*Biogeochemistry* **30:** 77–97, 1995.

# Colloidal and dissolved organic matter in lake water: Carbohydrate and amino acid composition, and ability to support bacterial growth

LARS J. TRANVIK[1] & NIELS O. G. JØRGENSEN[2]
[1] *Dept. of Ecology, Limnology, University of Lund, S-223 62 Lund, Sweden* [2] *Microbiology Section, Department of Ecology and Molecular Biology, Royal Veterinary and Agricultural University, Rolighedsvej 21, DK-1958 Frederiksberg C, Denmark*

Received 29 September 1994; accepted 15 May 1995

**Abstract.** Bacterial utilization of dissolved organic matter (DOM) was studied in water from a humic and a clearwater oligotrophic lake. Indigenous bacteria were inoculated into either 0.2 $\mu$m natural filtered lake water, or lake water enriched fivefold with colloidal DOM >100 kD but below 0.2 $\mu$m. Consumption of DOM was followed from changes in concentrations of total dissolved organic carbon (DOC), dissolved combined and free carbohydrates and amino acids (DCCHO and DFCHO, and DCAA and DFAA, respectively) and by uptake of monosaccharide and amino acid radioisotopes. DCCHO and DCAA made up 8% (humic lake) to 33–44% (clear-water lake) of the natural DOC pools, while DFCHO and DFAA contributed at most 1.7% to the DOC pools. Addition of >100 kD DOM increased the DOC concentrations by 50% (clearwater lake) to 92% (humic lake), but it only resulted in a higher bacterial production (by 63%) in the humic lake. During the incubations 13 to 37% of the DOC was assimilated by the bacteria, at estimated growth efficiencies of 4–8%. Despite the measured reduction of DOC, statistically significant changes of specific organic compounds, especially of DCCHO and DCAA, generally did not occur. Probably the presence of high molecular weight DOC interfered with the applied analytical procedures. Addition of radiotracers indicated, however, that DFAA sustained 17–58% and 29–100% of the bacterial carbon and nitrogen requirements, respectively, and that glucose met 1–3% of the bacterial carbon requirements. Thus, our experiments indicate that radiotracers, rather than measurements of concentration changes, should be used in studies of bacterial utilization of DOC in freshwaters with a high content of humic or high molecular weight organic matter.

**Key words:** amino acids, carbohydrates, colloidal organic matter, dissolved organic matter, lake water bacteria

## Introduction

High molecular weight compounds often constitute a large fraction of dissolved organic matter (DOM) in lake water (Münster 1985; Tranvik 1990). In addition, a fraction of the DOM (generally defined as organic matter not retained by certain filters) consists of colloidal aggregates (Burnison & Leppard 1983; Koike et al. 1990; Wells & Goldberg 1991).

Concentration and composition of colloids found in freshwater imply that substantial amounts of potential bacterial substrates will be in a colloidal state. Among possible colloidal compounds available to bacteria are amino acids and carbohydrates. Colloidal amino acids have been found to constitute from 0.3 to 15 mg C/l (Peake et al. 1977; Sigleo et al. 1983; Tucshall & Brezonik 1980) and make up about 30% and 70% of the carbon and nitrogen content of the colloids (Sigleo et al. 1983). Carbohydrates of high molecular weight may be another potential source of carbon, comprising from about 0.2 to 4.4 mg C/l in lakes (Münster & Chrost 1990). In bogs about 15% of the DOC may consist of carbohydrates >100 kD (Satoh et al. 1987). Polymer carbohydrates appear especially important when more labile substrate pools are depleted (Middelboe & Søndergaard 1993).

In lakes, the high molecular weight proportion of DOM increases with the content of humic matter (Aho 1986; Tranvik 1990). Some studies indicate that high molecular weight DOM inhibits microbial metabolism (Ford & Lock 1987, Freeman & Lock 1992). However, these high molecular weight compounds may also be of nutritional value for pelagic bacteria, as suggested by the large fraction of bacterial growth in humic waters due to such compounds (Meyer et al. 1987; Tranvik 1990).

Here, we examine the importance of colloidal and low molecular weight substrates for bacterial growth. We manipulated the concentration of colloids <0.2 m but larger than 100 kD, in order to study the effect of colloidal DOM on bacterial substrate utilization. The study reported here involves both a humic lake, and a clearwater lake. High molecular weight compounds (>10 kD, possibly to a great extent consisting of colloidal aggregates) have been shown to be of increasing importance as bacterial substrates with increasing humic content of lakes (Tranvik 1990). Other investigations of bacterial utilization of molecular weight classes of DOM in humic lake water focus on organic compounds released by phytoplankton (Sundh 1989, 1991). However, these studies do not consider the overall composition of the DOM, the bacterial substrates originating from other sources than the indigenous primary production which are important energy sources for bacteria in humic lakes (Tranvik 1989), or the larger colloids.

Our results show that colloids are a more important food source to bacteria in the humic lake, i.e. that enhancement of the colloid concentration stimulates bacterial growth to a larger extent in the humic water than in clear water. In addition, we present information about the amino acid and carbohydrate content of dissolved and colloidal organic matter, and the contribution of glucose and amino acids to bacterial carbon and nitrogen demand.

## Materials and methods

### *Experimental design*

Water was sampled in June 1992 from 1 m below the surface of two oligotrophic south Swedish boreal forest lakes, Klintsjön (clear water, low DOC) and Skärshultsjön (humic brown water, high DOC; both lakes have previously been described in Tranvik [1989]), and brought to the laboratory in acid-rinsed 20 liter polycarbonate carboys.

The water was filtered through precombusted Gelman type A/E (diameter, 142 mm) glass fiber filters, which were replaced frequently to prevent clogging and capture of particles smaller than the nominal pore size of the filter (approximately 1 $\mu$m). Subsequently, the water was filtered through 0.2 $\mu$m Durapore filters by tangential flow using a Minitan apparatus (Millipore, USA). This method removes particles of bacterial or larger size, but lets through a substantial amount of colloids in the <0.2 $\mu$m but >100 kD range (Tranvik 1994). Thereafter, <0.2 $\mu$m material with nominal molecular weights >100 kD were concentrated in the tangential flow filtration apparatus by the use of polysulfone ultrafilters (PTHK, Millipore), to obtain a fivefold concentration of colloids >100 kD but <0.2 $\mu$m. For each lake, triplicate samples were set up in acid-rinsed, autoclaved 2.5 liter polycarbonate flasks with 1.5 liters of 0.2 $\mu$m filtered water or 0.2 $\mu$m filtered water enriched with colloids. Each flask was inoculated with bacteria by adding 150 ml of lake water filtered through a precombusted Whatman GF/F glass fiber filter. The flasks were incubated in the dark, and samples were retained at various times for analysis of bacterial biomass and activities, dissolved organic carbon, and concentration of specific organic compounds. Temperature during the incubation varied between 19.4 and 21.8 °C.

### *Bacterial biomass*

Bacteria were enumerated with a Nikon Optiphot epifluorescence microscope. Subsamples were stained for about 10 minutes with DAPI (Porter & Feig 1980) at a final concentration of 10 mg/liter, and filtered onto black polycarbonate filters (Poretics, 0.2 $\mu$m). From each slide, at least 250 bacteria and 10 fields of view were counted. Bacterial cell volume was estimated from measurements on photomicrographs according to Tranvik (1988). Bacterial carbon biomass was calculated assuming 0.3 pg C per $\mu m^3$ of bacterial volume (Fry 1988). Bacterial N content was calculated assuming a bacterial N:C ratio of 1:5 (Nagata 1986).

