Vol. 19: 37–45, 1999 | AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol | Published September 6

Bacterial utilization of humic substances from the Arctic Ocean

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ABSTRACT: The main water exchange between the Arctic and the Atlantic oceans takes place at the Fram Strait. Humic substances (HS), derived from the whole Arctic, are included within the water entering the Atlantic. Batch experiments were set up to investigate the bioavailability of the Arctic HS to bacteria and to characterize any controlling factors. HS were extracted with XAD resins 2 and 4, and served as the sole carbon source for bacteria. Leucine incorporation rate, oxygen consumption, bacterial cell counts and dissolved organic carbon (DOC) concentrations were monitored for the experiments. Overall, 11% of the initial carbon was consumed and a turnover time of 220 d was extrapolated for marine HS in polar surface waters and a profound temperature dependence was observed (Q_{10} = 2.9 to 4.8). Comparing the bioavailability of HS from surface water with that of sea water-DOC revealed no significant differences, with both showing the same quality as a bacterial carbon source. Additionally, the bioavailability of HS from surface water was compared with that of HS from deep water. This comparison revealed that surface HS are of significantly better bacterial food quality than deep water HS. The experiments indicated that marine HS play an active role in bacterial carbon cycling in the Arctic Ocean.

KEY WORDS: XAD-resin · DOC · Fram Strait · Bioavailability

INTRODUCTION

Humic substances (HS) constitute the largest fraction of dissolved organic matter (DOM) in most natural waters (Thurman 1985) and for a long time have been considered biologically inert. This assumption originates mainly from freshwater and soil studies, where HS may even have inhibitory effects on bacterial growth (Kontchou & Blondeau 1990). Recent studies from the marine environment, however, show that HS can be used as a carbon source by bacteria, with all the associated biogeochemical implications (Moran & Hodson 1994, Bano et al. 1997). Most studies on the bioavailability of HS have focused on estuaries or coastal areas with high allochthonous input of HS. Less attention has been paid to areas with low terrestrial input and correspondingly autochthonous marine HS. In contrast to terrestrial-derived HS with high phenol and lignin content (Opsahl & Benner 1995), seawater HS are essentially aliphatic and considered to be of phytoplankton origin (Ishiwatari 1992).

The Fram Strait, located between Greenland and Svalbard, is an area of intensive exchange of water masses between the Polar Ocean and the North Atlantic. The upper layer of the Arctic Ocean is characterized by cold, low salinity water (polar mixed surface water). The polar mixed surface water of the Fram Strait is composed of water masses of different origin, e.g. returning Atlantic water and Pacific water (Rudels 1989, Jones et al. 1998), superimposed with strong local influences such as melting ice floes and an enhanced primary production (Smith et al. 1985, Thomas et al. 1995). Therefore the terrestrial influence of the large Siberian rivers is considered to be minor in the surface waters of the Fram Strait. A well-developed halocline separates the surface layer from the large body of Arctic Ocean deep water. The outflow of the saline Arctic

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Ocean deep water to the Greenland Sea, through the western Fram Strait, provides a direct connection between the deep thermohaline circulation of the Arctic Ocean and the seas to the south (Aagaard et al. 1991). As DOM is also encompassed in this water mass exchange, it is therefore important to know more about the characteristics and the bioavailability of the Arctic-derived DOM entering the Atlantic.

This study focuses especially on the bacterial utilization of HS, as a presumably stable part of Arctic DOM being exported into the Atlantic. Batch experiments were set up to investigate the mechanisms regulating the bioavailability of HS in a polar marine system with low concentrations of total DOM and low temperatures.

MATERIAL AND METHODS

From August to September 1997, RV 'Polarstern' crossed the Fram Strait from Greenland to Svalbard (Krause 1998) at about 80° N (Table 1). Water samples (20 l) were taken with Niskin bottles from a rosette and transferred immediately to a dark, constant-temperature room (0°C), where the samples were processed. All experiments were carried out on board.

Bacteria were extracted from 20 l of seawater using 2 stirred ultrafiltration cells (Amicon 8400). The autoclaved cells were connected with Teflon tubing. The first cell served as a prefilter (0.8 µm) to remove most grazers, with the second filter (0.2 µm) concentrating bacterial cells. The filtration was gravity driven by placing the sample container and the filtration cells on different levels, ca 1 m apart. To prevent the filters from clogging, the water was stirred slowly all the time. Filtration was done at 0°C in the dark with a flow rate of ca 1 l h^{-1}. The first 4 to 5 l were discarded. At the end of the extraction process, the supernatant of the 0.2 µm filter (generally 200 ml) was taken as inoculum. Bacterial concentration in the experiments was ca 10% of the naturally occurring concentration.

The incubation medium was artificial seawater and contained the following salts (g l^{-1}): 24 NaCl; 5.3 $MgCl_2 \cdot 6\ H_2O$; 7 $MgSO_4 \cdot 7\ H_2O$; 0.7 KCl; 0.1 $CaCl_2 \cdot 2\ H_2O$. Trace element solution was added at a concentration of 1 ml l^{-1} (Pfennig et al. 1981). KNO_3 (400 µM) served as a nitrogen source and the medium was buffered with 200 µM PO_4 (Na_2HPO_4/KH_2PO_4) and 200 µM $NaHCO_3$ (all final concentrations) to a pH of 7.3 to 7.5.

