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Rhamnose and hydrolysis of MUF-α-L-rhamnopyranoside coupled with producers of rhamnose-rich extracellular polysaccharides in a hypereutrophic reservoir

Armando Augusto Henriques Vieira*, Vanessa Colombo-Corbi, Inessa Lacativa Bagatini, Maria José Dellamano-Oliveira, Leticia Piton Tessarolli

Department of Botany, Federal University of São Carlos, Via Washington Luís km 235, PO Box 676, São Carlos 13565-905, São Paulo, Brazil

ABSTRACT: Water collected from several depths of the tropical hypereutrophic Barra Bonita Reservoir (Brazil) was analyzed monthly from August 2002 to January 2004. The concentration of rhamnose ranged from 0 to 3.8 µmol l^{-1}, representing 28 % of total dissolved neutral carbohydrates (TDnC) on average. Rhamnose concentration was linearly correlated with TDnC concentration and with the abundance of *Microcystis aeruginosa*, which releases a rhamnose-rich extracellular polysaccharide (EPS). This correlation was slightly stronger when the cell densities of *Aulacoseira granulata, Cryptomonas tetrapyrenoidosa* and *Anabaena spiroides*, also rhamnose-rich EPS producers, were included. The enzymatic activity on methylumbelliferyl (MUF)-α-L-rhamnopyranoside (EARh) ranged from 0 to 57.8 nmol MUF l^{-1} h^{-1}. Glucose concentrations were found to vary from 0 to 4.15 µmol l^{-1} (27.5 % of TDnC), and the enzymatic activity on MUF-β-D-glucopyranoside (EAGl) ranged from 7.4 to 116.9 nmol MUF l^{-1} h^{-1}. Variation of EARh in time was directly correlated with the density of *M. aeruginosa*, and with rhamnose concentration in TDnC, while EAGl activity was correlated with density of *M. aeruginosa* only for variation with depth. Analysis of rhamnose, glucose, TDnC, EARh, EAGl, and bacterial density at each depth individually provided evidence that EPS released was employed as bacterial substrates, despite the permanent presence of TDnC from surface to bottom, owing to its constant production. These results also showed strong evidence that both enzymes were induced by EPS. Our results strongly suggest that the predominant phytoplankton species, known producers of rhamnose and glucose-rich EPS, α-L-rhamnosidase(s) activity and rhamnose concentration in the Barra Bonita Reservoir were coupled.

KEY WORDS: Rhamnose · MUF-α-L-rhamnopyranoside · α-L-rhamnosidase(s) · *Microcystis aeruginosa* · Extracellular polysaccharides

INTRODUCTION

Even though polysaccharides are generally considered less significant in freshwater than in marine habitats (Hoppe et al. 2002), increasing eutrophication of freshwater environments has enhanced the growth of the efficient producers of extracellular polysaccharide (EPS), as noted for the cyanobacteria *Microcystis* spp., *Anabaena* spp., *Pseudoanabaena* spp. and others (Tien et al. 2002). Consequently, it is increasingly relevant to study the role of these polysaccharides as substrates for heterotrophs (Freire-

*Email: ahvieira@ufscar.br

Nordi & Vieira 1996, Giroldo et al. 2005, Choueri et al. 2007). However, many other functions can also be performed by EPS in the ecology of eutrophic freshwater environments, e.g. facilitating aggregation of cells (Vieira et al. 2008), transparent exopolymeric particle formation (Bittar & Vieira 2010) and metal complexation (Lombardi et al. 2002, Gouvêa et al. 2005). Despite the ubiquitous occurrence of phytoplanktonic producers of rhamnose-rich EPS (De Philippis et al. 1998, Paulsen et al. 1998, Giroldo et al. 2003, Vieira et al. 2008) in freshwater environments around the world, there are no reports on the abundance of this monosaccharide. In fact, most of the literature concerning monosaccharide abundance and extracellular enzymatic activity in aquatic ecosystems focuses solely on glucose and α- and β-D-glucosidase, owing to the high concentrations of glucose-rich substrates. Nonetheless, while glucose is always present in natural water bodies, and often in large amounts, it is just one of a wide range of monosaccharides that can be used as substrates by heterotrophic microorganisms (Cowie & Hedges 1984). Since microorganisms can also utilize pentoses and deoxyhexoses as their sole carbon source, rhamnose must also be considered in studies involving carbon sources and adaptive metabolic routes in different bacterial populations (Watanabe & Makino 2009).