### *Assimilation of glucose, starch and free amino acids*

Bacterial production was measured by incorporation of L-[$^3$H]leucine into bacterial proteins according to Kirchman et al. (1985) and Jørgensen (1992). For each measurement, triplicate 5 ml water samples and a control sample with 2% final concentration of formaldehyde were amended with 100 nM leucine (10% [$^3$H]leucine (45–65 Ci/mmol, DuPont NEN Research Products, USA) and 90% non-radioactive leucine). After addition of leucine the samples were incubated for 30 min at the *in situ* temperature in the lake. The incubations were terminated by addition of 5 ml cold 10% trichloroacetic acid (TCA) and filtered through 0.2 $\mu$m membrane filters after 30 min of extraction. Radioactivity of the filters was assayed with liquid scintillation counting (LSC). Bacterial production was calculated using the conversion factors of Simon & Azam (1989).

Bacterial incorporation and respiration of glucose and starch were measured with uniformly labeled D-[$^{14}$C(U)]glucose (250–360 mCi/mmol) and [$^{14}$C(U)]starch (1.5–2.0 mCi/mg), both from DuPont NEN Research Products (USA). Water samples of 20 ml were amended with about 6 nCi [$^{14}$C]glucose or [$^{14}$C]starch and incubated at *in situ* temperature for 45 to 75 min. The incubations were stopped with buffered (pH 8.5) formaldehyde to a 2% final concentration. For measurement of respiration, the incubation bottles were closed with stoppers with rubber septa. Plastic cups with accordion-folded paper wicks were attached under the stoppers. The wicks were soaked with 500 $\mu$l of Carbosorb $CO_2$ absorber (Packard Instruments, the Netherlands), added to the cups with a hypodermic needle. With another hypodermic needle, 20 $\mu$l 85% $H_3PO_4$ was added to the water sample in each bottle. After 1 h on a shaking table, the $CO_2$-traps were transferred to 20 ml scintillation vials and radioassayed by LSC. Bacterial incorporation of glucose was determined by 0.2 $\mu$m membrane filtration of the acidified water samples after removal of the $CO_2$-traps. The filters were radioassayed by LSC. Addition of the glucose isotope increased the natural glucose concentration by a maximum of 1.8 nM, corresponding to $\leq 8\%$ of original glucose concentration.

Incorporation and respiration of free amino acids was determined from addition of 9 nCi of an equimolar mixture of L-[$^{14}$C(U)]amino acids (glutamic acid, serine, glycine and alanine; 58–282 mCi/mmol (Amersham, UK)). Otherwise the procedure for amino acid assimilation was identical to that used for glucose and starch. Addition of $^{14}$C amino acids increased the natural DFAA concentration by a maximum of 2 nM ($\leq 1\%$ of original DFAA concentration). For calculation of the total DFAA flux, assimilation of the four amino acids was assumed to represent assimilation of all DFAA.

The isotopic labels were added 29 and 53 hours after inoculation of the cultures, i.e. in early and late exponential growth (cf. Fig. 1).

### *Dissolved organic carbon*

Samples for dissolved organic carbon (DOC) were kept frozen in precombusted (500 °C, over night) glass vials with teflon lined screw caps. Organic carbon of the thawed samples was analyzed as $CO_2$ after platinum-catalyzed high temperature oxidation using a Shimadzu TOC 5000 total carbon analyzer. Inorganic carbon was purged from acidified samples (pH 2, HCl) with $CO_2$-free air before analysis. Prior to analysis, samples were sonicated in an ultrasonic bath to destroy any flocculates of DOM that may have formed due to freezing and thawing. For each analysis, three replicate injections were made on the carbon analyzer, usually resulting in a coefficient of variation of less than 2%. Milli-Q (Millipore) water was used as a blank, which was subtracted from the samples. At most, it corresponded to 11% of the sample signal. Later studies employing the automatic blank checking procedure of the Shimadzu TOC 5000 indicates water blanks and instrument blanks (sensu Benner & Strom 1993) for our procedures of about 180 $\mu$g C/l and 70 $\mu$g C/l, respectively.

### *PAD-HPLC analysis of saccharides*

Concentrations of mono- and disaccharides were determined by pulsed amperometric detection (PAD), combined with high performance liquid chromatography (HPLC) according to Jørgensen & Jensen (1994). The equipment consisted of the following Millipore/Waters (USA) equipment: two 510 pumps, a 712 WISP autosampler, a 464 pulsed amperometric detector with a gold electrode and a Maxima 820 or Millenium data acquisition and processing module. For separation of the saccharides a CarboPac PA1 250 × 4 mm column and a CarboPac PA 52 × 3 mm guard column (Dionex Corporation, USA) were used. In order to increase the detection level of the PAD-HPLC analysis, all samples for measurements of free mono- and disaccharides (DFCHO) were concentrated by a factor of 10 to 13.3 before injection. Samples of 15 ml 0.2 $\mu$m filtered water were freeze-dried and redissolved in Milli-Q water (Millipore). Precipitates in the redissolved samples were removed by filtration through 0.2 $\mu$m filter mini-cartridges.

Individual saccharides of combined carbohydrates (DCCHO) were determined in 1.0 ml 0.2 $\mu$m filtered water samples. The samples were transferred to glass ampoules and Suprapur HCl (Merck, Germany) was added to a final concentration of 1.5 M. The ampoules were flushed with $N_2$, sealed and heated to 100 °C for 4 hours. After cooling the hydrolyzed samples were frozen and freeze-dried to remove HCl and water. The samples were redissolved in Milli-Q water, gently sonicated and filtered through 0.2 $\mu$m membrane filters. Simultaneous with each series of hydrolyzed samples, 1.0 ml Milli-Q water was hydrolyzed and treated as above. Content of saccharides in the Milli-Q water served as a blank and was subtracted from the content of the lake water samples.

### *HPLC analysis of DFAA*

Dissolved free amino acids were analyzed as fluorescent o-phthaldialdehyde derivatives by HPLC according to Lindroth & Mopper (1979) and Jørgensen et al. (1993).

## Results

### *Occurrence and composition of DOM/DOC*

In water from the clear lake, the fivefold increase of the concentration of >100 kD DOM resulted in an almost doubled DOC concentration, and an increased concentration of dissolved combined carhohydrates (DCCHO, by 109%) and amino acids (DCAA, by 70%) (Table 1).

In the humic lake cultures, the enhanced colloid concentration raised the DOC concentration by 50% (Table 1). As in the cultures from the clear lake, there was a large increase in DCCHO (69%) but the colloid enrichment did not significantly affect the concentration of DCAA in this lake. Unlike in the clearwater cultures, the concentration of dissolved free carbohydrates (DFCHO) was markedly increased (by 715%) upon colloid enrichment (Table 1).