HS were extracted with XAD 2 and 4 resins (Serva), with pore sizes of 9 and 5 nm respectively. The resins had been cleaned intensively by Soxlett distillation according to Malcolm (1989). Each resin (30 ml) was packed into a liquid chromatography column. The filtrate from the bacteria concentration was used for HS extraction after adjusting the pH to 2 with HCl. Within 24 h, the filtrate ran through the XAD columns—first XAD 2 followed by XAD 4. Thereafter, the columns were rinsed with 200 ml of 0.01 N HCl to remove salts. The HS were finally eluted with 200 ml of 0.1 M NaOH for the acid fraction (Hb A), followed by 200 ml methanol for the neutral fraction (Hb N). To eliminate MeOH, the sample was processed 3 times in a rotary evaporator with Milli-Q water. The hydrophilic fraction (H I) was the effluent of the resins, taken after approximately 10 l had passed through the columns. For the experiments, the buffers were added immediately to the eluate and the pH was adjusted to pH 7. Preliminary experiments with Arctic surface water had shown that about 40% of the DOC was retained and could be eluted from the resins. Blanks with Milli-Q water showed no increase of DOC in neither the effluent nor in the eluate (author's unpubl. data).

Five experiments were set up to assess the bioavailability of Arctic HS (Table 1). In Expt 1, to assess the influence of carbon concentration (HS concentration) on carbon consumption rate (HS consumption), the concentration of HS ranged from 18 to 168 µM C. Temperature dependence of the HS degradation was investigated in Expt 2, with temperatures ranging from −1 to 10°C. Expt 3 compared the different HS fractions

Table 1 The different experiments, location of the sample stations and the natural concentrations of DOC and humic substances (HS) (n = 3)

Expt		Position	Water depth	HS fraction	DOC (µM C)	HS[b] (µM C)	% HS[b] of total DOC
1	Substrate concentration	77° 55' N, 5° 18' W	26 m	Hb A	110 ± 5	18 ± 5	16
2	Temperature[a]						
3	Different HS fractions	80° 53' N, 2° 34' W	Surface	Hb A, Hb N, H I, Seawater	122 ± 8	27 ± 3	22
4	Deep water	81° 05' N, 2° 02' W	3394 m	Hb A	47 ± 2	17 ± 4	36
5	Surface water	81° 02' N, 7° 47' W	20 m	Hb A	83 ± 0	22 ± 1	27

[a]For Expt 2 the same material was taken as for Expt 1
[b]For Hb A only

with total seawater. In this experiment, the seawater, and the Hb A and Hb N fractions were diluted with artificial seawater to obtain the same initial DOC concentrations as in the H I fraction. In Expts 4 and 5 the bioavailability of surface versus deep water HS was compared.

Incubations were done at 0°C (unless otherwise stated) in 1 l acid-rinsed glass bottles. Bacteria were added to the medium immediately after filtration. The experiments started with the addition of the HS to the prepared bottles. In general, the concentration of the HS was set at 2.5 times the natural concentration. Samples for cell counts, dissolved organic carbon (DOC) concentration, leucine incorporation rate and oxygen consumption were taken over a time period of up to 400 h. All settings were run in duplicate (a/b).

Negative controls were set up without bacteria or without HS. Positive controls were prepared and grown with glucose and acetate (150 µM each) as the labile carbon source and also with HS added to the glucose and acetate.

Samples for DOC determination were stored frozen in precombusted glass ampoules. The samples were not filtered and therefore included bacterial carbon. Back in the laboratory, DOC was analyzed by high temperature combustion, using a Shimadzu TOC 5000 analyzer equipped with an auto sampler. Potassium hydrogen phthalate was used as the standard substance. Before injection, samples were acidified with HCl to pH 2 and sparged for 10 min to remove the inorganic carbon as CO_2. Samples from 1 time series were measured on the same day, each series starting with the lowest concentrations.

Measurements of ^{3}H-leucine incorporation rate (LEU) were carried out, according to the methods of Chin-Leo & Kirchman (1988). Leucine with a specific activity of 58.0 Ci mmol^{-1} (Amersham) was used and added to a final concentration of 10 nM. To increase the sensitivity, 20 ml samples were incubated for 6 h at 0°C with the incubations done in triplicate. Tests showed that LEU was still linear over 6 h incubation time, but with a lower variability. Formalin-killed samples served as blanks. Incubation was stopped by filtration onto nucleopore filters and, after extraction of proteins with ice cold 5% TCA, the filters were frozen. In the laboratory, the filters were transferred into Filter Count Scintillation Cocktail (Packard) and radio-assayed in a Packard TriCarb liquid scintillation counter. Quench correction was performed by automatic external standardization. Initial LEU (pmol l^{-1} h^{-1}) is the rate at the start of the experiment (t = 0). Cumulative LEU $\int_{0h}^{200h}$LEU in nmol l^{-1} is the rate integrated over 200 h (the shortest duration of all experiments).

For oxygen consumption rates, incubations were done in triplicate 50 and 100 ml BOD bottles. Oxygen concentrations were determined by the Winkler method with a Mettler DL 21 auto-titrator with potentiometric end-point detection (Granéli & Granéli 1991). Respiration rates were calculated from the slope of a linear curve of O_2 concentration versus incubation time. The lag phase, before a measurable O_2 consumption started, was omitted.

Growth yield was calculated using the formula GY (%) = $prod/(prod + resp) \times 100$. *Prod* being the bacterial carbon produced, based on LEU, which was converted into bacterial carbon (Simon & Azam 1989), and *resp* being the bacterial carbon respired, based on oxygen consumption, which was converted into carbon consumption with the ratio 1:1 (Pomeroy et al. 1995).

Acridine orange direct counts (AODC) of bacteria were determined by epifluorescence microscopy. The samples were fixed with 1% formaldehyde (final concentration), filtered onto black 0.2 µm nucleopore filters (Costar) and subsequently stained with 0.01% acridine orange. Microscopic analyses were carried out with a Zeiss Axioskop 20. Cell counts were further used to calculate the growth rate ($\mu = [\ln N t_2 - \ln N t_1]/t_2 - t_1$), doubling time ($t_D = 1/\mu$) and lag time ($t_L = t_2 - [\{\ln N t_2 - \ln N t_1\}/\mu]$) according to Schlegel (1985). N being the number of cells at sampling point t_1 or t_2.