In the tropical Barra Bonita Reservoir, São Paulo State, Brazil, there are frequent blooms throughout the year of prolific producers of EPS, particularly the cyanobacteria *Microcystis aeruginosa* (Kützing) and *Anabaena spiroides* (Klebahn), but also the cryptophycean *Cryptomonas tetrapyrenoidosa* (Skuja) and the diatom *Aulacoseira granulata* (Ehrenberg) Simonsen (Calijuri et al. 2002). Their EPS, including released free colloids, capsules of *A. spiroides* and colonial matrices of *M. aeruginosa*, are important sources of rhamnose (Colombo et al. 2004, Giroldo et al. 2005, Gouvêa et al. 2005, Vieira et al. 2008). Released polysaccharides may induce an increase in the activity of glycosidases, α-L-rhamnosidase(s) among them, as already demonstrated for some species of phytoplankton (Chróst 1991, Vrba et al. 1992).

The aims of the present study were to determine rhamnose concentration and to test for enzymatic activity on 4-methylumbelliferyl (MUF)-α-L-rhamnopyranoside (EARh) in Barra Bonita Reservoir, emphasizing the importance of this abundant monosaccharide in freshwater hypereutrophic environments, and to investigate a possible correlation of these measurements with the abundance of the rhamnose-rich EPS phytoplanktonic producers. Beside rhamnose, EPS from the 4 microalgae listed above are also glucose-rich polymers, so the occurrence of glucose and the enzymatic activity of β-D-glucosidase(s) (EAGl) was also studied, mainly as a source of comparative material.

MATERIALS AND METHODS

Study site

Barra Bonita Reservoir is located in São Paulo State, Brazil (22° 29′ S, 48° 34′ W), and is formed by the confluence of rivers Piracicaba and Tietê, both of which are hypereutrophic and polluted by industrial and agricultural activity. The reservoir is shallow (average depth: 10.2 m), hypereutrophic and polymictic. Constant horizontal water flux promoted by hydroelectrical power generation and the prevailing wind, which blows along the reservoir's longitudinal axis, ensures that the reservoir water column is rarely stratified. The seasons are not very pronounced in terms of temperature variation (20 to 28.4°C in the water at the surface during the present study), but the hottest period (December to February) is very rainy, while the winter (May to July) is the dry season, with little or no rain (Dellamano-Oliveira et al. 2007).

Sampling

Water samples were collected with a PVC Niskin sampler once a month, from August 2002 to January 2004, at different depths of the water column: surface, 1, 3, 5 and 10 m, and bottom (18 ± 2 m). The euphotic zone extends down the water column from the surface to the depth where photosynthetic available radiation (PAR) is 1% of that measured at the surface. This was determined with a LI-250 quantameter (LI-COR) with a spherical sensor LI-193AS (Li-COR). During the study period, the euphotic zone depth was usually ~3.0 m. Therefore, in the results, values for the euphotic zone correspond to the mean of the values measured at 0, 1 and 3 m, while values for the whole water column are the mean of all sampled depths. The sampling station was in the pelagic zone, where the water from both rivers is thoroughly mixed, 3 km upstream from the dam and 20 km from the point where the rivers meet. Immediately after sampling, aliquots from each sampled depth were fixed for phytoplankton counting. For glycolytic assays, the collected water was filtered through a 70 µm nylon net to remove zooplankton and large

detritus, and transported in sterilized glass containers to the laboratory, 2 h away. Water samples for bacterial counting were collected from each depth in a sterilized glass bacteriological sampler.

For convenience, to describe temporal variation, 'monthly mean' was used. This was calculated from values measured in the water column (euphotic or total) for a specified month. 'Depth variation' was described using the mean values measured during the whole sampling period for a given depth, called 'depth mean'.

Phytoplankton and bacterial analysis

Phytoplankton cells fixed with Lugol solution were counted in 5.0 ml sedimentation chambers after settling for 8 h. All counts were done at 95% confidence limits, SD < 5% (APHA 1998), with an Axiovert Zeiss inverted microscope. Samples for bacterial counts were fixed in 3.6% (final concentration) formaldehyde. Aliquots (1 to 2 ml) of the fixed bacterial samples were stained with 4′,6′-diamidino-2-phenylindole (DAPI) (Sigma) for 30 min and filtered onto a black 0.2 µm-pore polycarbonate filter, as described by Porter & Feig (1980). Five hundred cells were counted in 20 to 30 microscopic fields (SD < 10%) with a Zeiss Axioplan 2 epifluorescence microscope.