DFCHO and DFAA contributed at most 1.7% of the DOC in all treatments (Table 2). In clearwater cultures, 24 to 33% and 9.3 to 9.9% of DOC consisted of combined carbohydrates and amino acids, respectively. In humic cultures, less DOC occurred in these fractions (ca. 5% and $\leq$3%, respectively). All the analyzed DOC fractions (DFCHO, DCCHO, DFAA, DCAA) together constituted 36 to 43% of the DOC in the clearwater lake, and about 8% in the humic lake. There were no obvious differences in the percentage of total DOC occurring in each of these fractions between water with and without enhanced concentration of >100 kD colloids (Table 2).

### *Composition of DFCHO and DCCHO*

In the clearwater lake dissolved free arabinose, glucose, and mannose were rather similar in quantitative importance (Fig. 1a). Fructose was the most abundant sugar, while galactose was of less importance. In addition to the five monosaccharides shown in Fig. 1a, four unknown sugars occurred in

*Table 1.* Bacterial carbon biomass, DOC, and the various fractions of DOC in the different treatments. NS; change during experiment not significant (paired t-test, $p < 0.05$). Significant differences (t-test, $p < 0.05$) between lake water with and without added colloids in bacterial C increase, decrease of DOC, and in initial concentrations of DOC, CHO and AA, are indicated by an asterisk (*). Values are average (±, $n = 3$).

| | Clearwater lake (Klintsjön) | | Humic lake (Skärshultsjön) | |
|---|---|---|---|---|
| | natural | +colloids | natural | +colloids |
| **DOM compounds** | | | | |
| DOC, mg/l | | | | |
| 9 h | 3.13 ± 0.18 | 6.00 ± 0.12* | 10.30 ± 0.38 | 15.40 ± 0.11* |
| 53 h | 2.24 ± 0.01 | 3.76 ± 0.18 | 8.92 ± 0.57 | 12.60 ± 1.1 |
| decrease | 0.89 ± 0.2 | 1.84 ± 0.30* | 1.36 ± 0.22 | 2.87 ± 1.2* |
| DFCHO, μg C/l | | | | |
| 9 h | 8.72 ± 0.30 | 18.2 ± 9.6 | 14 ± 12 | 114 ± 15* |
| 53 h | 6.47 ± 0.84 | 8.17 ± 4.0 | 9.74 ± 5.8 | 22.1 ± 1.0 |
| decrease | 2.26 ± 0.63 | NS | NS | 92.3 ± 15 |
| DCCHO, μg C/l | | | | |
| 9 h | 766 ± 32 | 1830 ± 258* | 459 ± 62 | 776 ± 117* |
| 53 h | 824 ± 85 | 1573 ± 157 | 462 ± 67 | 797 ± 93 |
| decrease | NS | NS | NS | NS |
| DFAA, μg C/l | | | | |
| 9 h | 44.30 ± 34 | 31.0 ± 8.9 | 34.2 ± 12 | 32.0 ± 16 |
| 53 H | 0.91 ± 0.6 | 2.60 ± 0.97 | 1.53 ± 1.8 | 3.57 ± 5.0 |
| decrease | NS | 28.35 ± 7.9 | 32.7 ± 10 | NS |
| DCAA, μg C/l | | | | |
| 9 h | 306 ± 83 | 520 ± 61* | 327 ± 182 | 301 ± 67 |
| 53 h | 237 ± 56 | 291 ± 95 | 260 ± 28 | 548 ± 279 |
| decrease | NS | 229 ± 34 | NS | NS |
| **Bacterial biomass, μg C/l** | | | | |
| 9 h | 4.2 ± 0.7 | 3.9 ± 0.3 | 4.4 ± 0.7 | 5.0 ± 0.7 |
| 29 h | 19.9 ± 1.2 | 25.4 ± 2.0 | 37.2 ± 1.0 | 44.4 ± 4.6 |
| 53 h | 75.8 ± 4.5 | 79.4 ± 4.0 | 107 ± 6.5 | 173 ± 18 |
| increase | 71.6 ± 4.5 | 75.6 ± 4.2 | 103 ± 7.0 | 168 ± 19* |
| growth efficiency (%) | 8.2 ± 1.1 | 4.2 ± 0.6 | 7.7 ± 1.5 | 6.8 ± 3.4 |

most samples. The concentration of these sugars was determined assuming a peak response similar to that of glucose. The four sugars made up 22 to 40% of all the DFCHO. The DFCHO spectrum of the humic lake resembled that

*Table 2.* Initial carbon concentration of DFCHO, DCCHO, DFAA, and DCAA as a percentage (±SD, $n = 3$) of total DOC in the cultures.

| | Clearwater lake (Klintsjön) | | Humic lake (Skärshultsjön) | |
|---|---|---|---|---|
| | natural | +colloids | natural | +colloids |
| DFCHO | 0.3 ± 0.006 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.7 ± 0.09 |
| DCCHO | 24 ± 1.8 | 33 ± 5.1 | 4.5 ± 0.8 | 5.0 ± 0.7 |
| DFAA | 1.4 ± 1.0 | 0.6 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 |
| DCAA | 9.9 ± 3.0 | 9.3 ± 1.2 | 3.1 ± 1.7 | 2.0 ± 0.4 |

of the clear lake. In both lakes the colloidal matter led to a larger increase of fructose, and a reduction of the relative mannose abundance (Fig. 1a). The four unknown sugars in this lake were more dominant than in the clear lake and constituted from 39 to 57% of the DFCHO pool.

The DCCHO composition in natural water from the two lakes was dominated by glucose, followed by either galactose (clear lake) or mannose (humic lake) (Fig. 1b). The contribution of fructose was severely underestimated, as fructose is degraded during the hydrolysis (Jørgensen & Jensen 1994). DCCHO of the colloidal matter had a lower relative abundance of glucose than DCCHO in the natural water (Fig. 1b). The four unknown sugars made up 18 to 20% of the DCCHO in both lakes.

### *Composition of DFAA and DCAA*

The composition of both DFAA and DCAA was fairly homogeneous in the different samples, irrespective of addition of colloidal DOM. Dominant DFAA (in a decreasing rank) were serine, glycine, alanine and aspartic acid. The DCAA pool of both lakes consisted of glycine, serine, alanine, glutamic acid, aspartic acid, leucine and in the humic lake also valine.

### *Microbial utilization of DOM*

In all cultures, an exponential bacterial growth occurred 20 to 50 h after inoculation, whereafter a stationary phase was established (Fig. 2).

In the clearwater lake cultures the fivefold addition of colloids did not affect the stationary phase of the bacterial biomass (Fig. 2, Table 1) or growth rate during the exponential phase (Fig. 3a). However, bacterial production, as measured by leucine incorporation integrated over 29 to 53 h, was 49% higher at the increased colloid concentration (Fig. 3b).