The Q_{10} values were determined for the temperature range −1 to 10°C and calculated from the slope of the conventional Arrhenius plot (the natural logarithm of the rate versus the inverse of absolute temperature) using the expression $Q_{10} = \exp(-10 \times m/T^{-2}_{is})$ (Raven & Geider 1988), with T_{is} being the *in situ* temperature (0°C) in Kelvin, and m as the slope of the regression line of the Arrhenius plot.

Statistical analyses were performed with StatView 4.5 for Macintosh (1994). Regression analysis was performed after log transformation and Student's t-tests after rank transformation of the data to obtain a normal distribution (Bortz 1985).

RESULTS

DOC concentrations in surface seawater in the Fram Straight varied between 83 to 122 µM, while deep water had a lower concentration, at 47 µM C (Table 1). Concentrations of HS for all stations ranged from 17 to 27 µM C. HS therefore accounted for 16 to 27% of the total surface DOC, and 36% of the 1 deep water sample (Table 1).

Controls showed that the addition of the HS concentrate (with its high salt concentration) had no effect on bacterial growth, as there was no significant difference (Wilcoxon signed rank test, p = 0.92, n = 2 × 6) of LEU

in the presence or absence of HS (with glucose/acetate as labile carbon source). Sterile controls showed no change of DOC over time, indicating no detectable uptake of DOC out of the surrounding air. Bacterial cell counts in controls without any added carbon source were in the range of 4 to 11% of the counts in the corresponding sample (data not shown). Samples were not corrected for these controls.

To investigate the influence of carbon concentration (HS concentration) on the carbon (HS) consumption rate, DOC data from Expt 1 (Table 1) and all DOC data from experiments with surface Hb A at 0°C were pooled (n = 10). Initial HS concentrations ranged from 18 to 168 µM C, and the corresponding consumption rates varied between 0.003 and 0.09 µM C h^{-1}. HS concentrations were far from reaching substrate saturation kinetics, thus a linear relationship was assumed (Fig. 1). HS consumption rate was linearly dependent on the carbon concentration (r^2 = 0.66, p = 0.004). Assuming a mean natural concentration of 22 µM C (Table 1), the HS consumption at this natural concentration was calculated to be 0.1 µM d^{-1}.

The initial HS concentration was not always in the same range in the experiments. To correct for this, I looked for relationships between initial HS concentration and the other parameters. But, apart from cumulative LEU, none of the parameters (t_D, t_L, initial LEU and O_2 consumption) showed a significant relationship to the initial HS concentration. The cumulative LEU depended linearly on the initial HS concentration (r^2 = 0.32, p = 0.0059, n = 22, log-transformed data of all experiments). Thus, for the 'Discussion' and Tables 2 & 3 only 'cumulative LEU' was corrected for different HS concentrations.

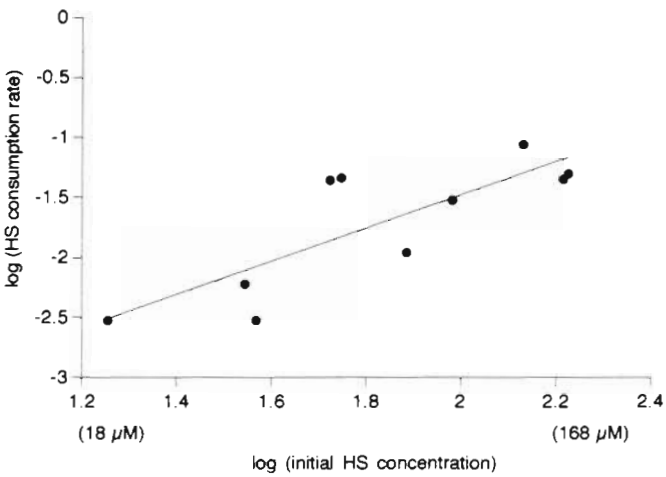

Fig. 1 Dependence of the humic substance (HS) consumption rate on the initial HS concentration, with log-transformed data. The numbers in parentheses show the actual concentrations

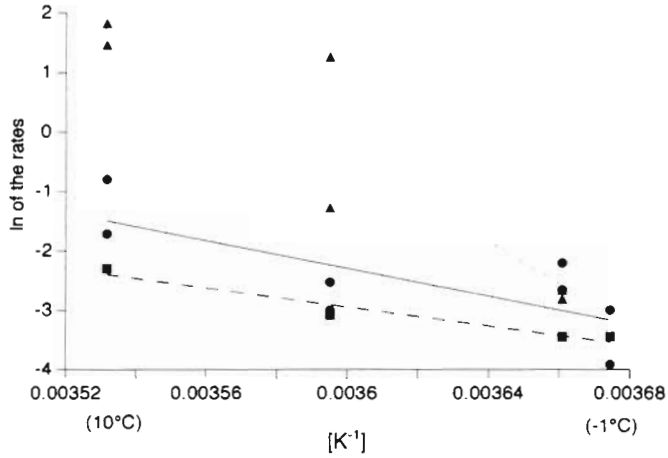

Fig. 2. Temperature dependence of oxygen consumption, initial leucine incorporation and growth yield. Measurements were made at –1, 0, 5 and 10°C. The Arrhenius plot shows the inverse absolute temperature versus the natural logarithm of the rates as follows: (●) leucine incorporation rate, (■) oxygen consumption rate and (▲) growth yield

To assess the influence of temperature on HS degradation (Expt 2), incubations were set up at –1, 0, 5 and 10°C. The Q_{10} was calculated for the O_2 consumption rate and initial LEU (Fig. 2). Q_{10} ranged from 2.90 ± 1.20 for O_2 consumption to 4.81 ± 1.72 for initial LEU. There was also a significant temperature dependence for growth yield (Fig. 2), but the slope was too steep to calculate a sensible Q_{10}. For AODC-based variables, no temperature dependence could be calculated, as for the '–1°' and '0°' settings no increase of cell numbers was detected.

