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RELATIONS BETWEEN BACTERIAL EXTRACELLULAR ENZYME ACTIVITIES AND HETEROTROPHIC SUBSTRATE UPTAKE IN A BRACKISH WATER ENVIRONMENT

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ABSTRACT - Extracellular enzymes mediate the decomposition of polymeric organic compounds in natural waters. In many cases these enzymes comprise a component linked to the bacterial fraction of the aquatic community and react in close association with these cells. An annual survey of microbial activities in a brackish water fjord exhibited an excellent correlation between V_m of proteases (ability to split the substrate analogue methylumbelliferyl-leucine) and V_m as well as T_R for leucine uptake by microorganisms. Hydrolysis rates (H_R) for the decomposition of naturally occurring competitive substrate analogues of the model substrates (methylumbelliferyl-(MUF)-leucine, MUF-α-D-glucose, MUF-N-acetyl-glucosamine, MUF-phosphate) drastically declined after chlorophyll *a* and bacterial abundance had dropped in autumn. H_R for protease and phosphatase were higher in offshore areas than in polluted inshore waters, whereas the opposite was true for α-glucosidase and glucosaminidase.

Key words : extracellular enzymes, bacteria, heterotrophic substrate uptake

RÉSUMÉ - Les enzymes extracellulaires permettent la décomposition des composés organiques polymèriques en milieu marin. Très souvent, une partie de ces enzymes est liée à la fraction bactérienne et réagit en association étroite avec ces cellules. Un suivi annuel de l'activité bactérienne dans l'eau saumâtre d'un fjord a montré une excellente corrélation entre le V_m des protéases (capacité à couper un analogue de substrat, la méthylumbelliferyl-leucine) et le V_m ou le T_r d'absorption de la leucine par les bactéries. Les taux d'hydrolyse (H_R) pour la décomposition de substrats naturels analogues du substrat testé (méthylumbelliferyl)-(MUF)-leucine, MUF-α-D-glucose, MUF-N-acétyl-glucosamine, MUF-phosphate) ont fortement diminué après la chute de la teneur en chlorophylle *a* et des numérations bactériennes, qui a lieu en automne. Les H_R des protéases et phosphatases ont été plus élevés dans les eaux du large que dans les eaux côtières polluées, alors que l'inverse s'est produit pour l'α-glucosidase et la glucosamine.

Mots clés : enzymes extracellulaires, bactéries, absorption hétérotrophe du substrat.

INTRODUCTION

Considerable amounts of dissolved organic compounds in sea water are in the form of polymeric, oligomeric or dimeric molecular structures often requiring enzymatic breakdown prior to their incorporation into the bacterial cell. The fraction of combined organic molecules from total DOC has only occasionally been determined and results cover a wide range. The latter observation is not surprising, because ratios of molecular size fractions may depend on factors such as organic nutrient supply, aging and chemical conversion. Through acid hydrolysis of sea water samples it has been shown that the concentration of dissolved combined amino acids is about 5 times that of dissolved free amino acids (DFAA) (Degens, 1970). From culture experiments with water of an eutrophic fjord Bauerfeind (1982) found that the ratio between dissolved free monosaccharides

(DFMS) after acid hydrolysis of particle-free water and naturally occurring DFMS may decline from 10 to approx. 1 within a 14 day incubation period. Data from lakes (Hama and Handa, 1980) suggest that only 20 % of total DOC is of polymeric nature (carbohydrates, proteins).

The question is how these materials, *a priori* unsuitable for direct use by heterotrophic bacteria, are introduced to the aquatic food chain and/or the pool of dissolved organic monomers. Hollibaugh and Azam (1983) and Hoppe (1983) demonstrated through different methodological approaches that products resulting from enzymatic splitting of polymers are incorporated by bacteria. However, a varying fraction also contributed to the DOC pool of small molecules.

In light of these general findings we constructed a few experiments on bacterial extracellular enzymatic activity concerning the following aspects: i) Origin of extracellular enzymatic activity investigated via fractionated filtration. This involved the abundance of free dissolved enzymes. ii) Extracellular enzymatic properties of bacteria pure cultures and axenic algal cultures were assessed in order to verify field observations where normally little extracellular enzymatic activity was found in the algal fraction. iii) Since extracellular enzymatic activity seems to be closely related to the bacterial fraction of aquatic organisms, the relationship between enzymatic and heterotrophic activity was investigated during an annual survey in two different biotopes of an eutrophic brackish water fjord. Special emphasizes was placed on the relationship with phytoplankton development, a possible key factor in the supply of polymeric organic substances in the investigated area.

