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Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake

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Abstract

Concentrations and bacterioplankton uptake of dissolved free monosaccharides (DFCHO) in relation to bacterioplankton production and phytoplankton biomass were studied in mesotrophic Lake Constance, Germany, from August 1995 to December 1996. Concentrations of total DFCHO, measured by high-performance liquid chromatography (HPLC) and pulsed amperometric detection, ranged from <20 to 440 nM and exhibited pronounced spatiotemporal variations. Enhanced concentrations of DFCHO were often associated with the breakdown of phytoplankton blooms and periods of low phytoplankton growth. Bacterial uptake rates of DFCHO were also enhanced during such events, as well as at peaks of bacterioplankton production (BP). Ratios of uptake of DFCHO/BP, however, indicated that DFCHO concentrations were relatively more important as substrates during periods of low bacterial growth than during phases of intense bacterial growth. Ratios of bacterial uptake of DFCHO/BP from spring to fall ranged from 0.22 to 0.39 in the layer 0–10 m, from 0.17 to 0.56 at 20 m, and from 0.22 to 0.46 at 50 m, respectively. Glucose, galactose, and mannose dominated the DFCHO pool and constituted ~55 to ~70 mol%. The analysis of individual turnover times of five DFCHO concentrations from August to November 1995 at 3, 10, and 50 m revealed that the glucose pool turned over most rapidly, followed by galactose and mannose. Glucosamine and fructose always exhibited substantially longer turnover times. Comparisons to previous studies show that DFCHO concentrations are of similar significance for bacterioplankton growth as dissolved free amino acids (DFAA), even though temporal uptake patterns of both substrates are different.

The large pool of dissolved organic matter (DOM) in aquatic ecosystems consists of a labile fraction, which is turned over rapidly, i.e., within days or weeks (Münster 1993; Søndergaard and Middelboe 1995; Amon and Benner 1996), and a recalcitrant fraction with a turnover time of years, decades, or longer (Hedges 1992; Münster 1993). The most important components of the labile DOM readily released by phytoplankton and consumed by bacterioplankton are DFAA and combined amino acids (DCAA) and DFCHO and combined monosaccharides (DCCHO; Sundh 1992; Münster 1993; Biddanda and Benner 1997; Simon et al. 1998). Many studies conducted during the last decade have examined the bacterial consumption and turnover of DFAA and DCAA over space and time in various aquatic ecosystems (e.g., Jørgensen 1987; Coffin 1989; Fuhrman 1990; Simon and Rosenstock 1992; Jørgensen et al. 1993; Keil and Kirchman 1993; Rosenstock and Simon 1993; Kroer et al. 1994; Middelboe et al. 1995; Simon 1998). Much less information, however, is available on the turnover and bacterial consumption of dissolved carbohydrates, even though they comprise the largest fraction of the DOM pool so far identified (Benner et al. 1992; Pakulski and Benner 1994) and usually exceed the concentration of DCAA (Münster 1993; Jørgensen et al. 1998). In lacustrine as well as in marine pelagic environments, it has been shown that highest concentrations of dissolved carbohydrates occur at the end of phytoplankton blooms (Ittekkot et al. 1981; Chróst et al. 1989; Mopper et al. 1995). A few studies have demonstrated that consumption of DCCHO can comprise a substantial fraction of the bacterial carbon demand in lakes (Jørgensen and Jensen 1994; Hanisch et al. 1996; Jørgensen et al. 1998), but only scarce information is available on the significance and seasonal dynamics of DFCHO for bacterioplankton growth. Jørgensen (1990) and Jørgensen and Jensen (1994), working with freshwater mesocosms from eutrophic lakes, showed that DFCHO can be an important substrate for bacterioplankton growth. Rich et al. (1996) reported that DFCHO supports 14–85% of the bacterial biomass production in the equatorial Pacific, depending on the season and depth. In the Arctic Ocean, uptake of DFCHO was found to contribute a substantial fraction of the bacterial carbon demand, even though uptake of DFAA contributed more (Rich et al. 1997). The reports of Rich et al. (1996, 1997) are the only ones about concentrations of DFCHO and uptake by bacterioplankton in relation to their C demand over space and time and conducted in marine systems, but there is no comparable information available for lacustrine environments.

A method for studying bacterioplankton turnover of DFCHO was lacking until the recent development of a reliable method for measuring concentrations of DFCHO in the nanomolar range, as they occur in natural waters, without preconcentrating the samples. Based on anion exchange chromatography at high pH in combination with pulsed amperometric detection (PAD; Rocklin and Pohl 1983; Andrews and King 1990; Johnson and LaCourse 1990), Mopper et al. (1992) demonstrated that precise measurements of DFCHO in unconcentrated water samples of various origins are possible. This very promising method has been applied

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in the above-mentioned studies on the turnover of DFCHO but still is not yet widely used.

We applied the PAD-HPLC technique to measure spatio-temporal dynamics and bacterioplankton turnover and uptake of DFCHO in Lake Constance, a mesotrophic lake, during a 16-month study in 1995 and 1996. The results show that DFCHO concentrations are important bacterial substrates, accounting for ~30% of the carbon demand for biomass production, and that they become relatively more important during the breakdown of phytoplankton blooms and when rates of BP are relatively low.

