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Microbial activities on *Trichodesmium* colonies

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ABSTRACT: During a cruise from 20 May to 19 June 1994 in the tropical North Atlantic Ocean between 30 and 10°N and in the Caribbean Sea, heterotrophic bacterial activities on colonies of the filamentous cyanobacterium *Trichodesmium* spp. were investigated. Thymidine incorporation rates between 0.011 and 0.045 pmol h^{-1} $colony^{-1}$ and leucine incorporation between 0.007 and 0.332 pmol h^{-1} $colony^{-1}$ were found. Turnover times of glucose varied between 31 and 267 d $colony^{-1}$ The hydrolysis rates (Hr) determined at trace concentrations of substrates ranged between 0.88 and 16.92% h^{-1} $colony^{-1}$ for phosphatase activities, between 0.01 and 0.87% h^{-1} $colony^{-1}$ for α-glucosidase activities, between 0.03 and 1.24% h^{-1} $colony^{-1}$ for β-glucosidase activities, between 0.02 and 0.58% h^{-1} $colony^{-1}$ for β-glucosaminidase activity, and between 0.40 and 7.60% h^{-1} $colony^{-1}$ for leucine peptidase activities, respectively. The quotient of glucose turnover and hydrolysis rate suggests that large quantities of released matter were not taken up by adherent bacteria. Most likely this dissolved material diffuses into the surrounding water and is available for free living bacteria. Thymidine incorporation of free living bacteria in surrounding water ranged between 0.977 and 18.49 pmol h^{-1} l^{-1} and for leucine incorporation between 21.44 and 127.67 pmol h^{-1} l^{-1} The turnover time of glucose of free living bacteria was between 24 and 109 d. Since abundance of colonies was approximately 7 colonies l^{-1}, this indicates that the activity of free living bacteria was substantially higher than that of attached bacteria on *Trichodesmium* colonies in the same volume of water

KEY WORDS: *Trichodesmium* · Cyanobacteria · Bacterial degradation · Thymidine incorporation · Leucine incorporation Extracellular enzyme activity

INTRODUCTION

The diazotrophic, bloom forming cyanobacterium *Trichodesmium* spp. is common in the euphotic zone of oligotrophic regions of tropical and subtropical oceans. *Trichodesmium* spp. are capable of N_2-fixation in the light under aerobic conditions. The nitrogen input by N_2-fixation of *Trichodesmium* comprises a quarter of the annual N_2-fixation of the ocean (Ohuki & Fujita 1988, Capone et al. 1990, Bergman & Carpenter 1991, Paerl et al. 1994). In the tropical North Atlantic Ocean (south of 30° N) and in the Caribbean Sea, the largest part of N- and C-input is caused by *Trichodesmium*. During different cruises and in different seasons between 1000 and 5500 *Trichodesmium* colonies m^{-3} were counted, which constituted approximately 61% of the total biomass (Carpenter & Price 1977, Carpenter & Romans 1991). *Trichodesmium* colonies caused a nitrogen input of 10 to 50 mg N m^{-2} d^{-1} by N_2-fixation and a carbon input of 55 to 275 mg C m^{-2} d^{-1} (Carpenter & Romans 1991).

Two different morphological types of *Trichodesmium* colonies can be found in natural samples: a filamentous type (tufts) and a spherical type (puffs). According to Borstad et al. (1993), the colonies can change their structure from tufts to puffs. In contrast to other phytoplankton blooms, it seems that *Trichodesmium* colonies do not sink to deeper layers (Pomeroy & Wiebe 1993). The highest abundances of colonies were counted in surface layers between 10 and 50 m. Below 150 m no *Trichodesmium* colonies were found (Carpenter & Price 1977). From these observations it can be concluded that substantial degradation of colonies occurs in the surface layer of the water column. During degradation of colonies part of the nitrogen bound by N_2-fixation will be transferred to the pool of dissolved nitrogen and can be used by other autotrophic and heterotrophic organisms (Carpenter & Price 1977).