A strong correlation between protease activity and heterotrophic leucine uptake has already been computed by Somville and Billen (1983) from data of different aquatic biotopes. However, it is an open question as to whether an uncoupling of these processes may occur seasonally and with changing environmental conditions.

The application of fluorigenic methylumbelliferyl-substrates (Hoppe, 1983) has proven to be an useful tool in the study of extracellular enzymatic activities of bacteria in the field as well as in experimental situations. The sensitivity of methods employing these substrates seems to be on the same order of that using radioactively labelled polymers (Hollibaugh and Azam, 1983). Results from both approaches, however, were not always comparable. This may be a consequence of the great variety of combined amino acids ranging from simple dimers to polymers of very different structure.

METHODS

Sampling

Water samples were taken from the pier of the Institut für Meereskunde (Kiel, FRG) and from the adjacent brackish water Kiel Fjord. Stations were located in the polluted harbour area (station Hauptpost) and in a more offshore position in front of the fjord's mouth (station Feuerschiff). Sterile 2 l-bottles in a surface sampler were used for water collection.

Measurement of extracellular enzymatic activity:

For the determination of enzymatic activities fluorigenic methylumbelliferyl (MUF) -linked substrates were used (MUF-α-D-glucopyranoside, MUF-N-acetyl-glucosamide, MUF-phosphate and L-leucyl-methylcoumarin-HCl = MUF-leucine, provided by Sigma or Fluca). Substrates of this nature were used for enzymatic measurements in ecological studies by Petterson and Jansson (1978), Hoppe (1983) and Somville (1984).

Dimeric coumponds of comparable composition as well as (in some cases) polymeric substrates have been shown to be competitors of MUF-Substrates. Molecule structure of MUF-substrates and mechanisms of their enzymatic breakdown are demonstrated in detail by Hoppe (1983).

Stock solutions were prepared by dissolving 2 μM of MUF-substrate in 1 ml Methylcellusolve (ethyleneglycol monomethylether). For some of the MUF-substrates water may be used as a solvent as well. Aliquot parts of the stock solution (1 to 400 μl) were added to 20 ml subsamples of the original water sample, yielding final MUF-substrate concentrations of 0.1 - 40 μM per l. Appropriate dilutions or more concentrated stock solutions may be used in order to gain a more similar solvent level in the subsamples. However, the influence of the solvent concentrations applied here on enzymatic activity proved to be negligible. For the substrate MUF-α-D-glucopyranoside additional final concentrations as low as 0.01 μM per l were applied, because saturation levels are occasionally much lower than for the other substrates.

The fluorescing compound methylumbelliferon is liberated from the combined molecular complex exclusively by extracellular enzymes. This has been proven by epifluorescence microscopy, where methylumbelliferon did not penetrate the interior of bacterial cells, while a strong blue fluorescence was observed in the surrounding medium. It is, however, admitted that the verification of this statement needs further investigation. Fluorescence intensity was recorded at 365 nm (excitation) and 455 nm(emission) in a Jasco spectrofluorimeter at the beginning and after 3 h of incubation. Previous time series experiments exhibited strict linearity of enzymatic activity vs. time over several hours. The fluorescence of methylumbelliferon is pH-dependent and, hence, 2.5 ml subsamples for measuring fluorescence intensity were adjusted to pH 10.3 with a commercial buffer (Merck). Controls were run with water samples boiled for 20 min in a water bath prior to addition of the MUF-substrates. Incubation was performed in a water bath at 20°C or at ambient water temperatures during the annual survey.

Extracellular enzymatic activities are expressed in terms of velocity of hydrolysis V_m (μgC l^{-1} h^{-1}) and hydrolyzation rate (% h^{-1}).
Generally in experiments involving fractionated filtration only three MUF-substrate concentrations were used and results are presented in units of relative fluorescence.
For calibration of methylumbelliferon (which is liberated from the complexes in equimolar concentrations) a calibration curve with different small amounts of methylumbelliferon was established. Calibration was repeated before the experiments from time to time. The standard deviation of extracellular enzyme activity measurements, as derived from experiments in different aquatic regions, was in the range of $\pm$ 5 % from the mean value.