In the third experiment the bioavailability of the seawater DOC was compared to the different HS fractions (Hb A, Hb N and H I). The experiment started with an initial cell concentration of 3.4 × 10^3 cells ml^{-1} (Fig. 3A). After 400 h the stationary phase was reached, with cell counts ranging from 6.57 × 10^4 (Hb Ab) to 1.36 × 10^6 (Hb Aa), with a mean of 3.8 × 10^5. Growth rates (0.02 h^{-1}) were about the same for all samples (Table 2). Lag time was highest for Hb Aa (85 h), while H Ia/b and Hb Nb grew exponentially from the beginning of the experiment (Table 2). Initial LEU differed by 1 order of magnitude between 0.06 and 1.69 pmol Leu l^{-1} h^{-1}. After an exponential increase, a mean value (± SD) of 200 ± 39 pmol Leu l^{-1} h^{-1} was reached in all fractions (Fig. 3B). A mean DOC concentration of 60 ± 14 µM was measured at the beginning of the experiment, excluding Hb Aa, which started with 135 µM DOC. Except for this sample, no significant decrease of DOC could be detected over time (Fig 3C). The main decrease of O_2 occurred after 73 h (Fig. 3D) and the O_2 consumption rate then varied between 0.06 and 0.24 µM h^{-1} (Table 2).

Table 2. Growth parameters for bacteria growing on seawater DOC (SW) and on different HS fractions (Hb A, Hb N and H I) for the 2 replicates a/b

	Growth rate μ (h^{-1})	Doubling time t_D (h)	Lag time t_L (h)	O_2 consumption[a] ($\mu M\ h^{-1}$)	Initial LEU[b] (pmol $l^{-1}\ h^{-1}$)	$\int_{0h}^{200h}$ LEU[c] (nmol l^{-1})	Growth yield[d] (%)
SW a/b	0.02/0.02	59/55	41/58	0.10 ± 0.06	1.10/1.30	6/7	6/7
Hb A a/b	0.02/0.02	60/56	85/44	0.24 ± 0.04	0.14/0.77	4/4	5/20
Hb N a/b	0.02/0.02	60/73	74/0	0.12 ± 0.02	– /0.62	6/4	6/9
H I a/b	0.02/0.01	62/57	0/0	0.06 ± 0.02	1.69/0.06	9/14	20/10

[a]For oxygen consumption rates the replicates a/b were pooled and 3 BOD-bottles were measured at each time point. The standard error of the slope of the regression line is shown
[b]Initial LEU is the leucine incorporation rate at the beginning of the experiment
[c]Cumulative LEU is the leucine incorporation rate integrated over 200 h. The cumulative LEU of Hb Aa and Hb Ab at initial carbon concentrations of 37 and 135 µM C was corrected to the mean carbon concentration of 64 µM C
[d]For the calculation of the growth yield, LEU was converted into bacterial carbon according to Simon & Azam (1989) O_2 consumption was converted into carbon consumption using a ratio of 1:1

Table 3. Growth parameters for bacteria growing on HS from surface and deep waters for the 2 replicates a/b

	Growth rate μ (h^{-1})	Doubling time t_D (h)	Lag time t_L (h)	Initial LEU[b] (pmol $l^{-1}\ h^{-1}$)	$\int_{0h}^{200h}$ LEU[c] (nmol l^{-1})	Growth yield[d] (%)
Surface a/b	0.01/0.01	154/113	11/39	0.21/0.20	1.0/3.5	2/5
Deep a/b	0.01/0.01	158/114	70/56	0.02/0.03	2.8/2.8	2/1

[b,d]As in Table 2
[c]The cumulative LEU of deep a/b at initial carbon concentrations of 29 and 19 µM C was corrected to a cumulative LEU at mean surface concentration of 54 µM C

The bioavailability of HS extracted from surface water was compared with HS from deep water (Expts 4 and 5, Table 1). Starting with the same concentration of bacteria, the surface HS reached much higher cell counts with 9×10^6 cells ml^{-1} compared to the 3.8×10^4 cells ml^{-1} reached with the deep water sample (Fig. 4A). Growth rates (0.01 h^{-1}) were the same for all samples. However, exponential growth in the deep water sample started later (70/56 h), compared to a lag time of only 11/39 h for the surface samples (Table 3). Except for the initial LEU with 0.20 pM Leu h^{-1} for surface HS compared to 0.02 for deep water HS (Table 3), few differences in LEU were found (Fig. 4B). A DOC decrease of 0.045 µM h^{-1} was detected for surface HS, but not for deep water HS (Fig. 4C) (as the experiments were set up on board without the opportunity to measure the DOC concentrations, the carbon concentrations of the deep water settings were lower than the surface ones, reflecting their real concentrations).

