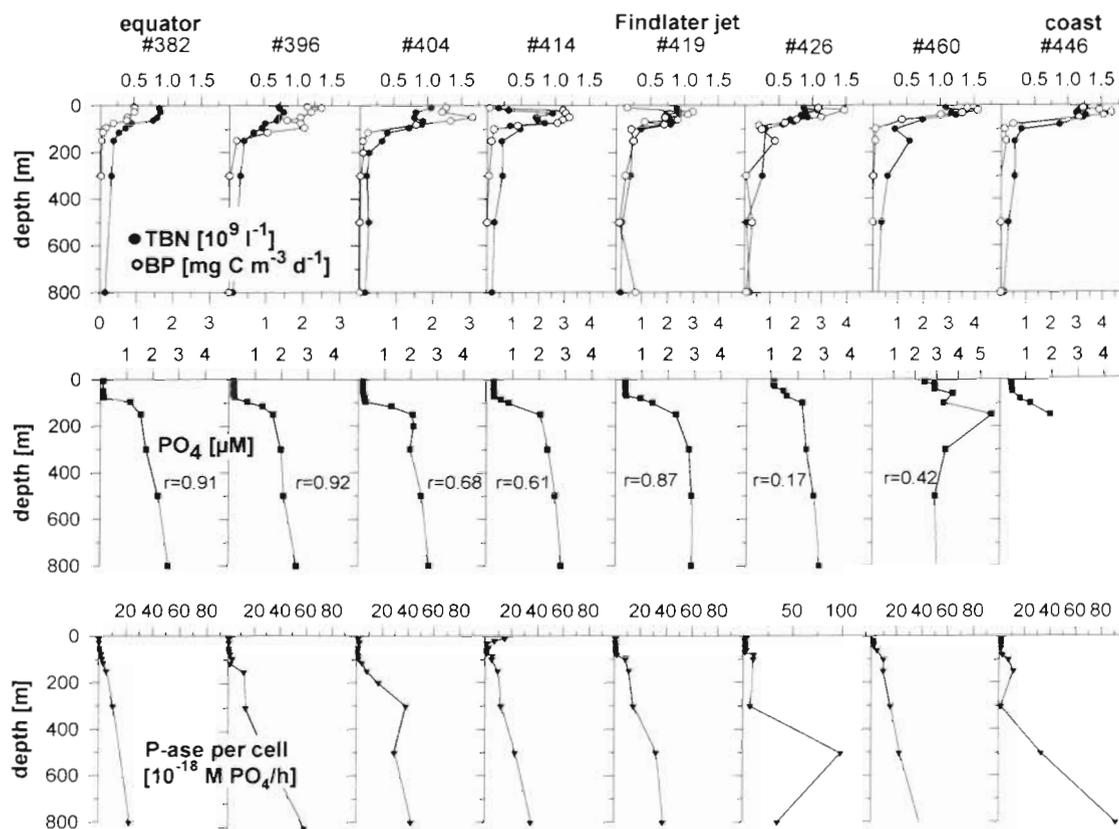


Fig. 3. Selected depth profiles of bacterial counts ( $\text{TBN}^+$ ; upper scale), bacterial secondary production ( $\text{BP}^+$ ; lower scale), phosphate concentration and phosphatase (P-ase) activity ( $V_m$ ) per bacterial cell (in terms of phosphate remineralised per hour) along the transect from the equator to the inshore upwelling region at the shelf edge off Oman. Correlation coefficients ( $r$ ) indicate the correlations between phosphate concentration and P-ase activity as presented in Fig. 2. Results from the Meteor cruise 32/5