Methods used for determination of additional parameters: Saprophytes were grown on ZoBell-agar with a salinity of 15 ‰. After acridine orange staining the total number of bacteria was counted in an epifluorescence microscope according to Zimmermann and Meyer-Reil (1974).

Heterotrophic uptake potential for ^{3}H-leucine was determined according to Hoppe (1978). Inorganic chemical (NO_3^-, NH_3, PO_4^{3-}), physical (T, S) and planktological (chlorophyll a, b, c, species distribution) analyses of water samples were conducted according to standard methods (Grasshoff, 1976 ; SCOR-UNESCO recommandation, 1966).

RESULTS

Origin of extracellular enzymes

Water samples from different brackish water areas were size fractionated with Nucleo-

pore filters of different pore sizes and the hydrolysis of MUF-substrates for these fractions was measured. Because patterns of activity for all samples were very similar, only one example is presented here (Tab. 1). MUF-substrates, though of relatively small molecular size, do not penetrate microbial cells. Enzymatic activity on these substrates should, therefore, be attributable to extracellular enzymes. Of the enzymes tested most of the activity originated from fractions where bacteria were most abundant (0.2 - 1 - 3μm). There was little or no extracellular enzymatic activity associated with the larger size classes. This corresponded well with the relatively low degree of bacterial attachment to particulate organic/inorganic matter. With MUF-phosphate as a substrate the highest amount of extracellular activity was frequently found in the $< 0.2\,\mu$m fraction. This was regarded as hydrolytic activity originating from free dissolved enzymes because no bacteria could be grown from this fraction. Among the various enzymatic activities only phosphatase activity was also associated with phytoplankton size classes to a great extent. Enzyme activities of the different size classes are affected by increasing MUF-substrate concentrations in an irregular manner, which cannot be readily explained.

Substrate	Concentration μM	$< 0.2\,\mu$m	0.2-1 μm	1-3 μm	3-8 μm	8-56 μm	56-150 μm
MUF-leucine	0.5	28.6	24.1	2.5	11.1	21.1	12.6
	10	17.4	41.3	4.9	8.2	16.7	11.5
	125	13.6	62.1	5.6	4.1	14.6	0
MUF-α-glucoside	0.5	2.8	44.5	1.4	27.8	1	23.6
	10	0	67.3	17.3	0	0	15.4
	125	4.9	87.6	4.9	0	2.4	0
MUF-β-Glucoside	0.5	4.0	72.0	24.0	0	0	0
	10	21.7	18.8	49.3	0	0	10.1
	125	34.6	38.6	23.5	3.5	0	0
MUF-N-acetyl glucosaminid	0.5	3.9	34.6	61.5	0	0	0
	10	27.5	66.0	0	0	4.6	1.9
	125	69.8	19.6	0	0	0	10.0
MUF-phosphate	0.5	32.6	13.9	0	12.4	17.1	24.0
	10	53.5	11.9	0	2.3	12.1	20.2
	125	48.4	16.7	8.1	0	6.7	20.1
MUF-palmitate	0.5	15.5	38.9	0	2.6	5.7	37.3

Table 1 : Extracellular enzyme activities associated with size fractions of natural water, values in % of the total activity (relative fluorescence units).

Pure culture experiments

Pure bacterial and axenic algal cultures were tested for their ability to decompose MUF-substrates. Results for the bacteria are reported in this volume (Kim an Hoppe). The axenic phytoplankton cultures showed little or no extracellular enzymatic activity compared with that of the bacreria (Tab. 2). Complete exclusion of bacteria from algal cultures is, therefore, vital since even a small number of bacteria may lead to a drastic overestimation of extracellular algal enzymatic properties. Batch culture algae in the logarithmic phase of growth were washed and resuspended in a mineral medium supplemented with MUF- substrates. Most of the algae investigated exhibited extracellular phosphatase activity : (*Scenedesmus microsporina, Chlorella* (very weak activity), *Cyclotella cryptica, Phaeodactylum tricornutum, Anabaena* sp). A few of them showed pro-

tease (*Chlorella* (very weak), *Scenedesmus microsporina, Anabaena, Phaeodactylum, Cyclotella),* α-glucosidase (*Cyclotella cryptica, Anabaena)* and glucosaminidase (*Scenedesmus quadricaula)* activity. Experimental algal cultures were tested for sterility following incubation, and recorded extracellular enzymatic activities are therefore due only to algal cells. The possibility cannot be excluded that algal cell disruption occurred during the procedure with a consequent release of intracellular enzymes.