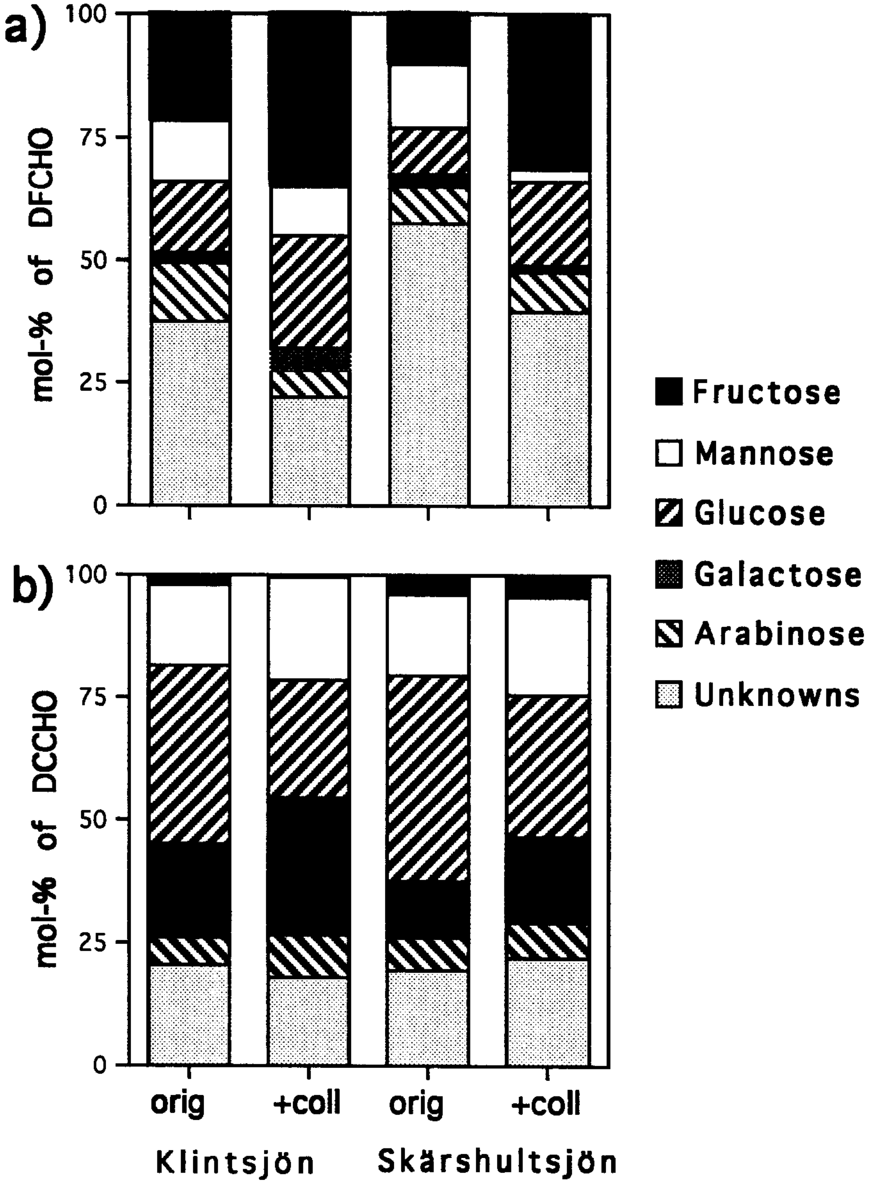

*Fig. 1.* Relative contribution of various individual sugars to DFCHO (a) and DCCHO (b).

The colloidal enrichment of the humic lake cultures led to a 62% increase in bacterial biomass during the stationary phase, relative to the natural cultures (Fig. 2, Table 1). Both bacterial exponential growth rate and leucine

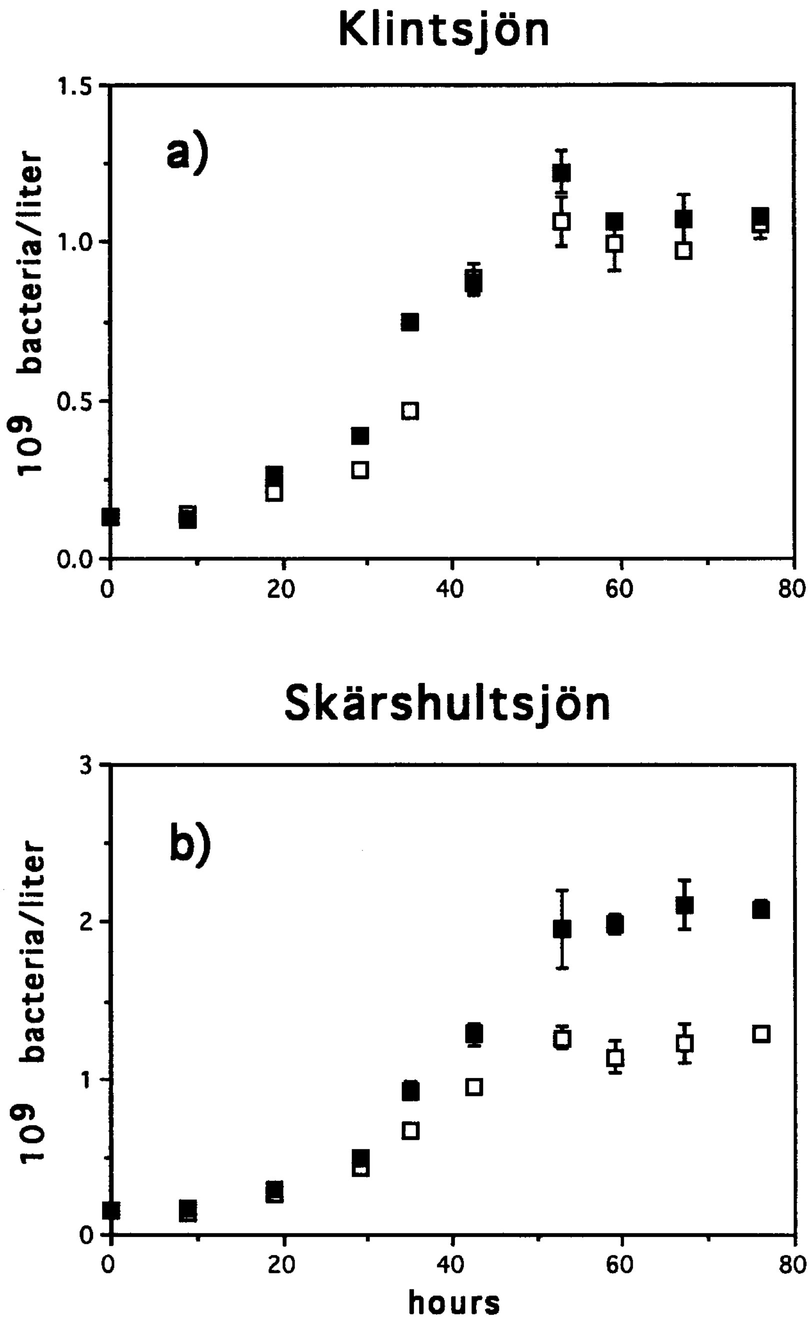

*Fig. 2.* Bacterial growth in cultures with original (open symbols) and enhanced (filled symbols) concentration of colloids >100 kD using water from clearwater Klintsjön (a) and humic Skärshultsjön (b). Error bars indicate ±1 SD for triplicate cultures.