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Dense bacterial colonization of around 50 000 bacteria per *Trichodesmium* colony were observed (Siddiqui et al. 1993), but very little is known about the nature of these bacteria (Zehr 1995). Bacteria are attached to the trichomes and also to the mucopolysaccarid layer surrounding the trichomes (Borstad 1978, Paerl et al. 1989). It has been proposed that the mucopolysaccarid layer has the function to protect the trichomes against the direct degradation by heterotrophic bacteria and may help to keep the trichomes in a colonial form (O'Neil & Roman 1992). On the other hand, bacteria may prevent the aggregation of phytoplankton by enzymatic degradation of the mucopolysaccarid layer (Azam & Smith 1991, Smith et al. 1995). According to observations of Borstad (1978), the attachment of bacteria to colonies is a characteristic feature of senescence, but these observations could not be confirmed by Paerl et al. (1989). They found that *Trichodesmium* colonies are densely colonized by bacteria during intense photosynthesis. Since bacteria are the main agents for degradation of particulate matter and the release of dissolved substances (Cho & Azam 1990, Azam & Smith 1991, Azam et al. 1992, Turley 1992), the colonization of *Trichodesmium* colonies may indicate an important transformation of organic matter produced by the cyanobacteria.

For the unproductive tropical and subtropical regions of the Atlantic Ocean there are only few data available about the activities of bacteria associated with particles and in the surrounding water The aim of this study was to investigate in detail the bacterial processes within *Trichodesmium* colonies.

METHODS

Trichodesmium spp. colonies were collected during the cruise with the RV 'Gyre' from 20 May to 19 June 1994 in the Atlantic Ocean between 30 and 10° N and in the Caribbean Sea (Fig 1). Colonies were collected by net tows (mesh size 243 µm) in depths between 10 and 30 m and individually picked up and transferred into 0.2 µm (sterile) filtered seawater by an inoculating loop.

Water samples for bacterial counts and estimation of cell volumes were preserved with 0.5% formaldehyde. Subsamples of 10 ml were filtered onto 0.2 µm black Nuclepore filters, stained with 4,6-diamidino-2-phenyl-indol (DAPI) solution for 5 min and mounted in immersion oil. Cells were counted and sized with an epifluorescence microscope (Zeiss) and an image analysis system (Photometrics) (Psenner 1993). Biomass calculations were performed as in Simon & Azam (1989) using the size dependent carbon content of bacterial cells.

Scanning electron microscopic investigations were performed with a CAM SCAN 44 'Analytic WEX' *Trichodesmium* colonies were fixed with 0.3% formaldehyde, dehydrated in a concentration series of ethanol (20 to 100%) and acetone, critical point dried and sputtered with gold.

Bacterial production was estimated by thymidine and leucine incorporation (Fuhrman & Azam 1982, Bell 1993, Kirchman 1993) with the 'dual label' method as in Chin-Leo & Kirchman (1988), Jonas et al. (1988), Simon et al. (1990) and Kirchman et al. (1993). Twenty colonies were transferred into 10 ml sterile filtered seawater containing ^{3}H-thymidine (Amersham, spec. activity 5 Ci mmol^{-1}) at a final concentration of 10 nM and ^{14}C-leucine (Amersham, spec. activity 300 mCi mmol^{-1}) at a final concentration of 100 nM. These samples were incubated in 20 ml plastic vials in the dark at *in situ* temperatures. After 2 h incubations they were stopped with 0.3% formaldehyde final concentration. Determinations were carried out in triplicate; formaldehyde prekilled samples were used as blanks. Samples were filtered onto 0.2 µm cellulose nitrate filters, rinsed 10 times with 1 ml 5% ice-cold trichloroacetic acid and radioassayed on board in an LKB Wallac liquid scintillation counter, using Ultima-gold XR (Packard) scintillation cocktail.

Thymidine and leucine incorporation of colonies was calculated as in Alldredge (1993):

$$\text{mol h}^{-1}\,\text{colony}^{-1} = (\text{dpm})(SA^{-1})(4.5 \times 10^{-13})/(N)(t)$$

where SA is specific activity of radioisotope solution (Ci mol^{-1}); 4.5×10^{-13} = Ci dpm^{-1}; N is number of aggregates; t is incubation time; and dpm (disintegration per minute) is activity in the sample.