	Extracellular enzyme (substrate)					
Species	protease (MUF-leucine)	α-glucosidase (MUF-α-glucoside)	β-glucosidase (MUF-β-glucoside)	N-acetyl-aminidase (MUF-glucosamine)	Glucos-phospatase (MUF-phospate)	lipase (MUF-palmitate)
Chorella spec.	+	-	-	-	+	-
Dunaliella fertiolecta	-	-	-	-	-	-
Phaeodactylum tricornutum	++	-	-	-	+++	-
Cyclotella cryptica	++	+	-	-	+++	-
Scenedesmus microsporina	++	+	+ (-)	-	+	-
Scenedesmus quadricaula	-	-	+ (-)	+++	-	-
Anabaena spec.	++	+	--	-	+++	-

- negativ, + very weak, ++ weak, +++ strong, ++++ very strong

Table 2 : Extracellular enzymatic properties of some axenic cultures of common phytoplanktonic algae, investigated by means of methylumbelliferyl - (MUF) - substrates

Field measurements of extracellular activity

Typical examples for the enzyme kinetics are presented by Hoppe (1983); here the results are presented in terms of mean-values in order to explain some principles of interaction between enzyme activity and organisms and/or substrate pools. Activities (V_m) during the course of the year were highest for extracellular proteases, α-glucosidase and phosphatase in July when water temperature, saprophytes and uptake of leucine reached their maximum. Total bacterial numbers and biomasses were also at their maximum (station Hauptpost) or very close to it (station Feuerschiff) at that time. Chlorophyll however, was not positively correlated with these enzyme parameters.

Extracellular glucosaminidase deviated from this pattern, with highest activity during the spring phytoplankton bloom in March. Minima for all extracellular enzymatic activities were measured in December, coinciding with lowest saprophytic bacterial counts, chlorophyll concentration and leucine uptake. Total numbers of bacteria and bacterial biomass were also low in December, although their absolute minima did not coincide with EEA (extracellular enzymatic activity) minima. Statistical analyses over the year revealed a significant correlation between enzymatic activities and total bacteria as well as saprophytes at the inner fjord station Hauptpost. At the station Feuerschiff a much higher significance was shown with the saprophytes. However, if the relationship between EEA and bacterial numbers is considered in terms of spatial distribution and not seasonally, a much higher dependency of EEA on total bacterial number becomes obvious. Hydrolysis

of glucosamine compounds, again, deviated from this pattern in that it only correlated with bacterial numbers at the station Hauptpost.

In both areas of investigation a wide range of EEA's was observed over the year. (Tab. 3). Generally an increase in V_m of EEA's was found in enclosed inshore waters. At the polluted inner fjord station annual average values of V_m of protease were 1.6 times higher than at the offshore station. The corresponding values were 3.1 for V_m of α-glucosidase, 1.5 for glucosaminidase and 1.9 for phosphatase (c.f. Table 4). This increase was proportional to that of total bacterial number, uptake velocity of leucine and chlorophyll *a* content of the water. Saprophytic bacteria numbers did not fit this correlation. For the hydrolyzation rates (H_R, % h^{-1}) of the different MUF-substrates this uniformity of response could not be detected. The hydrolyzation rates for MUF-leucine substrate analogues were 3 times higher, and those for MUF- phosphate were 5 times higher at the offshore station. In contrast H_R for MUF-α-glucoside substrate analogues were 5.5 times smaller and those for MUF-glucosaminide were 10 times smaller at the offshore station than at the polluted inshore station.

Parameter	Station Hauptpost (inshore)		Station Feuerschiff (offshore)	
	Minimum	Maximum	Minimum	Maximum
1) Saprophytes	2,600	283,300	100	12,600
2) AODC	1.4	9.6	0.5	2.5
3) V_m - leucine uptake	0.01	0.3	0.001	0.09
4) T_R - leucine	0.25	12.1	0.22	8.9
5) V_m MUF-leucine	0.93	22.4	0.29	14.1
6) V_m MUF-α-glucoside	0.02	1.06	0.01	0.17
7) V_m MUF-glucosaminide	0.07	0.55	0.08	0.53
8) V_m MUF-phosphate	0.004	0.16	0.002	0.07
9) H_R MUF-leucine	0.06	2.2	0.08	17.8
10) H_R MUF-glucoside	0.002	14.0	0.001	4.3
11) H_R MUF-glucosaminide	0.006	2.7	0.001	0.13
12) H_R MUF-phosphate	0.026	0.53	0.01	8.3
13) Chl. a	0.34	18.8	0.74	9.3