Materials and methods

The study was carried out between August 1995 and December 1996 at the center and deepest point of Lake Überlingen, the northwestern fjordlike arm of prealpine Lake Constance, adjacent to Austria, Germany, and Switzerland. Lake Constance is a mesotrophic and warm-monomictic lake with a surface area of 472 km^2 and maximum and mean depths of 253 and 101 m, respectively. Lake Überlingen has maximum and mean depths of 147 and 90 m, respectively. The lake has undergone oligotrophication during the last 15 yr due to a severe reduction of the P load (Güde et al. 1998). Plankton dynamics have been studied extensively in the recent past (e.g., Gaedke 1998; Haese et al. 1998; Simon et al. 1998; Weisse and Müller 1998). Samples were collected with a clean van Dorn bottle weekly to biweekly and at longer time intervals during the cold season at depths of 1, 3, 6, 10, 20, and 50 m. Samples were immediately transferred into clean 1-liter polyethylene bottles. Samples for measuring the in situ concentration of DFCHO were immediately filtered through thoroughly rinsed 0.2-μm Gelman Acrodiscs (Tuffrin) into carefully acid-rinsed 20-ml polyethylene vials. These filtrates and all other samples were brought to the lab in a cooler and further processed within 2 h. DFCHO samples were kept frozen at −20°C and analyzed within 3 months.

Analysis of monosaccharides—The concentration of DFCHO was determined by HPLC and PAD according to Mopper et al. (1992). As weak acids with a pK between 12 and 14, mono- and disaccharides react as anions in a strong alkaline environment and thus can be separated with an anion exchange column. For the analysis, we used a Dionex system consisting of a gradient pump (GP 40), an autosampler (AS 3500), the column CarboPac PA1 (4 × 250 mm), and an electrochemical detector (ED 40). Sodium hydroxide at a concentration of 45 mM (pH = 13.6) served as eluent. This concentration was established by mixing Milli-Q water and 200 mM NaOH, which was freshly prepared (<2 d prior to the analysis) from a carbonate-free 50% NaOH solution (Baker, Germany). Milli-Q water was degassed with helium for 30 min to remove carbonate prior to the first analysis, since carbonate interferes during the separation of anions in the column. The flow rate was 1 ml min^{-1}, and the column temperature was kept at 10°C, which gave best separations of the individual mono- and disaccharides. Glucose, galactose, and mannose, however, coeluted under these conditions. We tried to separate these three monosaccharides by lowering the NaOH concentration to 12.5 mM, thus extending the retention times. However, due to peak broadening, the separation did not improve, while the detection limit increased from 0.5 to >5 nM because of the less accurate integration of the peak area, which was problematic for samples with concentrations of some monosaccharides close to the detection limit. Monosaccharides were identified by retention time in a comparison to an external mixed standard composed of 14 monosaccharides run prior to and after every series of 16–24 analyses. We were able to identify all peaks, except three, which did not occur in every sample and contributed <10% to total DFCHO concentrations. The concentration of the unidentified monosaccharides, however, was included in calculations of total DFCHO. For routine analyses, subsamples of 1.8 ml were placed into combusted glass vials, degassed for 10 min with helium, and closed with screw caps. Samples of 1 ml were injected by the autosampler, and, after separation, mono- and disaccharides were detected by PAD. The column was cleaned after each run for 15 min with 200 mM NaOH and equilibrated for 10 min with 45 mM NaOH. When the separation deteriorated or the baseline became unstable, the column was washed for 15 min with Milli-Q water, 1 h with 1 M HCl, 15 min with Milli-Q water, and 15 min with 200 mM NaOH. The coefficient of variation (C.V., mean/SD) of triplicate analyses was usually <5%.

Turnover and uptake of monosaccharides—Bacterial turnover of monosaccharides was determined by measuring turnover rates of an equimolar mixture of ^{14}C-glucose (Glc, 320 mCi $mmol^{-1}$), ^{14}C-galactose (Gal, 315 mCi $mmol^{-1}$), ^{14}C-mannose (Man, 287 mCi $mmol^{-1}$), ^{14}C-fructose (Fru, 277 mCi $mmol^{-1}$), and ^{14}C-glucosamine (GlcN, 212 mCi $mmol^{-1}$) (Amersham). Four subsamples of 5 ml (most samples) or 10 ml (for 50-m samples and those collected during periods of low activity in November and December) were withdrawn into polystyrene test tubes and labeled with the mixture at a final total concentration of 5 nM (Glc + Gal + Man + Fru + GlcN). One sample was immediately fixed with 2% Formalin (final concentration) and served as a blank. All samples were incubated at in situ temperature in the dark, and activity was stopped after 1 h (most samples) or 2 h (low activity samples) by adding Formalin. The samples were then filtered onto 0.45-μm nitrocellulose filters (Sartorius, Germany), and, after rinsing with ice-cold particle-free lake water, the filters were radioassayed by liquid scintillation counting. Turnover rates of individual monosaccharides were determined in the same way but by labeling subsamples with one of the five ^{14}C-monosaccharides individually at final concentrations of 1 nM. Incorporation of monosaccharides into the macromolecular fraction of the bacterial cell was determined as described above, but, after filtration and rinsing with particle-free water, the filters were extracted with 5% ice-cold trichloroacetic acid (TCA) for 5 min and rinsed twice with ice-cold 5% TCA. Incorporation of monosaccharides into the protein–carbohydrate fraction was determined in the Formalin-fixed samples by adding TCA at a final concentration of 5% and heating them in glass test tubes with screw caps to 100°C for 15 min to hydrolyze the nucleic acids (Simon and Azam 1989). Thereafter, the

samples were chilled, filtered, and radioassayed as described above.

The bacterial turnover rate (r_T) and net uptake of DFCHO ($DFCHO_{up}$) per hour were calculated as $r_T(h^{-1}) = dpm_{uptake}/dpm_{added}$, and $DFCHO_{up}$(mol liter^{-1} h^{-1}) = concentration of DFCHO (nM) $\times$ r_T. The turnover time (T_1, single substrate approach) of all five or of individual monosaccharides was calculated as $1/r_T$(h).