For the estimation of bacterial production of free living bacteria, 20 ml of water sample were incubated

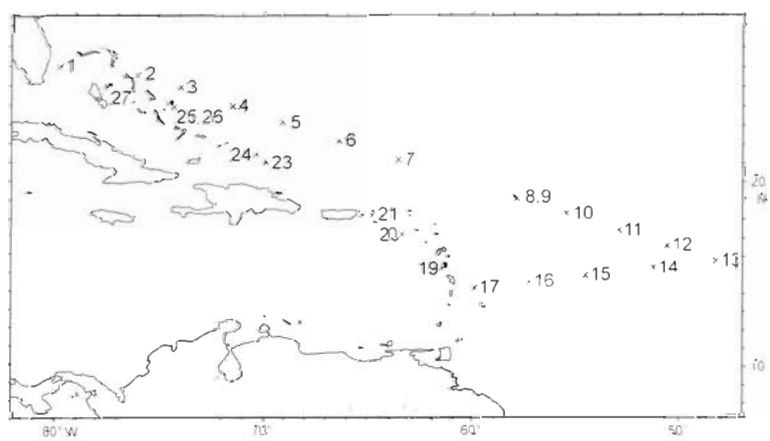

Fig. 1. Location of sampling stations during the cruise with RV 'Gyre' in May and June 1994

with 5 nM ^{3}H-thymidine and 50 nM ^{14}C-leucine as described above. Water samples were collected in 10 m depth with a rosette sampler. The bacterial biomass production was calculated from leucine incorporation using a conversation factor of 3.1 kg C mol^{-1} (Kirchman 1993).

For the determination of uptake of natural substrates (Gocke 1977, Güde 1988) the turnover rate (% h^{-1} colony^{-1}) of ^{14}C-glucose was measured. ^{14}C-glucose (Amersham, spec. activity 300 mCi mmol^{-1}) was added to 20 colonies in 10 ml sterile filtered seawater in concentrations of 33 nM and incubated as described above.

Bacterial extracellular enzyme activities on *Trichodesmium* colonies were determined according to the methods of Hoppe (1983, 1991, 1993). For the measurement of the hydrolysis rate (Hr) the following substrates were added: 4-methylumbelliferyl-(MUF)-phosphate, MUF-α- and MUF-β-glucoside, MUF-N-acetyl-β-glucosaminide, and leucine-methylcoumarinylaminide (leucine-MCA). All substrates were added in trace amounts of 50 nM except MUF-α-glucoside (100 nM) for the estimation of *in situ* hydrolysis of natural substrates. Tests showed that substrate saturation was reached at 12.5 µM for α-glucoside. The other substrates reached saturation concentrations at 50 µM. For each experiment 6 colonies were incubated in 1 ml sterile filtered seawater in the dark at *in situ* temperatures. Fluorescence readings were performed with a KONTRON-spectrofluorometer SFM 25 at 364 nm excitation and 445 nm emission. Calibration was performed with standard solutions of 4-methylumbelliferone and 7-amino-4-methylcoumarin in a range between 0.01 and 1.0 µmol l^{-1}.

For measurements of particulate organic carbon (POC) and nitrogen (PON) *Trichodesmium* colonies were collected on Whatman GF/F filters, which were precombusted at a temperature of 450°C. The samples on the filters were stored at −20°C until analysis in a CHN-analyser 'O-Rapid' (Haereus).

RESULTS

Trichodesmium colonies can be divided into sinkers and floaters. According to investigations of Romans et al. (1994), sinkers contain more carbohydrates than floaters. For this reason the colonies were separated at the first 2 stations into sinkers and floaters. However, sinkers and floaters did not show large differences particularly with respect to enzyme activities. Microscopic observations on board revealed that the trichomes of puffs were imbedded in mucopolysaccharides more strongly than those of tufts. Since this may affect bacterial activity, at the following stations the filamentous colonies (tufts) were separated from the spheric colonies (puffs). The colonization of *Trichodesmium* trichomes by bacteria observed by scanning electron microscopy showed coccoid and rod shaped bacteria attached to the trichomes (Fig. 2). In the mucus between the trichomes rod shaped bacteria seemed to dominate.

Thymidine and leucine incorporation in the whole area (except Stn 9) ranged between 0.011 and 0.045 pmol thymidine h^{-1} colony^{-1} and between 0.077 and 0.322 pmol leucine h^{-1} colony^{-1} (Fig. 3). Only at Stn 9 was incorporation substantially higher for thymidine (0.155 pmol h^{-1} colony^{-1} for tufts and 0.184 pmol h^{-1} colony^{-1} for puffs) and leucine (0.69 pmol h^{-1} colony^{-1} for tufts and 0.45 pmol h^{-1} colony^{-1} for puffs). At this station, the other parameters investigated were also higher than at the other stations. There was a significant correlation between thymidine and leucine incorporation ($r = 0.72$; $p = 0.01$). At some stations the thymidine incorporation was higher for tufts than for puffs; at other stations no differences could be observed. Leucine incorporation by puffs was generally lower than by tufts. On average leucine incorporation by tufts was twice that of puffs (Table 1).