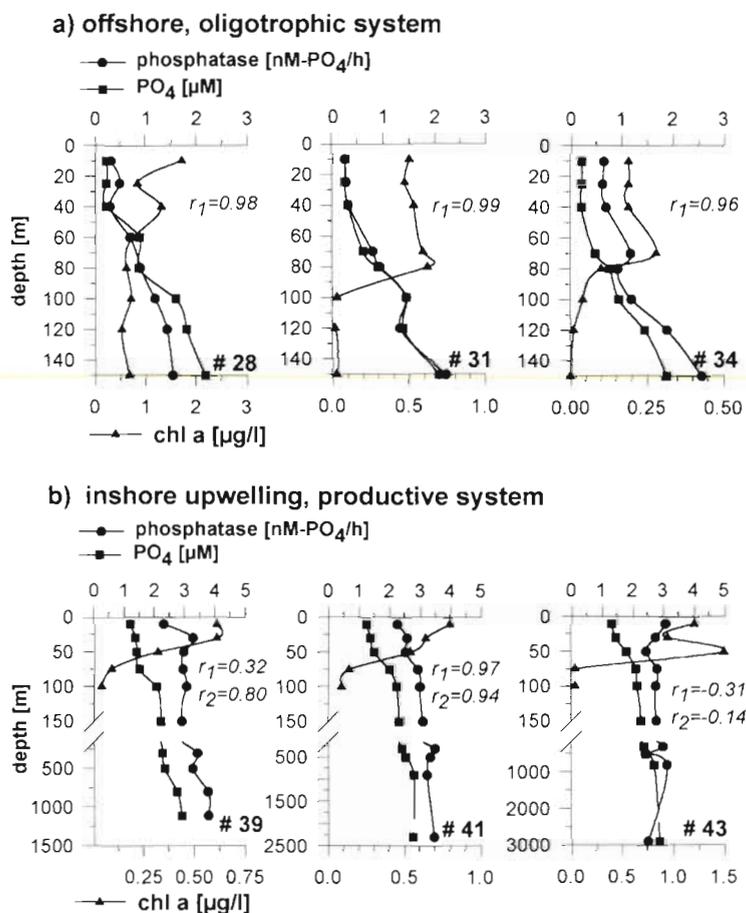


Fig. 4. Selected depth profiles of P-ase activity ( $V_m$ ) together with phosphate and chlorophyll *a* concentrations (a) offshore and (b) from the upwelling region at the shelf edge off Oman. Indicated correlation coefficients concern the relationship between P-ase activity and phosphate concentration:  $r_1$  from the surface to 150 m,  $r_2$  from the surface to the greatest depth. Results from the Sonne cruise 120

In the upwelling region at the shelf edge off Oman (Fig. 4b, the location is very deep due to the steep angle of the continental slope), P-ase activity in the euphotic zone was not as closely correlated with inorganic phosphate as it was in the oligotrophic offshore region. Chlorophyll *a* was not correlated with P-ase activity. This may be due to the relatively high concentrations of phosphate which excluded P limitation and its regulatory effect on P-ase activity.

In the upwelling region at the shelf edge off Oman both the  $V_m$  of P-ase and the  $V_m$  of  $\beta$ -glucosidase increased or remained fairly constant with depth. In contrast,  $V_m$  of aminopeptidase decreased strongly with depth (Fig. 5a). On the other hand (Fig. 5b), hydrolysis (= turnover) rates ( $H_t$ ) of both peptides and  $\beta$ -glucosides decreased with depth, while those of organic P compounds increased or remained fairly constant. In an evaluation of the *in situ* enzyme activity it should be

considered that the parameter  $H_t$  may provide a better measurement than  $V_m$ .

The relationship between  $V_m$  and  $H_t$  of P-ase ( $V_m/H_t$ , see 'Materials and methods') in the offshore region showed a very interesting pattern of distribution when compared to the inshore upwelling region (Table 1). In both regions, values of  $V_m/H_t$  increased or remained fairly constant with depth but they were generally much higher in the upwelling region than in the offshore region. This indicates an increased enzyme potential in the eutrophic system and in deep water. The relatively low hydrolysis rate of the pool of competitive substrates (preferentially phosphomonoesters) in the eutrophic systems may be due to the large size of this pool. In contrast, low hydrolysis rates in the deep water may be attributed to the inert nature of substrates.

## DISCUSSION

P-ase activity is widespread in marine bacteria (Kobori et al. 1979, Martinez et al. 1996). It is closely connected with both the P cycle and the C cycle (Siuda & Güde 1994). The second function can be important for C-limited bacteria in deep water. P-ase activities in the oceanic mesopelagic zone and deeper horizons have rarely been reported. Only 1 corresponding deep-sea investigation (Koike & Nagata 1998) is available for comparison with our data.