Units 1) saprophytes ml^{-1}
2) total bacteria numbers $ml^{-1} \times 10^6$; 3) ug C l^{-1} h^{-1} ; 4) % h^{-1} ; 5) - 7) μg Cl^{-1} h^{-1} ; 8) μM l^{-1} h^{-1} ; 9) - 12) % h^{-1} ; 13) μg l^{-1}

Table 3 : Extreme values of different parameters obtained from 12 monthly investigations.

Though pool sizes of polymers (including oligomers) and of monomers of the different substrates investigated are not known, some indication for the mechanism of equilibration between these pools may arise from the comparison of hydrolyzation rate (H_R, % h^{-1}) of the directly available part of the polymer pool and the turnover rate (T_R, % h^{-1}) of the corresponding monomer pool. However, one must bear in mind, that the polymer hydrolyzation rate as measured via MUF-substrates will, at best, provide a relative measure of that part of the polymer pool which is exposed to enzymatic splitting from the end points of the macromolecules.

Over the year the relation between T_R for leucine and H_R for MUF-leucine substrate analogues (we may call it the pool size ratio) varied between 2.3 and 24.5 at the inner fjord station. The lowest value was obtained during the warm season (21°C), while the greatest divergence was observed in late October (9°C) when values for bacteria numbers and activities as well as chlorophyll levels drastically dropped. Factors of approx. 10 were obtained during the cold season; from annual average values of T_R and H_R a relation of 5.6 was calculated. For the outer station (Feuerschiff) the relationship between T_R and H_R varied between 0.5 and 13.1. For annual average values it was approx. 1 (Tab. 4).

Parameter	Station Hauptpost (inshore) average	Station Feuerschiff (offshore) average	inshore offshore
1) Saprophytes	70,500	3,100	23
2) AODC	3.6	1.5	2.4
3) V_m-leucine uptake	0.08	0.03	2.3
4) T_R leucine	4.4*	2.6**	1.7
5) V_m MUF-leucine	7.35	4.46	1.6
6) V_m MUF-α-glucoside	0.26	0.08	3.1
7) V_m MUF-glucosaminide	0.23	0.16	1.5
8) V_m phosphate	0.03	0.015	1.9
9) H_R MUF-leucine	0.78*	2.86**	0.3
10) H_R MUF-α-glucoside	2.87	0.52	5.5
11) H_R MUF-glucosaminide	0.3	0.03	10
12) H_R MUF-phosphate	0.23	1.38	0.2
13) Chl. a	9.3	3.5	2.7

Units 1) saprophytes ml^{-1} ; 2) total bacteria numbers $ml^{-1} \times 10^6$; 3) $\mu g\ Cl^{-1}h^{-1}$; 4) $\%\ h^{-1}$; 5) - 7) $\mu g\ Cl^{-1}h^{-1}$; 8) $\mu M\ Pl^{-1}h^{-1}$; 9) - 12) $\%\ h^{-1}$; 13) $\mu g\ l^{-1}$.

* relation $T_R : H_R$ inshore : ~ 6 : 1,
** relation $T_R : H_R$ offshore : ~ 1 : 1.

Table 4 : Average values of different parameters obtained from 12 monthly investigations

Discussion

Biochemical properties of aquatic bacterial populations were investigated by means of selective agar media supplemented with polymeric nutrients such as starch, protein, and chitin (Sieburth, 1971 ; Kjelleberg and Hakansson, 1977). Through these studies information is provided about the distribution and enrichment of saprophytic bacteria with distinct biochemical activities in different aquatic biotopes and ecological niches (e.g. lipid films). Little is known, however, about the quantitative aspect of soluble polymer degradation in sea water and its importance for the substrate pool equilibrium. Recently a few attempts have been made to measure soluble polymer degradation at *in situ* conditions using highly sensitive fluorometric or radiometric methods (Hoppe, 1983 ; Somville and Billen, 1983 ; Hollibaugh and Azam, 1983).