Bacterial biomass production—Biomass production of heterotrophic bacterioplankton was determined by the incorporation of ^{14}C-leucine (Leu) according to Kirchman et al. (1985) and Simon and Azam (1989). Triplicates and a Formalin-killed control were labeled with ^{14}C-leucine (312 mCi mmol^{-1}, Amersham) at a final concentration of 30 nM. Experiments at various seasons showed that this concentration always maximized incorporation of Leu (Simon and Rosenstock 1992). Incubation, filtration, extraction, and the radioassay of the samples were determined in the same way as the incorporation of DFCHO into the bacterial macromolecular fractions (*see above*). Rates of bacterial biomass production (BBP-C, g C liter^{-1} h^{-1}) were calculated according to Simon and Azam (1989) as BBP-C = $Leu_{inc} \times 1{,}545 \times F_1 \times F_2 \times$ ID. Leu_{inc} is the amount of Leu incorporated into the ice-cold TCA precipitate (mol liter^{-1} h^{-1}), 1,545 is the conversion factor for Leu_{inc} into biomass produced (g C per mol Leu), F_1 is the ratio of cellular carbon/protein (0.86), and F_2 is the ratio of Leu_{inc} in the hot TCA precipitate (protein–carbohydrate fraction) over Leu_{inc} in the ice-cold TCA precipitate. Experiments at various seasons showed that F_2 ranged between 0.80 and 0.90. Therefore, a constant F_1 of 0.85 was used for the calculation. ID is the intracellular isotope dilution of Leu and was assumed to equal 2 (Simon and Rosenstock 1992).

Bacterial abundance and chlorophyll—The abundance of bacteria was enumerated by epifluorescence microscopy after staining with 4′, 6-diamidino-2-phenylindole (DAPI) according to Porter and Feig (1980). Chlorophyll *a* (Chl *a*) was determined after hot ethanol extraction spectrophotometrically as described in Simon and Tilzer (1987).

Seasonal dynamics and seasonal mean values of all parameters measured at 1, 3, 6, and 10 m are presented as mean values for the 0–10-m layer. The means were calculated as the arithmetic mean ± standard deviation.

Results

When the investigation started in late August 1995, Lake Constance was thermally stratified with a near-surface temperature of 20°C. In October and November, the thermocline gradually eroded, such that in January and February 1996, the temperature decreased to 4–5°C, and the lake turned over. From June until early September 1996, the lake became stratified again with a near-surface temperature around 20°C. Three major phytoplankton blooms occurred during the study period, one from late August until early October 1995, the typical spring bloom in April and May 1996, which occurred in several phases and was followed by the clear-water phase, and a summer bloom in July and August (Fig. 1).

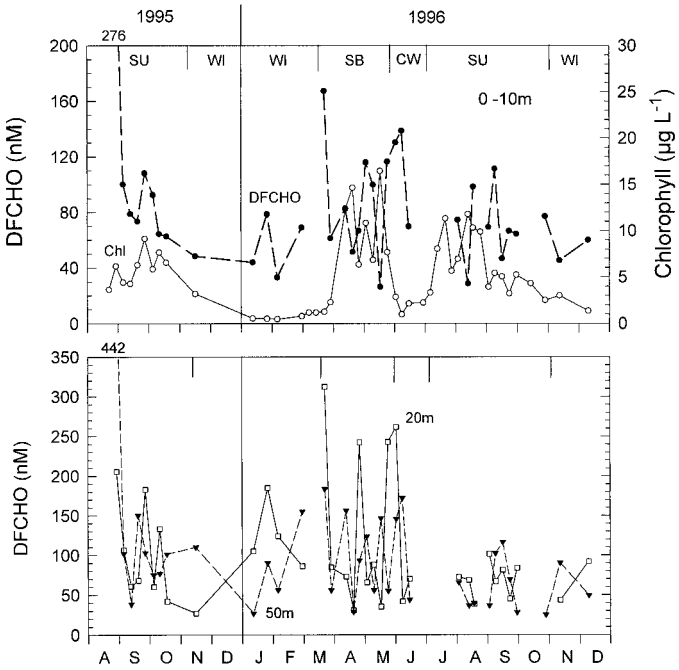

Fig. 1. Concentrations of DFCHO and Chl *a* (Chl) in the 0–10-m layer (upper panel) and of DFCHO at 20 and 50 m (lower panel) in Lake Constance between August 1995 and December 1996. The different seasonal periods as indicated in Table 1 are marked along the top line of the panels. Note the different scales for DFCHO in the upper and lower panels.

Seasonal dynamics of bacterial biomass production reflected that of the phytoplankton with elevated rates in August and September 1995 in the layer 0–10 m and high rates in early May during the spring bloom, in late June, and in August 1996 (Fig. 2). During the spring bloom and the clear-water phase, high rates of bacterial biomass production occurred also at 20 m, whereas at other times, only minor fluctuations were recorded at 20 and 50 m (Fig. 2). The pronounced differences in Chl *a*, BP, and also bacterial numbers during the various seasonal periods, with highest values of the bacterioplankton parameters in the clear-water phase, are also obvious from the means of these periods (Table 1).

Concentration and composition of DFCHO—Concentrations of DFCHO varied greatly vertically and seasonally. Highest concentrations of >200 nM occurred at all depths at the beginning of the study period in late August 1995 and also at 20 m during the spring bloom and the clear-water phase (Fig. 1). Lowest concentrations of <40 nM occurred at several instances in the 0–10-m layer and also at 20 and 50 m. During the phytoplankton blooms, concentrations of DFCHO in the 0–10-m layer usually ranged between 80 and 120 nM. Samples from mid-June until the end of July 1996 unfortunately were lost. During most of the study period, concentrations did not covary with any bacterioplankton or phytoplankton parameter measured. Only during the spring bloom were concentrations of DFCHO significantly correlated to bacterial numbers and production and inversely to Chl *a* (Table 2). The inverse relationship between concen-