Analysis of carbohydrates

Triplicate hydrolyzed (total dissolved carbohydrates) and non-hydrolyzed (free dissolved carbohydrates) samples of reservoir water were filtered through a 0.45 µm-pore membrane filter (Isopore™, Millipore) and analyzed for neutral polysaccharides and free monosaccharides, respectively, as described by Gremm & Kaplan (1997). Briefly, 10 ml of each sample were acidified with 300 µl of 8 M HCl and hydrolyzed at 100°C for 12 h for analysis of total dissolved carbohydrates. All samples were desalted on Bio Rad™ ion-exchange columns (AG2X8: anion exchange and AG50W: cation exchange). Possible losses during hydrolysis and desalting were tested with calibration standards (Sigma-Aldrich™). Total recovery of all monosaccharides was >85%. Monosaccharides were separated by HPLC-PAD on an anion-exchange analytical column, as described below, eluted with 18 mM NaOH (at 1.0 ml min^{-1}). The column was regenerated after each injection by washing with 200 mM NaOH. The monosaccharides detected and quantified in polymeric carbohydrates were arabinose, fucose, galactose, glucose, rhamnose and mannose co-eluted with xylose. Besides these major sugars, small amounts of fructose and ribose were also detected in the free dissolved monosaccharides. Mannose and xylose were co-eluted, and uronic acids and amino sugars were retained during desalinization and therefore not detected. Therefore, total dissolved carbohydrates in this assay account only for the neutral carbohydrates (TDnC). Further analysis by gas chromatography of the polymeric carbohydrates dissolved in reservoir water showed a mannose:xylose ratio of 0.77 and a mean contents of 3.4% glucuronic acid and 4.3% galacturonic acid in TDnC. Amino sugars occurred in very low amounts (0 to 0.3%) (Gouvêa et al. 2005, Colombo 2006).

High performance liquid chromatography and pulsed amperometric detection (HPLC-PAD) were performed, as described by Gremm & Kaplan (1997), in a Dionex HPLC-PAD DX500 system consisting of a PEEK GP40 gradient pump module, ED40 electrochemical detector, LC5 manual injector with a Rheodine 9125 valve and a 25 µl peak sample loop. A Dionex CarboPac PA-10 anion-exchange analytical column (4 × 250 mm), fitted with an appropriate guard column (4 × 50 mm), was used to separate monosaccharides.

Enzymatic activity

The enzymatic activity was analyzed using methods described by Hoppe (1983), with modifications by Chróst & Krambeck (1986), Hoppe et al. (1988) and Chróst & Velimirov (1991). Samples (2 ml) were filtered through a 70 µm nylon mesh, in triplicate, and treated with 0.25 ml 0.1 M phosphate buffer, pH = 7.0. A MUF substrate (4-MUF-α-L-rhamnopyranoside; Sigma-Aldrich), previously dissolved in 2.0 ml ethylene glycol monomethyl ether (Methylcellosolve™) was added to each sample, reaching a final concentration of 0.2 mM. Similarly, the substrate MUF-β-D-glucopyranoside was used to detect the enzymatic activity of β-D-glucosidase (EAGl) in water samples. The reaction mixtures were incubated for 30 min at 25°C (average temperature of Barra Bonita Reservoir). Incubation time was determined in preliminary experiments. After incubation, each sample received 100 µl of 5 M NaOH and the fluorescence was immediately measured. Three autoclaved samples with MUF substrate added were analyzed as 'killed' control and samples without MUF substrate were treated as background water control. Fluores-

cence was measured with a Jasco FP-6500 spectrofluorometer at 450 nm, with 365 nm excitation. Relative fluorescence readings were calibrated against a range (1.0, 5.0, 12.5, 25.0, 50.0, 100.0, and 150.0 nmol) of 4-MUF (Sigma-Aldrich) concentrations, allowing them to be expressed as nmol MUF l^{-1} h^{-1}.

Statistical analysis

All statistical tests were performed in the R computing environment, differences being treated as significant when $p < 0.05$. Raw data were tested for normal distribution with the Shapiro-Wilk test. For data that were not normally distributed, the Spearman rank correlation coefficient (r_s) was used to test linear correlation. Only the linear correlation between the monthly mean rhamnose concentration and enzymatic activity and between depth mean values, which were normally distributed, were tested using the Pearson correlation coefficient (r). The standard deviations for each month were calculated from pooled standard deviations, for 0, 1 and 3 m for the photic column, and 0, 1, 3, 5, 10 m and bottom for the total column, while the standard deviations for each depth, taken over the whole sampling period, were calculated from the pooled standard deviations for all months at the specified depth.