The method employed here for the determination of extracellular enzymatic activity relies on the acceptance of methylumbelliferon-linked monomers as substrate analogues for soluble macromolecules. It has been clearly shown that methylumbelliferyl (MUF)-

substrates compete with naturally occurring dimers and in some cases also with polymers (Hoppe, 1983; Somville, 1984). Furthermore, there was nearly no qualitative discrepancy in the growth of pure bacteria cultures with polymers and corresponding MUF-substrates. It is, however, clear that a much better working knowledge of the MUF-substrate properties is needed when extrapolation of results such as turnover rate or velocity of hydrolysis (V_m) in the natural pool of soluble polymers is desired. Nevertheless, such extrapolation is occasionally attempted here in a cautious manner, because correlation between extracellular enzymatic activity (EEA) and heterotrophic activity was convincing. Usually EEA on MUF-substrates followed first order enzyme kinetics as is also frequently reported for heterotrophic substrate uptake mechanisms. It may be suggested that application of enzyme kinetic approaches is even more suitable for studies on EEA than for heterotrophic uptake, because the function of extracellular enzymes seems to be more independent than the function of transport enzymes which are more closely linked to the cell metabolism (Krambeck, 1979). All the theoretical objections to the use of simple enzyme kinetics for multienzyme systems inherent with substrate uptake by different bacteria (Williams, 1973) may also be valid for EEA.

The results from culture experiments (Kim and Hoppe, this volume) were confirmed by EEA measurements of size fractions from natural waters. Size fractions dominated by free-living bacteria contributed most to EEA and there was little activity recorded from fractions >3 μm. Only phosphatases were more abundant in larger size classes (8 - 150 μm), where their activity was about twice as high as in the bacterial size classes. It may be that attached bacteria possess disproportionally higher polymer-degrading abilities than free-living ones, as suggested by Hollibaugh and Azam (1983) and Kim (personal discussion). However, in the investigated waters of the Kiel Bight (Baltic Sea) the fraction of attached bacteria is steadily less than 10 % of total bacteria and, thus, most EEA originates from free-living bacteria.

The existence of active free dissolved enzymes no longer closely associated with their sites (cells) of origin would be of considerable biochemical and ecological significance, since these enzymes could condition macromolecular DOC and organic surfaces for subsequent bacterial growth. Size fractionation experiments described here revealed different patterns for the abundance of free dissolved enzymes (found in 0.2 μm filtrates) dependent upon different MUF-substrates and substrate concentrations. Free proteases are generally believed to be scarce in marine waters. Our results suggest that the activity of free proteases may climb from 14 to 29 % of total activity of a water sample inversely depending on substrate concentration. The opposite was true for hydrolytic activities of β-glucosidase and N-acetyl-glucosaminidase, whereas no clear dependency on substrate concentrations could be observed for α-glucosidase and phosphatase.

From field measurements of EEA distribution it can be concluded that the turnover of α-glucosides and glucosaminides is much faster in polluted inshore areas than in offshore waters. Because V_m for α-glucosidase was also relatively high at the inshore station (c.f. Tab. 4) it may be suggested that this is due to a high abundance of bacteria specialized in the decomposition of α-glucosidic compounds. In the case of protein and organic phosphorous a much faster turnover was measured in the offshore region. This may be attributed to a smaller pool of these substrates and a relatively higher abundance of specialized bacteria in offshore waters.

The relation between the hydrolyzation rate (H_R) of the available polymer pool and the microbial turnover rate (T_R) of the corresponding monomers may provide some information on the coupling of these pools under natural conditions. For this it has to be accepted that the concentration of monomers in the water is kept rather constant over time and that monomers resulting from extracellular enzymatic decomposition of poly-

mers are immediately incorporated by microorganisms. In this case the relation between H_R and T_R would be equal to the relation between the pool of directly available polymers and the pool of monomers. In the present study we found that in a polluted inshore area the pool size of MUF-leucine substrate analogues (available protein pool) was about 6 times greater than the leucine pool. In offshore waters the corresponding pools were approx. of the same size. This means that the pool of available polymers, in the sense as it is used here, and the pool of the corresponding monomers is within the same order of magnitude.

It is suggested that besides exudation of phytoplankton, products resulting from extracellular enzymatic decomposition of soluble polymers by bacteria serve as an important source for bacterial nutrition in natural environments. The enzymatic hydrolyzation rate is strong enough to balance the pool of soluble monomers. It is expected that in sub-euphotic zones and at the bottom of water bodies extracellular enzymatic hydrolysis of polymers and oligomers may be even a key factor for the existence of heterotrophic bacteria.

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