DISCUSSION

This study demonstrates that even under polar conditions marine HS can be used by the natural bacterial population as their sole carbon source. Averaged over all experiments, 11% of the initial HS carbon was consumed, with consumption ranging from 4 to 28%. This is a higher percentage than the 3 to 11% reported by Moran & Hodson (1994), who had a greater terrestrial influence at their study site. However, Fig. 1 reveals a high variability in the data. This may have occurred because DOC measurements were not always sensitive enough to detect a DOC decrease over time, despite detectable bacterial growth rates. Assuming the same reactivity for all HS, an HS consumption rate of 0.1 µM d^{-1} at a mean surface concentration of 22 µM HS (Table 1), a turnover time of 220 d can be extrapolated for marine HS in polar surface waters. However, under *in situ* conditions, photooxidation may facilitate the production of labile DOC from refractory material (Moran & Zepp 1997) and, on the other hand, low inorganic nutrient concentrations may slow down the HS degradation process. Therefore, the calculated results should be used as an estimate, additionally influenced by environmental conditions.

Although both the HS consumption rate and cumulative LEU depended on the initial HS concentration, this was not the case for other parameters: t_D, t_L, initial LEU and O_2 consumption. Besides substrate concentration,

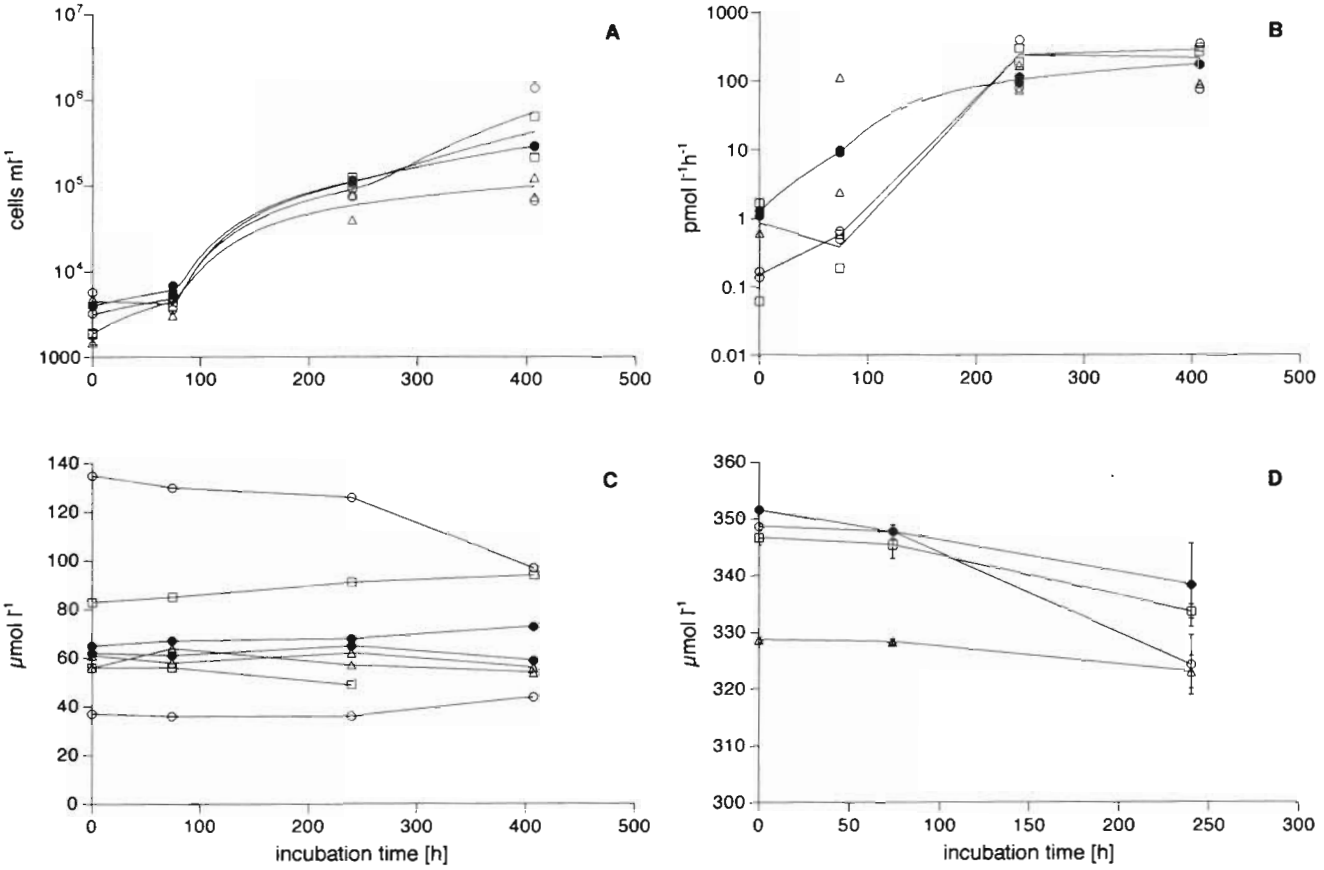

Fig. 3. Natural bacterial population, growing on different fractions of HS or seawater DOC as their sole carbon source. Showing the changes of (A) acridine orange direct counts (AODC) ml^{-1}, (B) leucine incorporation rate, (C) DOC, and (D) oxygen consumption. (Except for the O_2 consumption, error bars for the standard deviation were mostly within the symbols size on the logarithmic scale.) (○) Hb A a/b, (□) Hb N a/b, (△) H I a/b, and (●) SW a/b

there are other factors controlling bacterial growth, but there are no simple, non-complex generalisations possible (Moriarty & Bell 1993). Cell count based parameters depend more on the initial cell concentration of an experiment (Pitter & Chudoba 1990). Low growth efficiencies, as observed in this study, may indicate that bacterial production was limited by factors other than DOC (Kirchman et al. 1991). It has also been reported that, at low temperatures, bacterial production will respond more slowly and to a lesser extent to the addition of carbon than at higher temperatures (Kirchman & Rich 1997). Being restricted to macromolecules (HS) as the sole carbon source may additionally limit growth due to the rate of polymer hydrolysis (Moriarty & Bell 1993). All this may explain the uncoupling of initial LEU, t_D, t_L and O_2 consumption from the initial HS carbon concentration. However, integrating LEU over the whole incubation time, cummulative LEU is in the end dependent on the amount of carbon available.