The estimation of turnover of natural substrates via ^{14}C-glucose showed that at most of the stations less than 0.05% h^{-1} colony^{-1} of substrates were utilized (Table 1). Correspondingly the turnover times of glucose were very long: on average 142 d. This indicates that only very small amounts of material were taken up

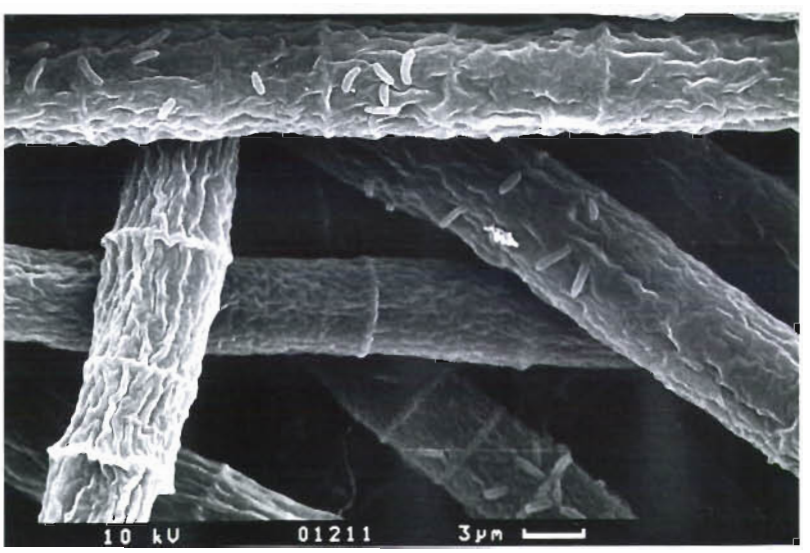

Fig. 2. Rod shaped bacteria associated with *Trichodesmium trichomes* (scanning electron micrograph) (R. Bahlo, Baltic Sea Research Institute, pers. comm.)

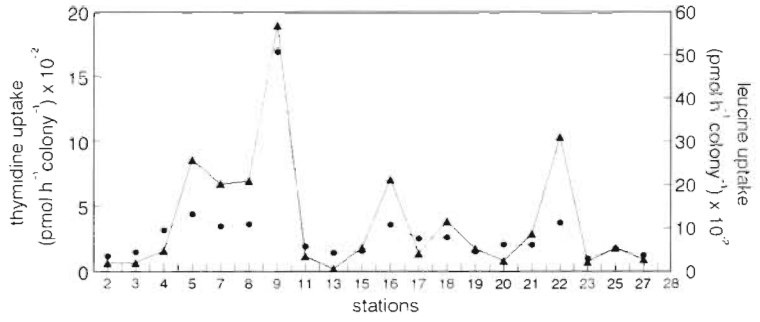

Fig. 3. Thymidine and leucine incorporation of *Trichodesmium* colonies at the stations (values are averaged for each station). (●) thymidine uptake, (▲) leucine uptake

by bacteria on *Trichodesmium* colonies. Table 1 shows that similar spatial trends as those found for bacterial production apply for glucose turnover rates, causing a significant correlation ($r = 0.94$; $p = 0.01$) between glucose turnover and bacterial production. No significant differences between glucose turnover of tufts and puffs could be detected.

At some selected stations bacterial counts, biomass, production and glucose turnover of free living bacteria in the surrounding water at a depth of 10 m were determined (Table 2). Thymidine incorporation ranged between 0.98 and 18.49 pmol h^{-1} l^{-1}; leucine incorporation ranged between 21.44 and 127.62 pmol h^{-1} l^{-1} The glucose turnover of free living bacteria ranged between 0.04 and 0.18% h^{-1} l^{-1}, corresponding to an average turnover time of 49 d. No correlation was found between bacterial activities in the surrounding water and on *Trichodesmium* colonies at different stations. During the cruise, on average 7 colonies l^{-1} were counted (E. J. Carpenter pers. comm.). The bacterial production on these colonies amounted to only 1–2% of the bacterial production of free living bacteria in the water. On the other hand the glucose turnover of bacteria on 7 colonies l^{-1} had the same range as that measured for the free living bacteria in 1 l of water

The hydrolysis rate of extracellular enzymes (Hr) for all substrates on average was higher for tufts than for puffs at many stations (Table 3). For the activities of enzymes the following sequence was found for tufts as well as for puffs: phosphatase > peptidase > β-glucosidase > β-glucosaminidase > α-glucosidase (Table 3). There was a significant correlation ($r = 0.61$; $p = 0.05$) between hydrolysis rate through peptidase and leucine incorporation (Fig. 4). The glucose turnover showed a significant correlation with the hydrolysis of α-glucoside ($r = 0.69$; $p = 0.05$), but not with β-glucoside (Fig. 5). The quotient between glucose turnover and the hydrolysis of carbohydrates (Tr/Hr) varied between 0.01 and 0.29.