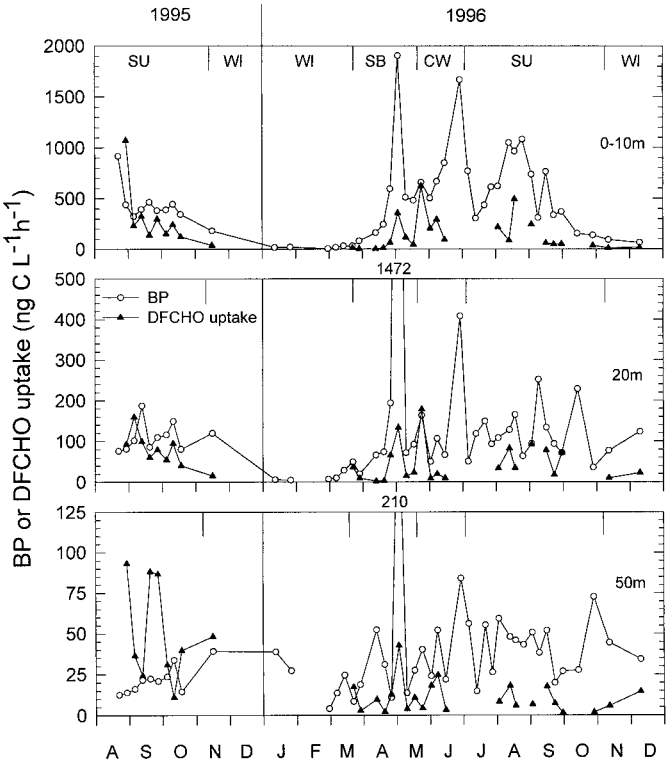

Fig. 2. Uptake rates of DFCHO and BP in the 0–10-m layer and at 20 and 50 m in Lake Constance between August 1995 and December 1996. The different seasonal periods as indicated in Table 1 are marked along the top line of the panels. Note the different scales.

Table 2. Correlation coefficient of linear correlation analyses between seasonal dynamics of concentrations ($DFCHO_{con}$) and uptake rates of DFCHO ($DFCHO_{up}$), the ratio of $DFCHO_{up}$/BP, and the parameters indicated below on the basis of mean values of 0–10 m ($P < 0.01$). Correlations were calculated for the total study and the following periods: fall 1995 (29 Aug–17 Nov 95), spring bloom 1996 (26 Mar–29 May), and summer and fall 1996 (summer/fall, 25 Jun–8 Oct 96).

X	*Y*	Total	Fall 95	Spring bloom	Summer/ Fall 96
$DFCHO_{con}$	Chl *a*	†	†	−0.53*	†
$DFCHO_{con}$	BN	†	†	0.62	†
$DFCHO_{con}$	BP	†	†	0.56	†
$DFCHO_{con}$	$DFCHO_{up}$	0.73	0.95	0.74	0.77
$DFCHO_{con}$	$DFCHO_{up}$/BP	0.53	0.95	†	−0.76
$DFCHO_{up}$	Chl *a*	†	†	†	†
$DFCHO_{up}$	BN	†	†	0.51	†
$DFCHO_{up}$	BP	†	†	0.44	†
$DFCHO_{up}$	$DFCHO_{up}$/BP	0.77	0.99	†	0.57
$DFCHO_{up}$/BP	Chl *a*	†	†	0.54	0.70
$DFCHO_{up}$/BP	BN	†	†	†	†
$DFCHO_{up}$/BP	BP	†	†	†	0.55

* Correlation for spring bloom and clear-water phase (26 Mar–25 Jun 96).
† Correlation not significant or with $r < 0.25$.

trations of DFCHO and chlorophyll is also obvious from the profiles on 14 and 21 May and 8 and 13 August 1996 (Fig. 3). In both cases, chlorophyll decreased from the first to the second week, whereas concentrations of DFCHO increased.

The differences in concentrations of DFCHO between the various seasonal phases also become evident from the mean concentrations of DFCHO at the three depth layers (Table

Table 1. Mean values ± standard deviation of Chl *a*, bacterioplankton numbers (BN), BP, concentrations ($DFCHO_{con}$) and uptake rates of DFCHO ($DFCHO_{up}$), and the ratio of $DFCHO_{up}$/BP at 0–10, 20, and 50 m for the total study and periods indicated.*