The bacterial efficiency of carbon turnover was quite low in this study. Growth yield ranged from 1 to 20%, with a mean of 8%. For oligotrophic oceanic waters and humic lakes, a range of 2 to 30% is given by Middelboe & Søndergaard (1993) for the growth yield, with about 4% calculated for the Polar Southern Ocean (Bjørnsen & Kuparinen 1991). Middelboe & Søndergaard (1993) showed a close coupling between growth yield and the labile part of DOC. Such a relationship was not detectable in this study, due mainly to the high variability (CV = 0.82) of the data.

Several studies have discussed the importance of temperature versus substrate availability. In temperate regions (<12°C), temperature is thought to be more important than substrate availability (Hoch & Kirchman 1993, Shiah & Ducklow 1994). In these studies the Q_{10} varied between 2.76 and 5.60. A Q_{10} of about 3 was found for the range 8 to 25°C (Barillier & Garnier 1993), but in contrast with the present study, growth yield was independent from temperature. Rivkin et al. (1996) focused on cold environments and reported a much lower temperature dependence with a Q_{10} of 1.4 to 1.5. As mean specific growth rates in cold and temperate regions were not significantly different, Rivkin et al. (1996) concluded that bacterial turnover in high

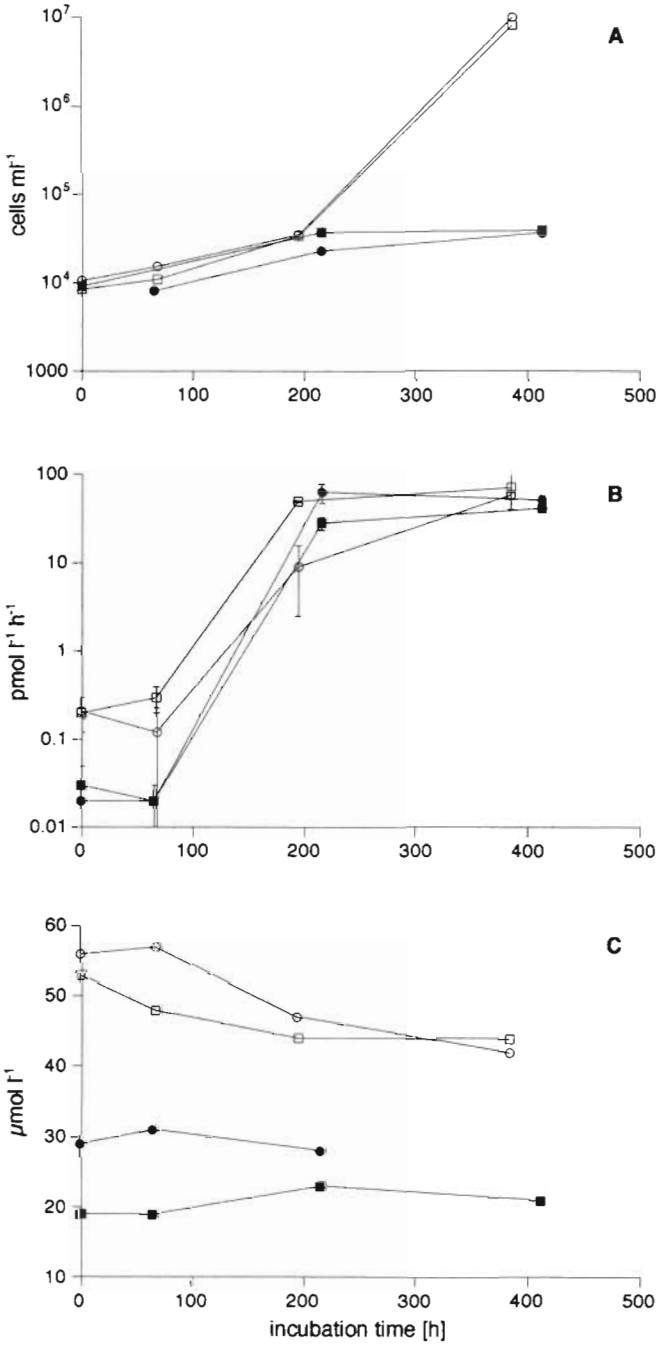

Fig. 4. Natural bacterial population, growing with HS as their sole carbon source. Showing the changes of (A) AODC ml^{-1}, (B) leucine incorporation rate, and (C) DOC. (Except for LEU, error bars for the standard deviation were mostly within the symbols size on the logarithmic scale). (○, □) Surface HS a/b, (●, ■) deep water HS a/b

latitudes is as important as in temperate regions, and is not hampered by low temperatures.

The observed values of Q_{10} in this study (2.9 and 4.8) are in the upper range of published data. However, since these experiments were carried out at low substrate concentrations, and taking into account reported reciprocal interactions of temperature and substrate concentration (Rivkin et al. 1996), the importance of temperature in this study was probably increased. Because of this reciprocal interaction, bacteria will grow only slowly at both low temperature and low substrate concentration (Wiebe et al. 1992), i.e. at low concentration of HS in polar waters.

Natural seawater includes all components of DOC, while the humic fractions are supposed to be composed of mostly refractory material. Thus the hypothesis was that carbon from seawater would be of better quality as a bacterial substrate than the carbon from the humic fractions alone. The better quality should have been reflected in a shorter lag time, a faster growth rate, higher LEU and O_2 consumption rates and a better growth efficiency.