Table 1 Thymidine and leucine incorporation as well as turnover rate and turnover time of glucose in *Trichodesmium* colonies of sinkers, floaters, tufts and puffs. SD: standard deviation

Stn	Thymidine uptake (pmol h^{-1} colony^{-1})				Leucine uptake (pmol h^{-1} colony^{-1})				Glucose turnover (% h^{-1} colony^{-1})				Turnover time (d)	
	Sinkers		Floaters		Sinkers		Floaters		Sinkers		Floaters		Sinkers	Floaters
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
3	0.013	0.003	0.018	0.001	0.021	0.020	0.019	0.008	0.020	0.002	0.023	0.005	209	182
4	0.038	0.002	0.026	0.002	0.052	0.019	0.045	0.020	0.041	0.001	0.022	0.004	100	190
	Tufts		Puffs		Tufts		Puffs		Tufts		Puffs		Tufts	Puffs
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
7	0.034	0.001	0.036	0.004	0.272	0.016	0.133	0.033	0.041	0.005	0.049	0.008	102	85
8	0.037	0.001			0.210	0.014								
9	0.156	0.072	0.184	0.018	0.699	0.410	0.452	0.206	0.127	0.007	0.133	0.029	33	31
11			0.020	0.002			0.036	0.013			0.028	0.003		222
13			0.015	0.001			0.007	0.002			0.018	0.002		229
15	0.016	0.001			0.055	0.017			0.033	0.008			127	
16	0.041	0.010	0.032	0.001	0.597	0.503	0.045	0.019	0.029	0.007	0.031	0.001	141	135
17			0.026	0.002			0.042	0.003			0.025	0.003		161
18	0.028	0.004			0.116	0.049			0.021	0.005			196	
19	0.016	0.002			0.053	0.035			0.015	0.001			267	
20	0.025	0.004	0.017	0.002	0.302	0.149	0.026	0.006	0.019	0.001	0.022	0.002	217	186
21	0.021	0.003			0.082	0.043			0.028	0.009			149	
	0.045	0.003	0.030	0.005	0.302	0.160	0.322	0.173	0.05	0.021	0.031	0.007	83	135
23	0.011	0.001			0.023	0.004			0.024	0.003			174	
25	0.018	0.002			0.055	0.025			0.047				87	
27	0.013	0.001			0.029	0.020			0.053	0.009			79	

Table 2. Bacterial counts, production and glucose turnover of free living bacteria in the water at 10 m depth at selected stations. Bacterial production was calculated using a conversion factor from Kirchman (1993). SD: standard deviation; nd: not determined

Stn	Bacterial counts ($\times 10^6$ l^{-1})	Bacterial biomass (µg C l^{-1})	Thymidine uptake (pmol h^{-1} l^{-1}) Mean	SD	Leucine uptake (pmol h^{-1} l^{-1}) Mean	SD	Biomass production (ng C h^{-1} l^{-1})	Glucose turnover (% h^{-1}) Mean	SD	Turnover time (d)
5	348	35.7	11.62	0.06	42.75	11.41	132.13	0.04	0.007	103
6	608	64.8	1.66	0.01	nd	nd		0.04	0.005	109
10	175		5.37	0.03	21.44	5.72	66.27	0.04	0.007	37
13	411	52.5	18.49	0.11	127.62	34.08	394.49	0.12	0.006	37
16	696	93.1	13.88	0.08	68.48	18.29	211.69	0.18	0.015	28
19	528	54.8	11.15	0.06	68.38	18.26	211.36	0.18	0.030	23
25	540	63.6	10.67	0.06	35.54	9.49	109.85	0.14	0.027	30
27	nd	nd	0.98	0.01	nd	nd		0.17	0.009	24

Table 3. Hydrolysis rate (Hr, in % h^{-1} colony^{-1}) of extracellular enzyme activities of bacteria associated with *Trichodesmium* colonies. SD: standard deviation