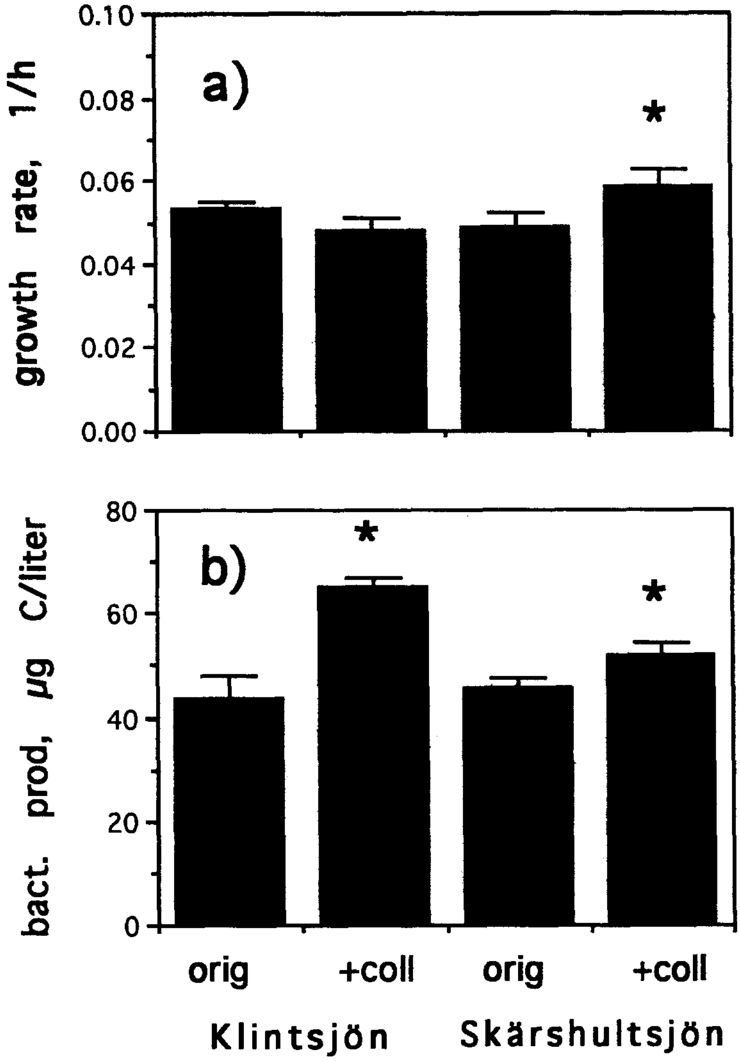

*Fig. 3.* Exponential growth rate of bacteria, as calculated from increase in cell numbers (a), and bacterial production during 29 to 53 h, as derived from leucine incorporation (b). Error bars are 1 SD for triplicate cultures. Asterisk (*) indicates significantly (t-test, $p < 0.05$) higher value for cultures with enhanced colloid concentration.

incorporation integrated over 29 to 53 h were slightly higher (18% and 14%, respectively) in cultures with added colloids (Fig. 3).

Bacterial production, determined by the leucine incorporation from 29 to 53 h (Fig. 3), amounted to 78 to 120% and 40 to 65% of the actual biomass accumulation during the same period in clear and humic cultures, respectively (Table 1).

*Table 3.* Bacterial incorporation of carbon from amino acids and glucose integrated over 29–53 h as a percentage (±SD, $n = 3$) of increase in bacterial carbon and nitrogen during the same period.

| | Clearwater lake (Klintsjön) | | Humic lake (Skärshultsjön) | |
|---|---|---|---|---|
| | natural | +colloids | natural | +colloids |
| Glucose C | 1.5 ± 0.09 | 1.3 ± 0.2 | 1.3 ± 0.3 | 3.0 ± 0.5 |
| Amino acid C | 16.9 ± 2.0 | 58.1 ± 3.6 | 30.0 ± 1.0 | 29.2 ± 6.3 |
| Amino acid N | 29.0 ± 3.4 | 100 ± 6.2 | 51.7 ± 1.7 | 50.2 ± 10.8 |

The DOC concentrations decreased by 13 to 37% during the bacterial growth (Table 1). Assuming that the decrease in DOC is due to consumption by bacteria, the net growth efficiency of bacteria (produced bacterial carbon according to the microscopic analysis relative to consumed DOC) varied between 4.2% and 8.2%, i.e. the decrease in DOC was at least an order of magnitude higher than the increase in bacterial C.

We detected a decrease in specific DOC components during the growth periods in only a few cases (Table 1). This occurred for DFCHO in clearwater cultures (the DFCHO decrease amounted to 3% of bacterial C increase) and in humic cultures with added colloids (55% of bacterial C increase), for DFAA in clearwater cultures (38% of bacterial C increase), and for DCAA in clearwater cultures amended with colloids (303% of bacterial C increase).

### *Microbial utilization of glucose, starch and amino acids*

Glucose utilization, derived from $^{14}$C-glucose utilization, was similar in clearwater cultures with and without enhanced colloid concentration, and in humic cultures without added colloids (1.3 to 1.5 $\mu$g C/l). It was markedly higher in humic + colloids cultures (3.8 $\mu$g C/l) (Fig. 4a). In all treatments, glucose carbon utilization corresponded to $\leq 3\%$ of bacterial carbon increase during the same period (Table 3).

Bacterial degradation of $^{14}$C-labelled starch was measured in the two lakes, but the ambient concentrations of starch were not measured (Table 4). In most cases, there was a shortened turnover time and a decreased fraction of utilized starch being respired towards the end of the experiment, indicating an increased capacity for degradation of $\alpha$-1,4-glucose polymers during the incubation.

In cultures from both lakes, addition of colloids resulted in increased utilization of DFAA (based on uptake of four $^{14}$C amino acids) (Fig. 4b). The

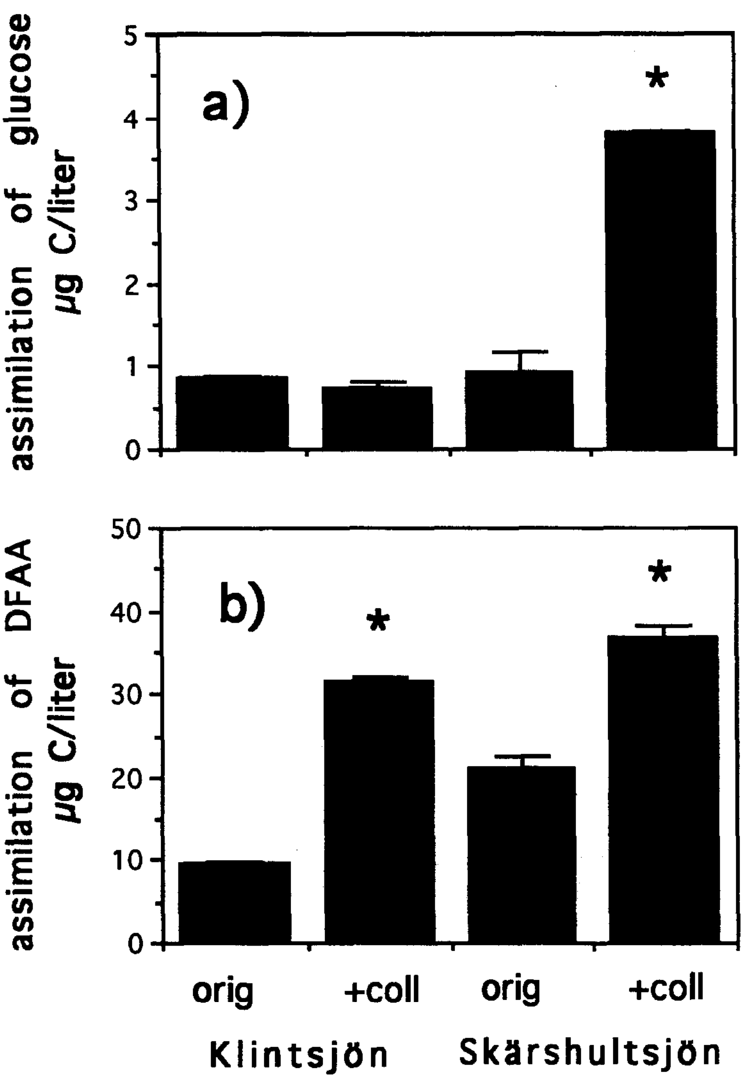

*Fig. 4.* Utilization of glucose (a) and amino acids (b), derived from isotope experiments and integrated over 29 to 53 h. Errors bars are 1 SD for triplicate cultures. Asterisk (*) indicates significantly (t-test, $p < 0.05$) higher value for cultures with enhanced colloid concentration.

utilized amino acids corresponded to 17 to 58% of bacterial C increase, and 29 to 100% of bacterial N increase (Table 3).