Period	Chl *a* (μg Chl *a* liter)	BN (10^6 ml^{-1})	BP (ng C liter^{-1} h^{-1})	$DFCHO_{con}$ (nM)	$DFCHO_{up}$ (ng C liter^{-1} h^{-1})	$DFCHO_u$/BP
0–10 m						
Total	5.50 ± 3.90	3.25 ± 1.59	440.2 ± 406.5	82.3 ± 44.0	194.5 ± 211.1	0.38 ± 0.39
Fall (29 Aug–17 Nov 95)	5.55 ± 1.56	3.48 ± 0.71	372.4 ± 90.7	96.7 ± 62.7	268.0 ± 262.6	0.67 ± 0.54
WI (17 Nov 95–26 Mar 96)	0.89 ± 0.31	1.07 ± 0.36	24.1 ± 10.6	56.1 ± 18.5	nd	nd
SB (26 Mar–29 May 96)	9.68 ± 4.43	3.71 ± 1.65	579.4 ± 537.7	77.6 ± 30.0	153.3 ± 208.4	0.22 ± 0.25
CW (29 May–25 Jun 96)	2.04 ± 0.67	4.98 ± 1.06	921.8 ± 447.5	112.8 ± 30.6	197.2 ± 81.9	0.39 ± 0.20
SU/Fall (25 Jun–8 Oct 96)	6.74 ± 2.89	3.93 ± 1.24	607.4 ± 288.3	70.0 ± 24.6	171.7 ± 151.6	0.26 ± 0.17
20 m						
Total	nd	1.95 ± 0.90	126.9 ± 211.4	103.6 ± 71.8	57.2 ± 46.6	0.46 ± 0.38
Fall (29 Aug–17 Nov 95)	nd	1.99 ± 0.61	114.1 ± 32.9	98.4 ± 59.6	77.1 ± 39.1	0.71 ± 0.39
WI (17 Nov 95–26 Mar 96)	nd	1.06 ± 0.26	19.4 ± 16.6	125.0 ± 37.2	nd	nd
SB (26 Mar–29 May 96)	nd	2.25 ± 1.08	286.9 ± 467.7	107.2 ± 80.2	53.4 ± 62.9	0.31 ± 0.33
CW (29 May–25 Jun 96)	nd	1.78 ± 0.42	157.5 ± 146.1	124.4 ± 97.6	12.4 ± 5.0	0.17 ± 0.02
SU/Fall (25 Jun–8 Oct 96)	nd	2.37 ± 0.98	124.6 ± 56.4	69.7 ± 19.2	58.4 ± 27.1	0.56 ± 0.31
50 m						
Total	nd	0.92 ± 0.33	35.3 ± 31.3	95.7 ± 73.8	24.1 ± 26.0	1.03 ± 1.59
Fall (29 Aug–17 Nov 95)	nd	0.87 ± 0.32	22.8 ± 8.1	133.2 ± 112.8	50.9 ± 28.9	2.65 ± 1.90
WI (17 Nov 95–26 Mar 96)	nd	0.95 ± 0.28	17.0 ± 11.4	82.0 ± 47.7	nd	nd
SB (26 Mar–29 May 96)	nd	1.23 ± 0.36	50.5 ± 61.7	89.4 ± 44.8	11.1 ± 12.5	0.32 ± 0.34
CW (29 May–25 Jun 96)	nd	0.90 ± 0.02	45.5 ± 25.2	120.5 ± 55.2	15.3 ± 9.0	0.46 ± 0.25
SU/Fall (25 Jun–8 Oct 96)	nd	0.81 ± 0.28	40.2 ± 14.1	61.8 ± 30.8	9.3 ± 5.8	0.22 ± 0.13

* WI, winter; SB, spring bloom; CW, clear-water phase; SU/FA, summer and fall; nd, not determined.

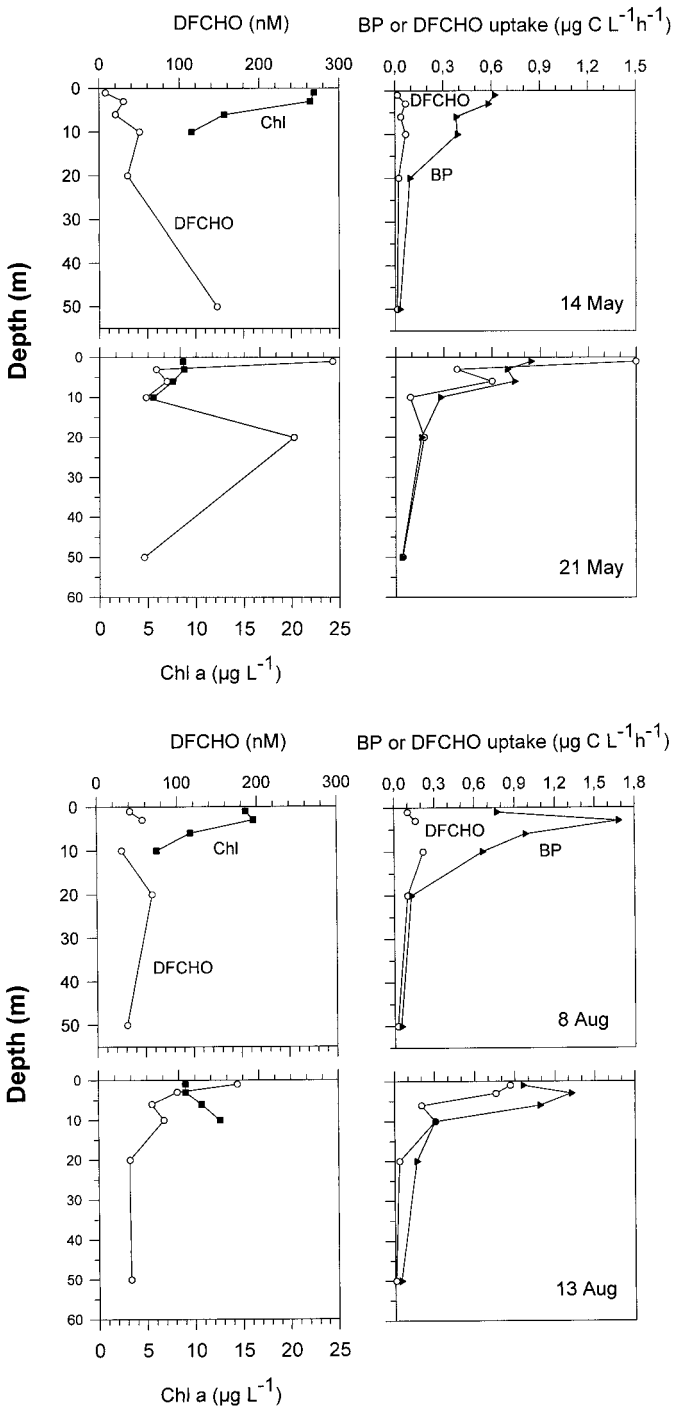

Fig. 3. Depth profiles of concentrations and uptake rates of DFCHO, Chl, and BP in Lake Constance on 14 and 21 May and 8 and 13 August 1996.