Stn	Phosphatase activity				α-glucosidase activity				β-glucosidase activity				β-glucosaminidase activity				Peptidase activity			
	Floaters		Sinkers		Floaters		Sinkers		Floaters		Sinkers		Floaters		Sinkers		Floaters		Sinkers	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2.20	1.40	3.60	1.60	0.01	0.01	0.03	0.01	0.42	0.3	0.03	0.01	0.02	0.02	0.12	0.12	2.60	0.86	0.40	0.10
2	4.80	2.10	3.00	0.88	0.05	0.04	0.02	0.01	0.60	0.60	0.12	0.05	0.04	0.02	0.18	0.06	2.00	1.2	2.64	0.08
3	3.80	1.12			0.04	0.03			0.20	0.12			0.18	0.12			0.40	0.22		
4	3.80	1.40			0.03	0.02			0.16	0.12							0.50	0.24		
	Tufts		Puffs		Tufts		Puffs		Tufts		Puffs		Tufts		Puffs		Tufts		Puffs	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
7	22.16	10.4	5.56	2.32	0.28	0.26	0.27	0.04	0.44	0.08	0.66	0.10	0.30	0.04	0.14	0.08	7.60	0.22	3.46	1.48
10	5.62	2.52	4.42	1.86	0.19	0.14	0.17	0.06	0.32	0.22	0.22	0.12	0.48	0.22	0.34	0.12	3.26	1.46	1.86	0.46
19	19.60	4.46	11.36	2.34	0.23	0.07	0.10	0.06	0.24	0.1	0.15	0.06	0.23	0.04	0.04	0.04	3.60	0.46	1.68	1.16
21	11.88	1.82	16.36	4.26	0.13	0.02	0.12	0.06	0.90	0.56	0.32	0.12	0.58	0.58	0.38	0.20	5.00	1.64	1.16	0.46
	5.12	3.2	10.02	1.9	0.08	0.02	0.14	0.10	0.82	0.42	0.24	0.08	0.20	0.16	0.08	0.02	3.70	1.62	1.14	0.02
23	10.32	1.54	5.54	0.90	0.09	0.06	0.18	0.10	0.96	0.22	0.88	0.44	0.28	0.28	0.16	0.12	1.14	0.78	1.02	0.26
25	16.92	1.96			0.55	0.41			1.04	0.46			0.10	0.06			3.22	0.34		
26	13.78	1.26			0.87	0.40			1.24	0.54			0.32	0.26			2.36	0.86		

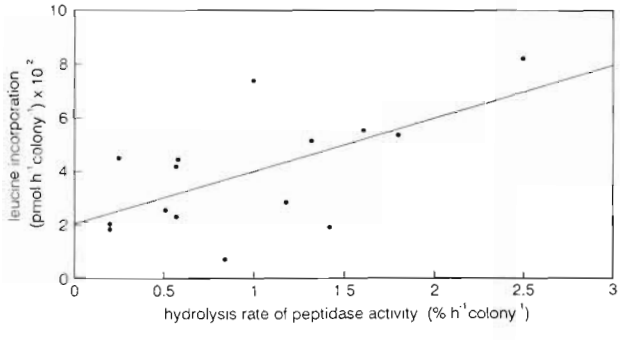

Fig. 4. Correlation ($y = 1.95x + 2.05$, r = 0.61, n = 15, p = 0.05) between hydrolysis of proteins and leucine incorporation of bacteria associated with *Trichodesmium* colonies, high value at Stn 9 excluded

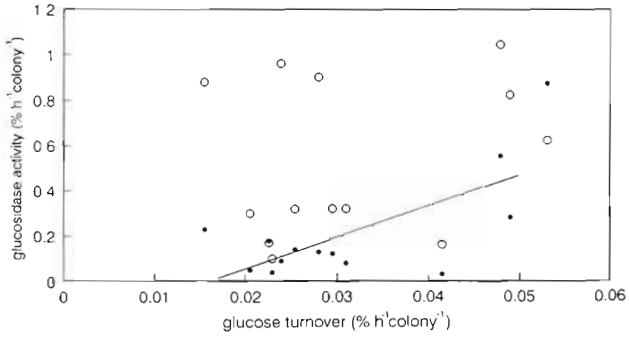

Fig. 5. Correlation between glucose turnover and hydrolysis rates of carbohydrates by α- and β-glucosidases: α-glucos.. $y = 13.69x - 0.22$, r = 0.69, n = 13, p = 0.01; β-glucos.: no correlation; (●) α-glucosidase; (○) β-glucosidase

The POC content of freshly isolated *Trichodesmium* colonies ranged between 4.0 and 21.8 µg C colony^{-1}, on average 9.1 µg C colony^{-1} PON values varied between 0.7 and 3.8 µg N colony^{-1}, on average 1.6 µg N colony^{-1}. The C/N ratio was relatively constant between 4.5 and 6.9, on average 5.7.