The bacterial respiration of glucose was on the average 50% of the total (incorporation + respiration) uptake. A lower respiration was observed for starch at 53 h (9 to 15% of the total uptake), except in the clearwater lake + colloids cultures (55%). The respiration of amino acids was significantly lower, with an average value of 8% of the total DFAA uptake. In cultures

*Table 4.* Turnover times (based on gross uptake, i.e. incorporation and respiration) and respiration of $^{14}C$-labeled starch. Values are average (±SD, $n$ = 3).

| | Clearwater lake (Klintsjön) | | Humic lake (Skärshultsjön) | |
|---|---|---|---|---|
| | natural | +colloids | natural | +colloids |
| Turnover time (h) | | | | |
| 29 h | 92 ± 1.7 | 104 ± 16 | 77 ± 8.0 | 26 ± 2.9 |
| 53 h | 13 ± 0.6 | 79 ± 15 | 19 ± 3.1 | 11 ± 0.6 |
| Respiration (% of gross uptake) | | | | |
| 29 h | 78 ± 14 | 44 ± 4.0 | 42 ± 3.2 | 15.0 ± 1.6 |
| 53 h | 13 ± 1.5 | 55 ± 8.1 | 15 ± 1.4 | 9.4 ± 0.7 |

with enhanced colloid concentration, the amino acid respiration was about 25% lower than in cultures without added colloids.

## Discussion

### *Concentration and composition of DOM/DOC in the two lakes*

The addition of colloids resulted in an almost twofold higher DOC increase in the clearwater lake than in the humic lake (increase by 92% and 50%, respectively). The DOC enrichment due to the colloids was also manifested by increased concentrations of combined carbohydrates in both lakes (Table 1). In addition, in the humic lake the colloids caused a severalfold increase in free mono- and disaccharides, possibly because analytically detectable monomers were adsorbed to the colloidal DOM (Carlson et al. 1985).

The larger relative increase in DOC in the clearwater lake than in the humic lake, due to addition of colloids, is not in accordance with previous observations. Typically a larger fraction of DOC is of high molecular weight in humic lakes (Aho 1986; Tranvik 1990). However, these previous investigations all involve DOC >10 kD, while the present study concentrates on the colloids >100 kD. Possibly, larger colloids (>100 kD) were of lower relative importance in the humic lake.

The measured concentrations of free and combined amino acids and carbohydrates in most cases fall within concentrations previously measured in freshwater, including humic lakes, see review by Münster (1993). However, compared to the oligotrophic, humic Lake Mekkojärvi (Münster 1992;

Münster et al. 1992), higher concentrations of DCCHO occurred in the natural water in our study (range in Lake Mekkojärvi was 0.5 to 113 $\mu$g C/l; in our cultures 459 to 766 $\mu$g C/l were measured (Table 1)). After addition of colloids, the only measured DOM fractions that exceeded the concentration range for Lake Mekkojärvi were DCCHO (1830 $\mu$g C/l in the present study) and DFCHO (114 $\mu$g C/l in the humic lake, compared to a natural range of 2 to 22 $\mu$g C/l in Lake Mekkojärvi).

The composition of DFCHO in the lakes (dominance of fructose and glucose, followed by arabinose and mannose, Fig. 1a) agrees with that measured in Danish lake water (Jørgensen & Jensen 1994), but it differs from the spectra measured in other lakes, in which glucose often has been found the single dominant monosaccharide (Münster 1984; Wicks et al. 1991). In these studies, however, a different water environment was studied (water from a macrophyte bed; Wicks et al. 1991), or a different analytical procedure (gas chromatographic detection including a derivatization step; Münster (1984)) was applied. Since monosaccharides are delicate components that are chromatographically difficult to separate and detect in low concentrations, the applied procedure may influence the measured concentrations.

The DCCHO composition of the two lakes (Fig. 1b) is comparable to that observed in other lakes and in fulvic acids from bog water (Wicks 1991; Ertel et al. 1993; Jørgensen & Jensen 1994). The identified saccharides, including xylose that may have co-eluted with mannose in our analyses, are also dominant components of cellulose and hemicellulose (Wicks et al. 1991). The similarity of carbohydrate profiles in lake water and in natural fulvic acids (major components of humic matter), suggests that a similarity in the DCCHO composition in humic and non-humic lakes can be expected. Enrichment with colloidal matter in water from both lakes caused an increased abundance of galactose, indicating that the colloids had a high content of this disaccharide than lake water components <100 kD.

The composition of DFAA (dominance of serine, glycine, and alanine) in both lakes resembled that typically measured in lake water (Jørgensen 1987; Simon & Rosenstock 1992). The addition of colloids did not significantly influence the DFAA composition. The occurrence of glycine, serine, alanine, and glutamic and aspartic acid as major DCAA largely agrees with the amino acid composition of colloidal matter reported by Sigleo et al. (1983), although the acidic amino acids were more abundant in their study.

### *Microbial growth parameters*

Enrichment of the present lake water cultures with fivefold >100 kD DOM indicated that colloids were a more important bacterial food source in the humic lake that in the clearwater lake. The added colloids increased the bacterial biomass during stationary phase in the humic lake cultures by 60% but no effects of the colloids were found in the clearwater cultures (Fig. 2). The simulation of the bacterial production was not due to a relatively larger colloidal fraction of DOM in the humic lake, since the colloids caused a two fold higher DOC concentration in the clearwater cultures than in the humic cultures.

The absence of a stimulating effect of colloids on the bacterial growth potential in the clearwater lake cultures is surprising, as the colloidal enrichment caused an increase in not only DOC, but also of DCCHO, and DCAA (Table 1). A possible cause for this may be limitation of the bacterial growth by factors other than availability of organic substrate, e.g. phosphorus limitation (Morris & Lewis 1992, Zweifel et al. 1993). In this study, we did not test whether phosphorus limitation occurred. Previous studies of the two present lakes indicate that addition of phosphate does not influence growth of the bacteria (Tranvik 1988), while addition of glucose alone stimulated growth (Tranvik 1990). Other studies of these lakes, however, indicate a bacterial phosphorus limitation (Tranvik in prep.). An alternative explanation for the lack of stimulation of bacteria by colloid enrichment in the clearwater cultures may be that the colloidal DOM, although analytically detectable as DOC, DCCHO, and DCAA, to some extent was recalcitrant towards microbial utilization.