### Methodological considerations

Measurements of enzyme activity followed experienced procedures which have been successfully applied by many investigators (e.g. Chróst 1991, Smith et al. 1992, Martinez & Azam 1993). All experiments were made with freshly prepared solutions of fluorogenic substrates. Blanks (boiled) did not show any abnormalities. Profiles of P-ase activity did not always increase with depth, but occasionally remained constant or even decreased, which excludes a systematic methodological error. Furthermore, similar results of P-ase activity increase with depth were obtained on the 2 subsequent cruises reported here, which excludes the possibility of it happening by chance. However, elevated P-ase activity in the deep may be influenced by unspecified reactions of enzymes (e.g. 5-NP-ase, Martinez et al. 1996) with the applied fluorogenic P substrate and by effects of de-pressurisation

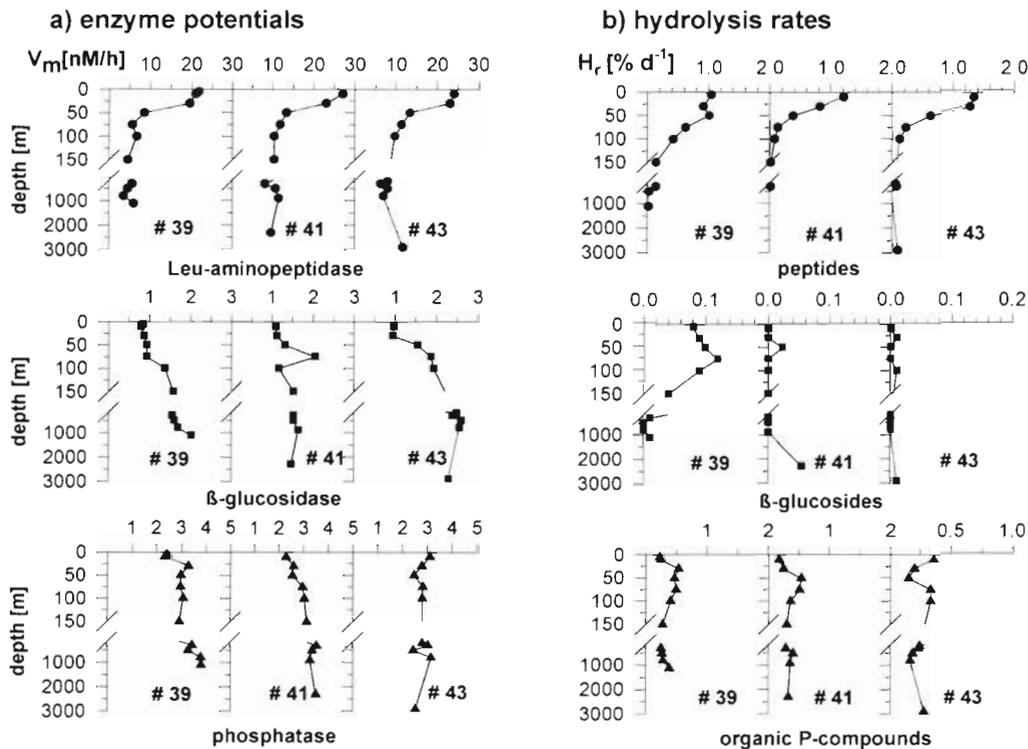


Fig. 5. Selected depth profiles of 3 enzyme activities from the upwelling region at the shelf edge of Oman. Comparison of (a) maximal velocity of hydrolysis ( $V_m$ ) and (b) hydrolysis rates ( $H_r$ , % hydrolysis per day) of the competitive natural substrate pools, which are peptides (substrates for Leu-aminopeptidase),  $\beta$ -glucosides (substrates for  $\beta$ -glucosidase) and organic P compounds, especially phosphomonoesters (substrates for P-ase). Results from the Sonne cruise 120

on the organisms during sampling. Another question concerns the suboxic conditions in the samples harvested from the oxygen minimum zone (OMZ) between approximately 120 and 1000 m water depth. In these samples oxygen decreased occasionally to  $0.1 \text{ mg O}_2 \text{ l}^{-1}$ , but never disappeared completely. The influence of suboxic conditions on the observed ectoenzymatic activities is regarded as slight or negligible because it has been demonstrated for marine and limnetic samples that only sulfide has a strong effect on enzyme activities (Hoppe et al. 1990).