DISCUSSION

Bacterial activities determined on *Trichodesmium* colonies can be influenced by the following factors related to colony morphology:

(1) *Trichodesmium* colonies consist of different numbers of trichomes.

(2) Trichomes in colonies have a different compactness causing different sizes of free spaces which are suitable for settlement of microorganisms.

(3) *Trichodesmium* colonies are embedded in a mucopolysaccharide layer which is a habitat for microorganisms. This mucopolysaccharide layer is differently developed in the colonies.

(4) Not only *Trichodesmium* colonies and the adherent bacteria were transferred into 0.2 µm filtered seawater, but also other organisms associated with the colonies, for instance bacteriovorous protozoa.

In this investigation the thymidine incorporation rates of bacteria on *Trichodesmium* colonies had the same range as those found by Alldredge et al. (1986) and Alldredge (1993) for marine snow in the southern North Atlantic. It has to be kept in mind, however, that marine snow particles mainly consist of detritus and only a few living algal cells, while *Trichodesmium* colonies are dominated by living, photosynthetically active cells. The high values at Stn 9 cannot be explained by environmental variables like temperature, salinity or chlorophyll fluorescence. Possible factors which could influence the measured bacterial activities on *Trichodesmium* colonies are the size and age of colonies, the bacterial populations which colonize them, as well as grazers of bacteria. According to Zehr (1995) the extent of bacterial colonization and the physiological state of *Trichodesmium* colonies may interact. These factors may be responsible for strong variations; however, we have no means to determine the physiological state of *Trichodesmium* colonies under natural conditions.

During our cruise the bacterial production in 1 l water was higher than that of bacteria on colonies contained in this volume. The biomass production of bacteria associated with *Trichodesmium* colonies using the conversion factor from Kirchman (1993) ranged between 0.07 and 2.17 ng C h^{-1} colony^{-1} or between 0.49 and 15.19 ng C h^{-1} for 7 colonies in 1 l of water. Using the same conversion factor, the bacterial production of free living bacteria ranged between 21.44 and 211.69 ng C h^{-1} l^{-1}. These observations of higher activity of the bulk of free living bacteria compared to the attached ones are corroborated by observations of Alldredge et al. (1986) and Alldredge (1993). Aggregates are inhabited by bacteria with abundances 2 to 5 times higher than in an equivalent volume of water. Nevertheless, through the low abundance of aggregates the number of attached bacteria represented only 0.1 to 4.4% of the total number of bacteria in the surface water of the North Atlantic. In Antarctic regions of the Atlantic Ocean about 20% of bacteria were associated with particles (Delille 1993), and in Chesapeake Bay less than 10% were found on particles (Griffith et al. 1994). On the basis of high ^{14}C-glucose uptake and low ^{3}H-thymidine incorporation, Kirchman (1983) concluded that bacteria on particles have a high metabolism but a low frequency of cell division. This is in accordance with our observations that in 1 l of water bacteria on *Trichodesmium* colonies had the same range of glucose turnover but a lower ^{3}H-thymidine incorporation than free living bacteria, indicating that Kirchman's (1983) conclusions may also be applicable to these colonies.

Colonization of *Trichodesmium* with microorganisms was previously described by O'Neil & Roman (1992), and Siddiqui et al. (1993). According to Carpenter & Price (1977) up to 8.3% (<1 to 8.3%) of *Trichodesmium* trichomes are populated with bacteria. Major groups of attached bacteria were identified as flavobacteria, enterics and β-proteobacteria (Zehr 1995).