To test the above hypothesis, all independent variables of Table 2 (t_L, t_D, initial LEU, cumulative LEU and O_2 consumption rate) were included in an analysis. Oxygen consumption rate was used instead of growth yield, because it was measured independently from LEU. Each column was rank transformed in such a way that the 'best' growing sample received the smallest number (for example a t_L of 0 h was assigned '1' vs '8' for 85 h; whereas a sample with an initial LEU 0.06 pmol l^{-1} h^{-1} was assigned '7' vs '1' for 1.69 pmol l^{-1} h^{-1}). For missing values, a neutral rank was inserted. After rank transformation (and thus also normalizing for the different units) all columns were pooled. The different fractions were compared with each other using an unpaired t-test. There was no significant difference between the different fractions as is illustrated by the mean ranks for each fraction: 4.3 (seawater), 4.8 (Hb A), 5.0 (Hb N) and 3.6 (H I), respectively.

The fact that the different HS fractions did not include more refractory material than the total DOM of seawater may have several explanations. XAD resins do not extract all humic-like compounds and additional non-humic compounds may have been concentrated on the resins (Aiken 1988). Most of the studies dealing with marine HS extract them with XAD 8, and allow for the problem of extreme bleeding from this particular resin. To circumvent this problem, which is especially severe in bioavailability studies, XAD resins 2 and 4 were used. Thus, not all HS from the seawater may have been extracted. However, there are several studies describing similar extraction characteristics for resins 2 and 8 in marine waters (for example, Esteves et al. 1996), without the problem of resin bleeding. XAD 4 was included in order to also extract small organic compounds (Aiken et al. 1992).

A more ecological point of view would lead to the question of the usefulness of XAD resins for the extraction of recalcitrant compounds. Such usefulness has

also been questioned by Tranvik (1988) with a different methodological approach. The fact that the present study not only focused on the Hb A fraction, but included all HS fractions (Hb A, Hb N and H I), supports his point of view that pelagic DOC comprises a continuum of fractions of varying recalcitrance which cannot be separated by XAD resins (Tranvik 1992). Such a continuum of recalcitrance of marine DOM is also postulated by Amon & Benner (1996), based on molecular weight classes.

DOM from surface water originates mainly from recent biological activities and is therefore considered to be of better food quality for bacteria when compared with the older material from deep water. Thus, the hypothesis was tested that surface HS are a better quality carbon source than deep water HS. As described above, this should have been reflected in a shorter lag time, faster growth rate, higher LEU and in a better growth efficiency. Reflecting the natural concentrations, the initial DOC concentrations from the surface settings were twice as high as in the deep water settings. However, as described in the 'Results', only cumulative LEU was influenced by the initial HS concentration, and thus was corrected (see Table 3).

To test the proposed hypothesis with the use of the independent variables in Table 3, each column was rank transformed as previously described. Then all columns were pooled, and surface HS versus deep water HS were tested (unpaired *t*-test). This showed that surface HS are of significantly better food quality than deep water HS ($p = 0.002$, $n = 16$). Surface HS revealed a mean rank of 1.9 compared to a mean rank of 3.1 for deep water HS.

Most studies on the bioavailability of marine HS were conducted with HS originating only from surface water (Tranvik 1993, Moran & Hodson 1994), but (to my knowledge) there are no studies on the bioavailability of deep water HS. DOC from deep water is known to be older than that obtained from surface waters and it is suspected to exhibit a more refractory humic character (Bauer et al. 1992). As well as the generally lower concentration of DOC in deep water than in surface waters, the chemical composition also differs. The deeper samples from the Atlantic and Pacific are dominated by humic-type fluorescence (Mopper & Schultz 1993), with these compounds being chemically different from surface water HS (Coble 1996). Furthermore, DOC of deep water is known to receive significant input from sedimentary organic carbon (Klinkhammer et al. 1997), probably even older than water column DOC.

To conclude, it was not surprising that this study found that HS from deep water were of lower quality as a bacterial carbon source than surface HS. Total DOC from deep water (including HS) has already been described as resistant to microbial oxidation (Barber 1968). The different quality of surface DOC versus deep water DOC is simply reflected in the HS fractions. This supports the previous findings that the different HS fractions have the same lability or recalcitrance as the total seawater DOC.

Organic material extracted from seawater with XAD resins, and therefore separated by different chemical characteristics, was shown to have no quality difference as a bacterial carbon source compared with total seawater DOC. Therefore, HS do play an active role in bacterial carbon cycling in the Arctic Ocean.

Acknowledgements. I wish to thank R. Engbrodt for co-operation in the humic extraction procedures, C. Lorenzen for assistance in cell counting, and R. Amon, P. Fischer and B. Rosenstock for helpful comments on earlier versions of the manuscript. This is publication number 1569 from the Alfred-Wegener-Institut.

LITERATURE CITED

Aagaard K, Fahrbach E, Meincke J, Swift JH (1991) Saline outflow from the Arctic Ocean: its contribution to the deep waters of the Greenland, Norwegian, and Iceland seas. J Geophys Res 96(C11):20433–20441

Aiken GR (1988) A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances. In: Frimmel FH, Christman RF (eds) Humic substances and their role in the environment. John Wiley & Sons Ltd, p 15–28

Aiken GR, McKnight DM, Thorn KA, Thurman EM (1992) Isolation of hydrophilic organic acids from water using non-ionic macroporous resins. Org Geochem 18:567–573

Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41:41–51

Bano N, Moran MA, Hodson RE (1997) Bacterial utilization of dissolved humic substances from a freshwater swamp. Aquat Microb Ecol 12:233–238

Barber RT (1968) Dissolved organic carbon from deep waters resists microbial oxidation. Nature 220:274–275