The observed DOM utilization may also have been influenced by differences in species composition of the originally added bacterial cultures from each lake. However, in a previous comparison of bacterial DOC consumption in a humic and a clear lake, the physiological response of the bacteria depended on the origin of the DOM and was independent on the origin of the bacterial inoculum (Tranvik & Höfle 1987). Thus, we suggest that the bacterial assemblage in this type of cultures adjust to the source of DOM by changing the species composition or by physiological adaptations.

The growth efficiency in the clearwater lake at the enhanced concentration of colloidal DOM was only half of the efficiency at natural colloidal concentration, although bacterial biomass development was not affected by the colloids (Table 1, Fig. 2). This is theoretically possible if colloidal DOM adsorbs labile bacterial substrates (Carlson et al. 1985) and makes them harder to take up (resulting in lower growth efficiency). However, it is likewise possible that the assumption implicit in the method, that decrease in DOC is entirely due to bacterial utilization, is not always valid. Due to the difficulties in keeping filtered lake water sterile for several days, like in most similar experiments, we had no sterile controls. However, DOC decline due to other factors than bacterial uptake, e.g. flocculation, may not only be an entirely abiotic process, but also dependent on the presence of bacteria as bacterial exopolymers can adsorb DOM (Decho 1990). Thus, abiotic control samples may not correctly account for changes in DOC other than bacterial uptake. There was no visible formation of particles during the incubation in our experiment. However, it is still possible that the colloidal DOM is more susceptible to aggregation into larger complexes, that are retained on the filters used for exclusion of particulate organic carbon (bacteria) prior to analysis of DOC.

Growth efficiency of bacteria growing on natural DOM, measured by decrease in DOC and increase in bacterial C, typically fall between 10% and 50% (e.g. Middelboe et al. 1992; Tranvik 1988; Zweifel et al. 1993), although some results in the 1 to 10% interval have been reported (Kirchman et al. 1991). Estimating bacterial growth efficiency by this approach is problematic due to methodological limitations in the measurement of bacterial C, but also due to the possibility that all bacterial production is not expressed as cell production. Some of the bacterial production may be secreted as extracellular polysaccharides (Decho 1990) or in other forms, e.g. as DCAA (Jørgensen et al. 1993).

Colloids generally affected the growth rate during the exponential phase less than stationary phase bacterial biomass (Figs. 2 & 3, Table 1). An exception to this was that the leucine-derived growth rate was higher in the presence of colloids in the clearwater lake, although there was no effect of colloids on the stationary phase biomass (determined from the number of bacteria) in this lake. Possibly, the leucine-to-bacteria conversion factor is different in the absence and presence of colloids. The background DOM may influence the conversion factor through interaction with leucine, e.g. by adsorption. The employed concentration of leucine (100 nM) is higher than the concentration used in most other studies but it has been found sufficient for obtaining saturation of uptake in a Finnish humic lake (Tulonen et al. 1993), as well as in the lakes of our study (Lindell, Granéli & Tranvik, manuscript). In batch cultures of indigenous lake water bacteria growing on natural DOM, it has previously been noticed that substrate quantity is poorly reflected by exponential growth rates, but it correlates well with achieved stationary phase biomass (Tranvik 1988; Tranvik 1994).

Measurement of microbial utilization of dissolved organic molecules relies on the detection of small relative changes in concentrations, as the DOM pools are large relative to the bacterial uptake. We detected only a few cases of changes in concentration of specific DOM fractions during growth of the cultures (Table 1). There was often a considerable variation among replicate samples. Possibly the increase in colloidal organic matter and high natural humic content (Skärshultsjön) interferred with the applied analytical procedures, e.g. through adsorption to filters and surfaces. In addition, net changes in concentration may not represent microbial uptake correctly. Excretion of

DOM by bacteria would result in underestimates of substrate consumption. A significant amount of the bacterial production may be excreted as extracellular polysaccharides (Decho 1990), a phenomenon that has received only little attention in studies of pelagic bacterial carbon dynamics. It has recently been indicated that considerable production of DCCHO occurs in bacteria-only cultures growing in lake water (Jørgensen & Jensen 1994). Accordingly, release of DCAA (probably proteins) has been measured in similar cultures of seawater (Jørgensen et al. 1993).

For these reasons, we consider the radioisotope approach more reliable for the measurement of microbial utilization of specific compounds. Colloid enrichment had only minor effects on the overall composition of DOM (Table 2), but had effects on the DOM being utilized by the bacteria, as indicated by the radioisotope uptake experiments. Hence, in the humic lake, the uptake of glucose was markedly increased in cultures receiving colloids (Fig. 4a), and in both lakes colloid enrichment resulted in increased utilization of amino acids (Fig. 4b). The contribution of glucose to bacterial biomass accumulation was minor ($\leq$3%, Table 3) in all cultures.

In addition to uptake of dissolved free glucose, the utilization of starch indicated that the bacteria increased their degradation of glucose-$\alpha$-1,4-polymers during the incubation (Table 4). This increased hydrolytic activity appeared not to be reflected in a generally higher capacity for degradation of polymer saccharides, as the ambient DCCHO concentrations only were reduced in the clearwater lake + colloids (Table 1). This observation may also indicate that glucose-$\alpha$-1,4-polymers were not commonly occurring DOM substances in the lakes.

Uptake of free amino acids contributed significantly to both bacterial carbon and nitrogen incorporation (Table 3). In the clearwater lake, this contribution was enhanced by the colloid enrichment, resulting in 17 to 58% of the bacterial carbon and 29 to 100% of the bacterial nitrogen being sustained by uptake of DFAA. This contribution of DFAA to bacterial C and N requirements agrees with previous studies of freshwaters, e.g. Lake Constance and various Danish lakes (Jørgensen 1987; Rosenstock & Simon 1993) and coastal, and oceanic ecosystems (Fuhrman 1990; Keil & Kirchman 1991).

## Summary

In summary, our study shows that natural colloidal organic matter may contribute to bacterial growth in humic water. Isotope experiments indicated that amino acids contributed to much of the carbon and nitrogen demand of the bacteria in both humic and clear water. Estimates of bacterial substrate consumption calculated from the decrease in concentration of specific

DOC fractions in response to growth was less successful than the radiotracer approach. Other studies have successfully employed a concentration approach, i.e. detected significant concentration changes in response to microbial growth (Jørgensen et al. 1993). Possibly, the humic matter in the lakes of our study, which frequently interferes with chemical analyses, caused variation between replicates which made the detection of significant concentration changes difficult. Nevertheless, the concentration data was a valuable component of our data, as it could be used in combination with radiotracer measurements to calculate the flux of glucose and amino acids into the bacteria. Thus, a combination of concentration measurements and radiotracer experiments may be a valuable tool for the understanding of interactions of bacteria and DOM.

## Acknowledgements

This study was supported by grants from the Swedish Natural Science Research Council to LJT, and from the Danish Natural Science Research Council to NOGJ. Regitze E. Jensen provided excellent technical assistance.