#### Comparison with other deep-sea investigations

Recently, Koike & Nagata (1998) measured potential P-ase activities together with other enzyme activities ( $\alpha$ - and  $\beta$ -glucosidase) on particles (collected on  $0.2 \mu\text{m}$  filters) in deep water of the Central North Pacific. Dissolved P-ases were not included in the activity measurements.

Their results indicate that P-ase activities at depths of 1000 to 4000 m can reach up to 50% of those measured in surface water, while the 2 glucosidase activi-

Table 1. Sonne cruise 120. Relationship between  $V_m$  (potential of P-ase) and  $H_r$  (hydrolysis rate of P-ase substrates) obtained from selected depth profiles of stations off the coast of Oman and from the inshore upwelling region

Offshore, oligotrophic						Inshore, upwelling, productive					
#26		#31		#34		#39		#41		#43	
Depth (m)	$V_m/H_r$	Depth (m)	$V_{max}/H_r$	Depth (m)	$V_m/H_r$	Depth (m)	$V_m/H_r$	Depth (m)	$V_m/H_r$	Depth (m)	$V_m/H_r$
10	1.3	10	0.9	10	2.0	10	9.0	10	12.9	10	8.6
25	1.1	25	0.9	25	2.0	30	6.1	30	10.3	30	13.5
40	1.2	40	0.8	40	1.9	50	6.3	50	4.7	50	15.7
60	2.7	70	1.9	70	2.7						
80	2.6	80	2.7	80	2.8	75	5.9	75	5.8	75	8.4
100	3.4	100	3.7	100	3.2	100	7.5	100	8.3	100	8.3
120	2.1	120	3.1	120	5.1						
150	3.3	150	3.6	150	8.6	150	10.2	150	10.1	150	11.3
						300	13.1	300	12.1	300	12.9
						800	12.9	900	9.0	800	18.4

ties decrease to less than 1%. High P-ase activities coincide with high phosphate concentrations (2.4 to 3.0  $\mu\text{M}$ ) in the deep water. The results were interpreted as a consequence of a P-ase import from the surface into the deep regions via rapidly sinking particles, rather than by the activities of the prevailing deep-sea microbial communities.

Our measurements from the Indian Ocean included the dissolved enzymes. Fractionation of particle-associated and dissolved enzymes was only occasionally made. P-ase activities down to 4000 m were equal to, or even higher than, those at the surface. Absolute values of P-ase activity at 800 m varied between 2 and 4.5  $\text{nM h}^{-1}$  (Fig. 2) while Koike & Nagata's (1998) measurements (1000 to 4000 m) were between 0.03 and 0.3  $\text{nM h}^{-1}$ . Both investigations generally confirm a different behaviour of P-ase from other hydrolytic enzymes in deep water. But there is a discrepancy concerning the relationship of P-ase activity at depth and in surface water, which was  $\geq 1$  in the Indian Ocean and  $\approx 0.5$  in the northern Pacific. Our attempts to explain this discrepancy are as follows: (1) The 2 investigated regions (Central Northern Pacific and Northern Indian Ocean during the SW Monsoon) are very different with respect to their productivity and vertical particle flux. Koike & Nagata (1998) measured only P-ase activities on particles while we measured total enzyme activity. (2) Allocating the observed P-ase activities in the deep Indian Ocean exclusively to bacteria would result in unrealistically high cell-specific P-ase activities (Fig. 3a). (3) Sinking particles can hardly be the only source of P-ase in the deep Indian Ocean because the number of (protein-containing) particles at 1500 m depth in the Arabian Sea amounts only to  $\sim 50\%$  of the surface values ( $2 \times 10^7 \text{ l}^{-1}$ ) (Long & Azam 1996) while P-ase activity increased several times. On the other hand, P-ase on organic aggregates collected from surface water frequently exhibited the highest concentration factor in comparison to P-ase in the surrounding water among a variety of hydrolytic enzymes (Smith et al. 1992). (4) If dissolved P-ases (originating from bacteria and other sources) were abundant in deep water, this could be a reason for the observed high P-ase levels.