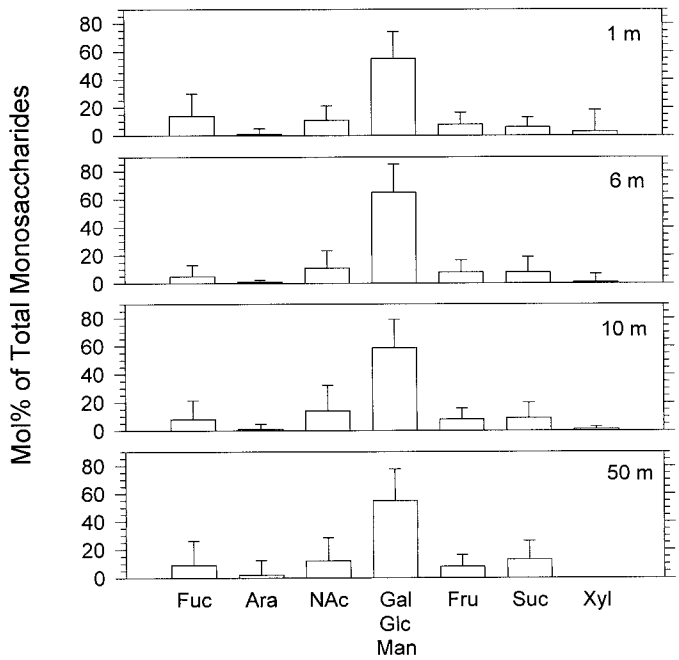

Fig. 4. Mol% of DFCHO in Lake Constance at 1, 6, 10, and 50 m. Fuc: fucose, Ara: arabinose, NAc: N-acetyl-glucosamine, Suc: sucrose, Xyl: xylose. Mean values ± standard deviation for the whole investigation period (August 1995–December 1996) are presented.

1). It is remarkable that there was no systematic decrease in concentrations with depth but rather, an increase, such as in fall 1995, in winter, and during the spring bloom. In winter during the overturn, the highest concentrations were even measured at 20 m. Only in summer and fall 1996 was there a tendency for decreasing concentrations with depth.

The composition of the DFCHO pool remained fairly constant during the study period. Mean for the four depths indicated that glucose, galactose, and mannose constituted 50–60 mol%, with only minor fluctuations vertically and seasonally (Fig. 4). N- acetyl-glucosamine comprised 10–14 mol%, and fucose, fructose, and sucrose each comprised 5–14 mol%. All other monosaccharides constituted <3 mol%. Xylose was not found in 1995. Only at the end of August 1995 was the mol% distribution different, with N-acetyl-glucosamine as the dominant monosaccharide comprising 35–65 mol% with increasing depth, followed by sucrose and glucose, galactose, and mannose with 14–30 mol%.

Turnover of individual monosaccharides—Unfortunately, we could not separate glucose, galactose, and mannose during the HPLC analyses and thus could not determine the relative concentration of these monosaccharides. To examine the relative contribution of these three monosaccharides and of glucosamine and fructose to the total turnover of DFCHO, we measured the turnover times of these compounds individually at 3, 10, and 50 m from August to November 1995 (Fig. 5). Glucose always exhibited the shortest turnover time, with mean values of 11 and 12 h from August to mid-October at 3 and 10 m, respectively. Galactose and mannose had mean turnover times of 31 and 39 h at these depths. At 50 m, all three monosaccharides turned over much slower, but glucose still exhibited the shortest turnover time. Glucosamine and fructose always turned over substantially slower than the three major monosaccharides, with mean values at 3 and 10 m between 56 and 118 h and at 50 m, of >600 h.

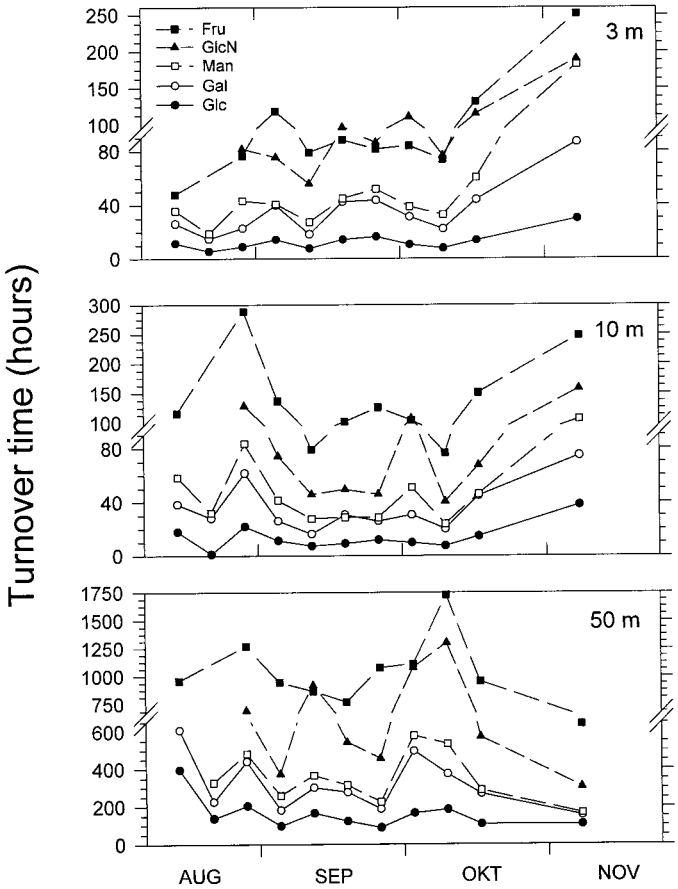

Fig. 5. Turnover times of individual monosaccharides in Lake Constance at 3, 10, and 50 m from August to November 1995. Note the different scales. The turnover time of glucosamine was not measured on the two first dates in August, and that of fructose was not measured on the second date in August.