Barillier A, Garnier J (1993) Influence of temperature and substrate concentration on bacterial growth yield in Seine River water batch cultures. Appl Environ Microbiol 59:1678–1682

Bauer JE, Williams PM, Druffel ERM (1992) ^{14}C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. Nature 357:667–670

Bjørnsen PK, Kuparinen J (1991) Determination of bacterioplankton biomass, net production and growth efficiency in the Southern Ocean. Mar Ecol Prog Ser 71:185–194

Bortz J (1985) Lehrbuch der Statistik: für Sozialwissenschaftler. Springer-Verlag, Berlin

Chin-Leo G, Kirchman DL (1988) Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. Appl Environ Microbiol 54:1934–1939

Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar Chem 51:325–346

Esteves VI, Cordeiro NMA, Duarte AdC (1996) Variation on

the adsorption efficiency of humic substances from estuarine waters using XAD resins. Mar Chem 51:61–66
Granéli W, Granéli E (1991) Automatic potentiometric determination of dissolved oxygen. Mar Biol 108:341–348
Hoch MP, Kirchman DL (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Mar Ecol Prog Ser 98:283–295
Ishiwatari R (1992) Macromolecular material (humic substance) in the water column and sediments. Mar Chem 39: 151–166
Jones EP, Anderson LG, Swift JH (1998) Distribution of Atlantic and Pacific waters in the upper Arctic Ocean: implications for circulation. Geophys Res Lett 25:765–768
Kirchman DL, Rich JH (1997) Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. Microb Ecol 33:11–20
Kirchman DL, Suzuki Y, Garside C, Ducklow HW (1991) High turnover rates of dissolved organic carbon during as spring phytoplankton bloom. Nature 352:612–614
Klinkhammer GP, Chin CS, Wilson C, Rudnicki MD, German CR (1997) Distributions of dissolved manganese and fluorescent dissolved organic matter in the Columbia River estuary and plume as determined by in situ measurement. Mar Chem 56:1–14
Kontchou YC, Blondeau R (1990) Effect of heterotrophic bacteria on different humic substances in mixed batch culture. Can J Soil Sci 70:51–59
Krause G (1998) The expedition Arktis-XIII/3 of RV 'Polarstern' in 1997. In: Riemann F (ed) Report to Pol Res No. 262. Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, p 13–14
Malcolm RL (1989) Factors to be considered in the isolation and characterization of aquatic humic substances. In: Allard B, Borén H, Grimvall A (eds) Humic substances in the aquatic and terrestrial environment. Springer Verlag, Berlin, p 9–36
Middelboe M, Søndergaard M (1993) Bacterioplankton growth yield: seasonal variations and coupling to substrate lability and b-glucosidase activity. Appl Environ Microbiol 59:3916–3921
Mopper K, Schultz CA (1993) Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. Mar Chem 41:229–238
Moran MA, Hodson RE (1994) Support of bacterioplankton production by dissolved humic substances form three marine environments. Mar Ecol Prog Ser 110:241–247
Moran MA, Zepp RG (1997) Role of photoreactions in the formation of biologically labile compound from dissolved organic matter. Limnol Oceanogr 42:1307–1316
Moriarty DJ, Bell RT (1993) Bacterial growth and starvation in aquatic environments. In: Kjelleberg S (ed) Starvation in bacteria. Plenum Press, New York, p 25–53
Opsahl S, Benner R (1995) Early diagenesis of vascular plant tissues: lignin and cutin decomposition and biogeochemical implications. Geochim Cosmochim 59:4889–4904
Pfennig N, Widdel F, Trüper HG (1981) The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer Verlag, New York, p 926–940
Pitter P, Chudoba J (1990) Biodegradability of organic substance in the aquatic environment, Vol 306. CRC Press, Boca Raton
Pomeroy LR, Sheldon JE, Sheldon WM, Peters F (1995) Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Mar Ecol Prog Ser 117:259–268
Raven JA, Geider RJ (1988) Temperature and algal growth. New Phytol 110:441–461
Rivkin RB, Anderson MR, Lajzerowicz C (1996) Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. Aquat Microb Ecol 10:246–254
Rudels B (1989) The formation of polar surface water, the ice export and the exchanges through the Fram Strait. Prog Oceanogr 22:205–248
Schlegel HG (1985) Allgemeine Mikrobiologie. Georg Thieme Verlag, Stuttgart
Shiah FK, Ducklow HW (1994) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Mar Ecol Prog Ser 103:297–308
Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213
Smith SL, Smith WO, Codispoti LA, Wilson DL (1985) Biological observations in the marginal ice zone of the East Greenland Sea. J Mar Res 43:693–717
StatView 4.5 for Macintosh (1994) Abacus Concepts Inc, Berkeley, CA
Thomas DN, Lara RJ, Eiken H, Kattner G, Skoog A (1995) Dissolved organic matter in Arctic multi-year sea ice during winter: major components and relationship to ice characteristics. Pol Biol 15:477–483
Thurman EM (1985) Organic geochemistry of natural waters. M Nijhoff, W Junk (publ), Dordrecht, p 275–361
Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16:311–322
Tranvik LJ (1992) Rapid microbial production and degradation of humic-like substances in lake water. Arch Hydrobiol 37:45–30
Tranvik LJ (1993) Microbial transformation of labile dissolved organic matter into humic-like matter in seawater. FEMS Microbiol Ecol 12:177–183
Wiebe WJ, Sheldon WM, Pomeroy LR (1992) Bacterial growth in the cold: evidence for an enhanced substrate requirement. Appl Environ Microbiol 58:359–364

Editorial responsibility: Fereidoun Rassoulzadegan, Villefranche-sur-Mer, France

Submitted: October 1, 1998; Accepted: February 9, 1999
Proofs received from author(s): August 17, 1999