Our hypothesis regarding the observed high P-ase potential in the Indian Ocean is that it represents the combined activities of dissolved enzymes of different origin and of particle-bound enzymes (attributed to free-living and attached bacteria). Minor contributions could arise from zooplankton grazing (Bochdansky et al. 1995), virus-mediated destruction of organisms (Middelboe et al. 1996) and particle-adsorbed enzymes of different origin. A high portion of dissolved P-ases has frequently been reported from different types of water (e.g. Hoppe 1986, Berman et al. 1990). In our

investigation 42% of total P-ase activity, 22% of total peptidase activity and 10% of total  $\beta$ -glucosidase activity were associated with the dissolved enzyme fraction  $< 0.2 \mu\text{m}$  (derived from samples down to 80 m, deeper samples were unfortunately not fractionated). The slow but steady action of these enzymes (together with the particle-associated enzymes) supplies and maintains the high inorganic P content of the deep sea and provides some support for the C demand of bacteria to mitigate C limitation.

However, the effect of increasing P-ase activity on bacterial C supply may be low because the maximal P-ase activities of  $\sim 4 \text{ nM h}^{-1}$  measured in the deep Indian Ocean are at the lower end of the ranges measured in other marine areas (for instance, the annual range in the Western Baltic Sea is between 2 and 160  $\text{nM h}^{-1}$ , Hoppe 1986). Furthermore it has to be considered that (1) concentrations of utilisable organic matter in deep water were likely very low (the recently reported patterns of abundance and utilisation of transparent exopolymer particles [TEP] in the mesopelagic Indian Ocean [Kumar et al. 1998, Ramaiah et al. 1999] may moderate this assumption), (2) a weak coupling between hydrolysis and substrate uptake may exist if dissolved enzymes dominate, and (3) the kinetics of dissolved enzymes may be different (slower) in comparison to enzymes situated in the cell envelope because the former are no longer in their optimal environment (with respect to pH and ion composition). Alteration of enzyme kinetics could also be the key for a better understanding of the observation that P-ases in deep water were not inhibited by the prevailing high phosphate concentrations.

### Considerations

The lack of experience with P-ase in deep oceanic water means that we can only speculate about the reason for the observed increase of P-ase in the Indian Ocean. Fig. 6 shows a conceptual model of the role of P-ase in deep water. Growth and maintenance of bacteria in the deep depends mainly on the quantity and quality of settling and suspended particulate organic matter (POM). The C:P ratio of POM changes with depth in favour of C (Copin-Montegut & Copin-Montegut 1983). But POM is a mixture of high and low C:P ratio molecules. An increase of the overall C:P ratio would suggest that combined C-P molecules of low C:P ratio were preferentially hydrolysed while the majority of the pure C molecules remained untouched. As such, remineralisation could also occur at high overall C:P (and C:N) ratios of POM (Tezuka 1990). However, the regenerated phosphate (and N) is only partly utilised in deep water because (1) there is no need for supply-