Bacterioplankton uptake of DFCHO—Uptake rates of DFCHO varied greatly seasonally and vertically. Highest rates of >500 ng C liter^{-1} h^{-1} occurred at the end of August 1995 and at the transition between the spring bloom and the clear-water phase at the end of May 1996 and in mid-August 1996 in the 0–10-m layer (Fig. 2). These maxima did not occur simultaneously with those of bacterial biomass production, even though at these dates, the latter was also enhanced. In fact, uptake rates of DFCHO varied fairly independently from bacterial numbers and production and also from Chl *a*. This is demonstrated by the profiles of 14 and 21 May and 8 and 13 August when uptake rates of DFCHO increased greatly from the first to the second date, whereas Chl *a* concentrations decreased, and BP remained fairly constant (Fig. 3). Uptake rates of DFCHO were not correlated to Chl *a* at all and were correlated to bacterial numbers and production only during the spring bloom (Table 2). In contrast to concentrations of DFCHO, uptake of DFCHO decreased strongly with depth (Figs. 2, 3; Table 1). Unfortunately, data on uptake of DFCHO were not measured from December until late March and from mid-June until the end of July 1996, including the late clear-water phase, which supported high rates of bacterial biomass production.

The ratio of DFCHO uptake to BP also varied greatly seasonally and vertically. Surprisingly high ratios exceeding 1 were recorded in late August and September 1995, particularly at 50 m (Fig. 2). At other times, it remained below 1, except at 20 m in late May 1996. The mean values of this ratio for the spring bloom, clear-water phase, and summer/fall period 1996 ranged from 0.22 to 0.39 in the 0–10-m layer, from 0.17 to 0.56 at 20 m, and from 0.22 to 0.46 at 50 m, indicating a greater variability and often higher values in greater depths. DFCHO uptake/BP exhibited no significant correlation to bacterial numbers and a weak correlation to BP in summer/fall 1996 but fairly strong correlations to Chl *a* during the spring bloom and in summer/fall 1996 and to DFCHO concentrations for the total study period, particularly in fall 1995 (Table 2).

As an overall mean, 88% of the DFCHO taken up was incorporated into the total macromolecular fraction of the bacterioplankton at 3 and 10 m and 76% at 50 m. Further, 62% of the DFCHO taken up occurred in the protein–carbohydrate fraction at 3 and 10 m and 48% at 50 m.

Discussion

Our results show that concentrations and bacterial uptake rates of DFCHO in Lake Constance varied greatly vertically and seasonally and that the significance of DFCHO as bacterial substrates increased during the breakdown of phytoplankton blooms and periods of reduced bacterioplankton growth. As an overall mean, the ratio of DFCHO uptake/BP was 0.38 in the 0–10-m layer and increased with depth. Concentrations and bacterial uptake rates of DFCHO exhibited weak positive correlations to bacterioplankton numbers and production but only during the spring bloom. Concentrations of DFCHO, however, were inversely correlated to Chl *a* during the spring bloom and the clear-water phase, suggesting that DFCHO concentrations were released to a greater extent by senescent phytoplankton and not during periods of active growth. This assumption is substantiated by the profiles analyzed in May and August (Fig. 3), which demonstrated that concentrations and bacterial uptake of DFCHO concurrently increased, whereas concentrations of Chl *a* decreased, even though rates of BP remained similar. By contrast, we did find positive correlations between the ratio of bacterial uptake of DFCHO/BP and Chl *a* during the summer and fall period 1996, indicating that the relative significance of DFCHO as bacterial substrates increased concurrently with Chl *a* during this period. Whether these relationships are a specific feature of Lake Constance or can be generalized to other pelagic ecosystems is not known.

The composition of the DFCHO pool was largely dominated by glucose, mannose, and galactose, which we unfortunately could not separate from each other in the HPLC analyses. That the turnover time of glucose at 3 and 10 m was threefold faster than that of mannose and galactose suggests that glucose also dominated the cycling and bacterial utilization of DFCHO in the epilimnion, even though a clear-cut proof needs a reliable separation of the three monosaccharides in the HPLC analyses. Rich et al. (1996, 1997) found that glucose always dominated the DFCHO pool in

the equatorial Pacific and the Arctic Ocean, whereas Jørgensen and Jensen (1994) reported roughly similar mol% of glucose, mannose, and galactose in a freshwater mesocosm.

Interestingly, because we did not have to desalt the samples prior to the analyses, we were able to detect N-acetyl-glucosamine in nearly all of our samples. On average, it was the fifth most abundant DFCHO, and, at the end of August 1995, it even dominated the DFCHO pool. This observation is important in light of the recent finding in oceanic waters of high amounts of bacterial peptidoglycan, which contains N-acetyl-glucosamine (McCarthy et al. 1998). We have no indication about the sources of N-acetyl-glucosamine, but we hypothesize that it originated from chitin rather than peptidoglycan. Chitin is the second most abundant biopolymer in nature after cellulose (Gooday 1990) and is therefore a prime source of N-acetyl-glucosamine. To determine the cycling of this monosaccharide and its significance in the cycling of total DFCHO and DCCHO, its turnover rate as well as the decomposition rate of chitin have to be known.

To our knowledge, no other study is available from pelagic ecosystems on seasonal dynamics of the turnover of DFCHO by bacterioplankton and on relationships to phytoplankton parameters such as Chl *a* to which our results can be compared. There are some reports on the dynamics of concentrations of DCCHO during phytoplankton blooms that indicate DCCHO release toward the end of the bloom (Ittekkot et al. 1981; Chróst et al. 1989; Mopper et al. 1995). A few reports are available on bacterial uptake of DFCHO from the equatorial Pacific and the Arctic Ocean (Rich et al. 1996, 1997) and freshwater mesocosms in eutrophic Danish lakes (Jørgensen 1990; Jørgensen and Jensen 1994). The equatorial Pacific study showed large spatial and seasonal variations of glucose uptake and that concentrations of DFCHO and the ratio of glucose uptake to BP varied in the same range as in our study (Rich et al. 1996). The study from the Arctic Ocean also found that glucose uptake rates varied greatly over a transect from the Chukchi Sea over the North Pole to the Nansen Basin, and ratios of glucose uptake to BP often exceeded 1 (Rich et al. 1997). Jørgensen (1990) and Jørgensen and Jensen (1994) reported concentrations, uptake rates of DFCHO, and ratios of DFCHO uptake/BP in the same range as ours, although these studies did not address seasonal dynamics of DFCHO turnover.