Alldredge (1993) discussed possibilities which may cause an underestimation of bacterial production on aggregates. One possibility is that the coupling of hydrolysis and uptake is so tight that no organic substances will be taken up from the surrounding water. Since in our investigations there was a correlation between hydrolysis of polypeptides or carbohydrates and the uptake amino acids or monosaccharides, we have to assume that the substrate uptake from the surrounding water was not prevented. Another possibility of underestimation is that caused by the density of trichomes. The diffusion of radioisotopes inside the colonies may be hindered, or dilution of radioisotopes may occur due to the release of dissolved organic matter from *Trichodesmium* cells. According to Capone et al. (1994) about 2 nmol d^{-1} colony^{-1} glutamate and glutamine may be released by exudation from *Trichodesmium* colonies. The lower bacterial activities of puffs relative to tufts of most of measured bacterial variables and the observation of higher mucus content of puffs suggest that the diffusion of added radioactive and fluorogenic substrates may be inhibited to a certain degree. An underestimation of our results cannot be completely excluded. However, since

bacterial production on colonies in general was lower by at least 1 order of magnitude compared to that of free living bacteria, the underestimation would have to be extremely high to change the conclusion that most activity was associated with free living bacteria.

Bacteria can only take up dissolved organic substances with low molecular weight; thus they hydrolyse high molecular weight substances by means of extracellular enzymes (Hoppe et al. 1988, Münster 1991, Overbeck 1991, Hoppe 1993). Only a small part of phosphatase activity is attributed to bacteria (Chrost 1991, Wetzel 1991) as it will be produced predominantly by phytoplankton if phosphate is limiting. The other enzyme activities are mainly associated with heterotrophic bacteria. The hydrolysis rates of substrates by extracellular enzymes is directly dependent on the pool of substrates and reflects the turnover of natural substrates. The quotient between substrate turnover and hydrolysis rate (Tr/Hr) describes the coupling between substrate hydrolysis and substrate uptake by bacteria (Hoppe et al. 1988, Hoppe 1991). A low Tr/Hr quotient may indicate that the hydrolysed substrates were not completely used by attached bacteria. Therefore, it can be assumed that a large portion diffuses into the surrounding water. A comparison of the hydrolysis rates of α- and β-glucosides for 7 colonies in 1 l of water (1.70% h^{-1} for α-glucosides and 4.21% h^{-1} for β-glucosides) and the glucose turnover of attached and free living bacteria in the same volume (0.28% h^{-1} and 0.11% h^{-1}, respectively) could demonstrate that only small parts of released substrates were taken up. This phenomenon of a loose connection between hydrolysis of high molecular weight substances and the uptake of low molecular weight substances by particle associated bacteria has also been described by Azam & Smith (1991), Smith et al. (1992, 1995) and Middelboe et al. (1995). With respect to free living bacteria, Chrost & Overbeck (1990) found a close coupling between the heterotrophic activity and the activity of β-glucosidase in limnic biotopes. In this investigation a correlation exists between hydrolysis of α-glucose and glucose turnover of attached bacteria. This indicates a partial coupling between substrate hydrolysis and substrate turnover by attached bacteria. According to Hoppe et al. (1988) in a balanced system there is an equilibrium between the release of monomers through enzymes and the uptake by bacteria. In an imbalanced system more monomers are produced than can be taken up by bacteria, causing a transient increase of low molecular substances in the dissolved organic carbon (DOC) pool. This was derived from a double labeling experiment with leucine-MCA as a peptide analogue and ^{3}H-leucine as the corresponding product of peptide hydrolysis.

Organic carbon will be released by glucosidase and peptidase activity, organic nitrogen by peptidase and β-glucosaminidase activity. Although the investigation of enzymatic hydrolysis with model substrates covers only a part of the total hydrolytic activities, the magnitude of carbon and nitrogen release from *Trichodesmium* colonies by enzyme activities was tentatively calculated. Values of potential PON and POC release by bacterial enzyme activity were calculated from PON and POC stocks of *Trichodesmium* colonies and the hydrolysis rate of proteins and carbohydrates (Hoppe et al. 1993). A theoretical C and N release between 30.52 and 1086.32 ng C h^{-1} $colony^{-1}$ and between 4.56 and 209.25 ng N h^{-1} $colony^{-1}$ was calculated. Given 7 colonies l^{-1} in our investigations, between 214 and 7460 ng C h^{-1} and between 32 and 1465 ng N h^{-1} could be hydrolyzed by enzymatic activities of the attached bacteria and potentially supplied to the surrounding water.

In summary, our results support the thesis that attached bacteria play an active role in the degradation of *Trichodesmium* colonies and the release of low molecular weight substances into the surrounding water This process is one possibility for the transformation of new nitrogen introduced by N_2-fixation in nutrient depleted subtropical and tropical waters, but it is limited by the low average abundance of colonies in the surface water.

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