## References

Aho J (1986) Size exclusion chromatograms of dissolved humic material in oligo-, meso- and polyhumic lakes and in ground water. Arch. Hydrobiol. 107: 301–314

Benner R & Strom M (1993) A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. Mar. Chem. 41: 153–160

Burnison BK & Leppard GG (1983) Isolation of colloidal fibrils from lake water by physical separation techniques. Can. J. Fish. Aquat. Sci. 40: 373–381

Carlson DJ, Mayer LM, Brann ML & Mague TH (1985) Binding of monomeric organic compounds to macromolecular dissolved organic matter in seawater. Mar. Chem. 16: 141–153

Decho AW (1990) Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Annu. Rev. 28: 73–153

Ertel JR, Caine JM & Thurman ME (1993) Biomarker compounds as source indicators for dissolved fulvic acids in a bog. Biogeochemistry 22: 195–212

Ford TE & Lock MA (1987) Epilithic metabolism of dissolved organic carbon in boreal forest rivers. FEMS Microbiol. Ecol. 45: 89–97

Freeman C & Lock MA (1992) Recalcitrant high-molecular-weight material, an inhibitor of microbial metabolism in river biofilm. Appl. Environ. Microbiol. 58: 2030–2033

Fry JC (1988) Determination of biomass. In: Austin B (Ed) Methods in Aquatic Bacteriology. Wiley, New York

Fuhrman J (1990) Dissolved free amino acid cycling in an estuarine outflow plume. Mar. Ecol. Prog. Ser. 66: 197–203

Jørgensen NOG (1987) Free amino acids in lakes Concentrations and assimilation rates in relation to phytoplankton and bacterial production. Limnol. Oceanogr. 32: 97–111

Jørgensen NOG (1992). Incorporation of [$^3$H]leucine and [$^3$H]valine into protein of freshwater bacteria uptake kinetics and intracellular isotope dilution. Appl. Environ. Microbiol. 58: 3638–3646

Jørgensen NOG & Jensen RE (1994) Application of PAD-HPLC in microbial ecology studies of fluxes of free monosaccharides and total carbohydrates in fresh water. FEMS Microbiol. Ecol. 14: 79–94

Jørgensen NOG, Kroer N, Coffin RB, Yang X-H & Lee C (1993) Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. Mar. Ecol. Prog. Ser. 98: 135–148

Keil RG & Kirchman DL (1991) Contribution of dissolved free amino acids and ammonium of the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. 73: 1–10

Kirchman DL, K'nees E & Hodson RE (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49: 599–607

Kirchman DL, Suzuki Y, Garside C & Ducklow H (1991) High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. Nature 352: 612–614

Koike I, Hara S, Terauchi K & Kogure K (1990) Role of sub-micrometre particles in the ocean. Nature 345: 242–244

Lindroth P & Mopper K (1979) High performance liquid chromatographic determinations of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Anal. Chem. 51: 1667–1674

Meyer JL, Edwards RT & Risley R (1987) Bacterial growth on dissolved organic carbon from a blackwater river. Microb. Ecol. 13: 13–29

Middelboe M & Søndergaard M (1993) Bacterioplankton growth yields seasonal variations and coupling to substrate lability and $\beta$-glucosidase activity. Appl. Environ. Microbiol. 59: 3916–3921

Middelboe M, Nielsen B & Søndergaard M (1992) Bacterial utilization of dissolved organic carbon (DOC) in coastal waters – determination of growth yield. Arch Hydrobiol. Beih. 37: 51–61

Morris DP & Lewis WM Jr (1992) Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol. Oceanogr. 37: 1179–1192

Münster U (1984) Distribution, dynamic and structure of free dissolved carbohydrates in the Plußsee, a North German eutrophic lake. Verh. Internat. Verein. Limnol. 22: 929–935

Münster U (1985) Investigations about structure, distribution and dynamics of different organic substrates in the DOM of lake Plußsee. Arch. Hydrobiol. Suppl. 70: 429–480

Münster U (1992) Microbial extracellular enzyme activities and biopolymer processing in two polyhumic lakes. Arch. Hydrobiol. Ergeb. Limnol. 37: 21–32

Münster U (1993) Concentrations and fluxes of organic carbon in the aquatic environment. Antonie van Leeuwenhoek 63: 243–274

Münster U & Chróst RJ (1990) Dissolved organic matter (DOM) in aquatic environments origin, distribution, composition and microbial utilization. In: Overbeck J & Chróst RJ (Eds) Advanced Biochemical and Molecular Approaches to Aquatic Microbial Ecology (pp 8–46). Brock/Springer Series in Contemporary Bioscience, Springer Verlag, New York

Münster U, Einii P, Nurminen J & Overbeck J (1992) Extracellular enzymes in a polyhumic lake important regulators in detritus processing. Hydrobiologia 229: 225–238

Nagata T (1986) Carbon and nitrogen content of natural planktonic bacteria. Appl. Environ. Microbiol. 52: 28–32

Peake E, Baker BL & Hodgson GW (1977) Hydrogeochemistry of the surface waters of the Mackenzie River drainage basin, Canada. II. The contribution of amino acids, hydrocarbons and chlorins to the Beaufort Sea by the Mackenzie River system. Geochim. Cosmochim. Acta 36: 867–883

Porter KG & Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943–948

Rosenstock B & Simon M (1993) Use of dissolved combined and free amino acids by planktonic bacteria in Lake Constance. Limnol. Oceanogr. 38: 1521–1531

Satoh Y, Shoji S, Satoh H & Takahashi M (1987) Dissolved organic matter in colored water from mountain bog pools in Japan. I. Seasonal changes in the concentration and molecular weight distribution. Arch. Hydrobiol. 110: 589–603

Sigleo AC, Hare PE & Helz GR (1983) The amino acid composition of estuarine colloidal material. Est. Coast. Shelf. Sci. 17: 87–96

Simon M & Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51: 201–213

Sundh I (1989) Characterization of phytoplankton extracellular products (PDOC) and their subsequent uptake by heterotrophic organisms in a mesotrophic forest lake. J. Plankton Res. 11: 463–486

Sundh I (1991) The dissolved organic carbon released from lake phytoplankton – biochemical composition and bacterial utilization. Acta Univ. Ups. Comprehensive summaries of Uppsala Dissertations from the Faculty of Science 324

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 (1989) Bacterioplankton growth, grazing mortality and quantitative relationship to primary production in a humic and a clearwater lake. J. Plankton Res. 11: 985–1000

Tranvik LJ (1990) Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear waters. Appl. Environ. Microbiol. 56: 1672–1677

Tranvik LJ (1994) Effects of colloidal organic matter on the growth of bacteria and protists in lake water. Limnol. Oceanogr. 39: 1276–1285

Tranvik LJ & Höfle MG (1987) Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. Appl. Environ. Microbiol. 53: 482–488

Tucshall JR & Brezonik PL (1980) Characteristics of organic nitrogen in natural waters: Its molecular size, protein content, and interaction with heavy metals. Limnol. Oceanogr. 25: 495–504

Tulonen T (1993) Bacterial production in a mesohumic lake estimated from $^{14}$C-thymidine incorporation rate. Microb. Ecol. 26: 201–217

Wells ML & Goldberg ED (1991) Occurrence of small colloids in sea water. Nature 353: 342–344

Wicks RJ, Moran MA, Pittman LJ & Hodson RE (1991) Carbohydrate signatures of aquatic macrophytes and their dissolved degradation products as determined by a sensitive high-performance ion chromatography method. Appl. Environ. Microbiol. 573: 135–143

Zweifel UL, Norrman B & Hagström Å (1993) Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar. Ecol. Prog. Ser. 101: 23–32