Carbohydrates make up a substantial fraction of the biomass of phytoplankton communities, roughly 20–45% of particulate organic carbon (POC) in phytoplankton cells and 20 to >90% of the dissolved organic carbon (DOC) freshly released by phytoplankton (Sundh 1992; Biddanda and Benner 1997). Because concentrations of DFCHO in our study were usually below 200 nM and concentrations of total dissolved carbohydrates (TDCHO) in Lake Constance ranged between 2 and 5 μM (Hanisch et al. 1996; Weiss and Simon 1999), 90 to >95% of the TDCHO in this lake are bound in DCCHO. Hanisch et al. (1996) and Weiss and Simon (1999), studying the seasonal dynamics of bacterioplankton utilization of TDCHO in Lake Constance, found that highest utilization rates of TDCHO and ratios of TDCHO utilized/BP occurred mainly during phases of low phytoplankton biomass such as during the breakdown of the spring bloom, the clear-water phase, and in late summer. Chróst et al. (1989) reported that uptake rates of glucose followed peaks of Chl *a* by a few days during the spring bloom in a eutrophic lake. These results are consistent with our observations on dynamics of the DFCHO turnover. Obviously, large quantities of carbohydrates become available to the bacterioplankton at the breakdown of phytoplankton blooms. In fact, Mopper et al. (1995) and Biddanda and Benner (1997) found that increasing amounts of carbohydrates bound in phytoplankton are released when cells become senescent. Also, Sundh (1992) reported that carbohydrates are the dominant release products in growing as well as senescent phytoplankton in Swedish lakes.

It is noteworthy, though, that the temporal patterns of phytoplankton release and bacterioplankton utilization of dissolved carbohydrates are different from those of amino acids. Several studies have shown that heterotrophic bacterioplankton prefer amino acids to DFCHO (Kirchman 1990; Schweitzer and Simon 1995; Rich et al. 1997). This observation may further explain why carbohydrates exhibit correlations to bacterioplankton and phytoplankton dynamics different from DFAA (Simon 1998) and dissolved proteins in Lake Constance (Rosenstock 1996) and in the Arctic Ocean, where the turnover of both substrate classes together with BP was studied simultaneously (Rich et al. 1997). The different utilization and turnover of DFAA vs. DFCHO also become obvious from the different turnover times of both pools. Rich et al. (1997) in the Arctic Ocean and Weiss and Simon (1999) in Lake Constance found that the turnover time of DFAA usually was significantly shorter than that of DFCHO. On the basis of mean values for various seasonal phases, however, ratios of bacterial uptake of DFCHO and of DFAA to BP are fairly similar in Lake Constance (Table 1 and Simon 1998).

One important factor controlling the bacterial uptake of DFCHO is the availability of a nitrogen source, usually ammonium. Kirchman et al. (1990) found that the availability of ammonium controls the uptake of glucose in the subarctic Pacific. Therefore, we can not rule out the possibility that a shortage in ammonium may have limited uptake of DFCHO in Lake Constance. In fact, Schweitzer and Simon (1995) demonstrated that the bacterioplankton may prefer ammonium + glucose to amino acids during certain situations if both types of N and C sources are available. In general, however, we do not think that the bacterial uptake of DFCHO in Lake Constance is controlled by the availability of ammonium. Rosenstock and Simon (1993) showed that during most of the growing season, bacteria regenerated ammonium due to high utilization rates of DFAA and DCAA. The availability of ammonium, however, may affect whether the DFCHO concentrations taken up by bacteria are preferentially used for biosynthetic requirements, i.e., amino acid and protein synthesis, or respired.

Surprisingly high ratios of DFCHO uptake/BP (often >1) were found from August to November 1995, suggesting that carbohydrates were the dominant substrates for bacterioplankton growth during this period. Concentrations of DFCHO were fairly high during this time at all depths, but particularly at 20 and 50 m, and turnover times of glucose, at least at 3 and 10 m, were fairly fast. We cannot rule out

the possibility that the high ratios of DFCHO uptake/BP may have been at least partially biased by high rates of extracellular polysaccharide production. During the fall period, microscopic observations indicated that bacteria often were surrounded by mucuslike material stained faintly with DAPI, and filtration rates also slowed down substantially at this time. It has been observed that bacteria excrete polysaccharides during the stationary phase when they are N and/or P limited (Jørgensen and Jensen 1994; Weiss and Simon 1999). Because the growth of bacterioplankton during most of the growing season in Lake Constance is limited by the availability of P or P + C (Schweitzer and Simon 1995), it is conceivable that the high uptake rates of DFCHO during this period led not to a net synthesis of biomass but mainly to the synthesis and subsequent excretion of mucuslike polysaccharides still associated with the cells. Other explanations for the high DFCHO uptake rates and high apparent ratios of uptake/BP include underestimation of BP rates because of an unrealistically low conversion factor from Leu incorporation into bacterial biomass and the fact that some of the DFCHO determined by PAD was not available to bacteria. However, we do not think it very likely that these latter two biases affected the results. Uptake rates of DFCHO determined at other seasons were consistent with the rates of BP, and the Leu conversion factor in Lake Constance has been found to remain fairly constant throughout the growing season (Simon and Rosenstock 1992). Rich et al. (1997) also reported that ratios of DFCHO uptake/BP as well as that of DFAA uptake/BP exceeded 1, but they did not discuss potential reasons for these high ratios.

In conclusion, we have shown that DFCHO concentrations are important substrates for the growth of planktonic bacteria in Lake Constance and presumably also in other pelagic environments. They support similar proportions of bacterial biomass production as DFAA but, in contrast to the latter, mainly during the breakdown of phytoplankton blooms and periods of low bacterial growth.

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