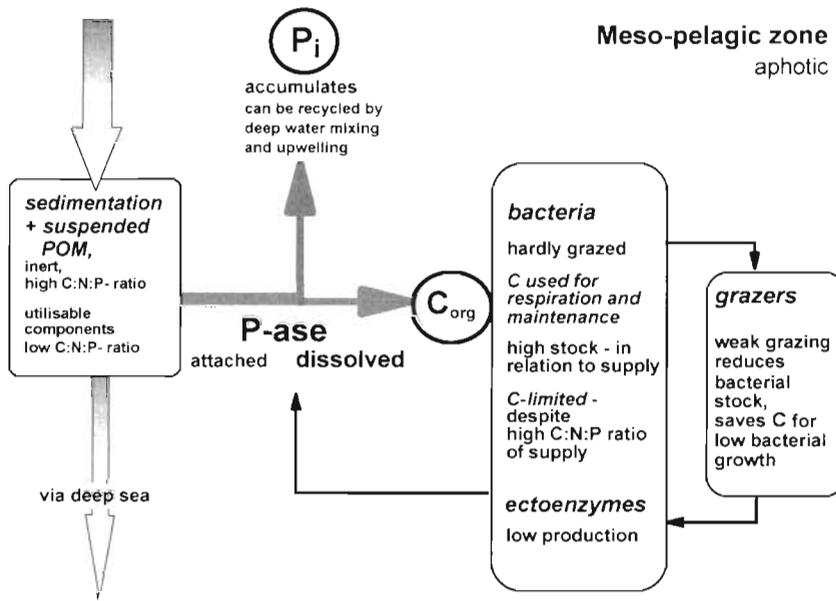


Fig. 6. A conceptual model of the different functions of P-ase and the C limitation of bacteria in the aphotic deep ocean. For explanation see 'Considerations'

ing phototrophs and (2) bacteria in the deep are strictly C limited (Tezuka 1990). This necessarily leads to the observed increase of phosphate (and inorganic N compounds) below the euphotic zone.

Strict C limitation of bacteria in the deep arises from the facts that only C is respired and that there is only very limited production of new bacteria which would require P and N. Thus the requirements for C are relatively high in comparison to P and N which could be supplied directly from abundant inorganic P and N pools (e.g. Ammermann 1991). The reduced C supply (by sinking and suspended POM) in the deep is mainly respired. If bacterial growth occurs at all in the deep this may be due to bacterial mortality caused by bacterivorous grazers. Grazing removes a part of the bacterial standing stock. This means that C which would have been potentially used up for respiration and maintenance of this part would then become available for the production of new cells.

Strict C limitation of bacteria could have been a reason for the observed induction of C-regenerating hydrolytic enzymes (such as P-ase) in the deep Indian Ocean.  $\beta$ -glucosidase activity was occasionally also elevated in the deep of the upwelling region at the shelf edge (Fig. 5a). Aminopeptidase activity was frequently low at depth, which could be due to the preferential use of N compounds already in surface water and probably also to the assumption that (surplus) N has to be excreted by the cells after incorporation of the N compounds. Nevertheless it has to be pointed out that absolute values of aminopeptidase activity in terms of

$V_m$  were always much higher than those of the other investigated enzymes.  $V_m$  of aminopeptidase was 4 to 21 times higher than  $V_m$  of P-ase at the surface and 3 to 5 times higher at 800 m depth. In comparison to  $\beta$ -D-glucosidase, aminopeptidase activity was 23 to 74 times higher at the surface and 59 to 434 times higher at 800 m depth. On the other hand turnover rates of substrates established by aminopeptidase were 9 to 28 times higher than those of P-ase at the surface but at 800 m turnover rates established by peptidase were up to 10 times lower than those of P-ase.

A prerequisite for our hypothesis to be viable is that P-ase substrates (preferentially phosphomonoesters) cannot be incorporated by bacteria without extracellular enzymatic hydrolysis. Glucose-6-P is known to be a suitable substrate for measuring P-ase activity.

Thus enzymatic hydrolysis even of

small phosphomonoesters seems to be necessary for the uptake of the constituents by algae and bacteria. Strong evidence for this assumption arises from the fundamental experiments with glucose-6-<sup>32</sup>P and <sup>14</sup>C-glucose-6-P conducted by Hernández et al. (1996). An exception is the *sn*-glycerol-3-phosphate-binding-protein of *Escherichia coli* which enables the incorporation of the complete P substrate (Argast & Boos 1980) into the cells, but this seems to be only valid at high substrate concentrations (Heath & Edinger 1990). A second prerequisite is that organic C regenerated by the hydrolysis of combined P compounds must be better utilised by bacteria than the bulk of pure C compounds. Indications for a preferential (or at least similar) uptake of compounds regenerated by hydrolysis in comparison to corresponding monomeric compounds arise from the investigations of Coffin (1989) and Kirchman & Hodson (1984).

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