RESULTS

The population of *Microcystis aeruginosa*, including large or small colonies and free cells, was at all times the most abundant, even when not forming blooms, except in January 2003, when the dominant species was *Cryptomonas tetrapyrenoidosa* at all depths (Fig. 1a). *Anabaena spiroides* and *C. tetrapyrenoidosa* showed higher abundance in the euphotic zone (Fig. 2). *Aulacoseira granulata* was dominant at the bottom of the euphotic zone, 10 m and at the bottom of the water column, except in November 2002, February 2003 and December 2003, when its distribution was almost uniform throughout the water

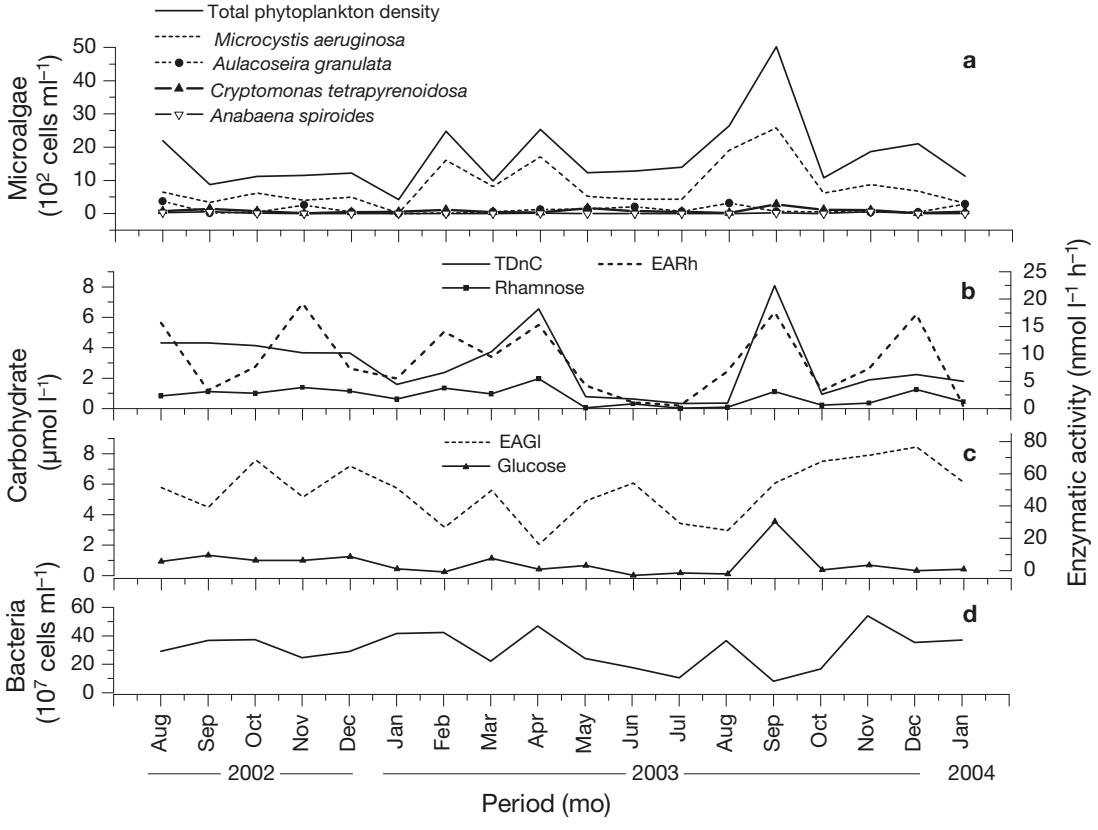

Fig. 1. Temporal variation of (a) density of main phytoplanktonic producers of rhamnose-rich extracellular polysaccharide (EPS) and total phytoplankton; (b) total dissolved neutral carbohydrates (TDnC), rhamnose concentration and enzymatic activity on methylumbelliferyl (MUF)-α-rhamnopyranoside (EARh); (c) glucose concentration and enzymatic activity on MUF-β-D-glucopyranoside (EAGl); (d) bacterial density, for a water column of Barra Bonita Reservoir (n = 6 depths)

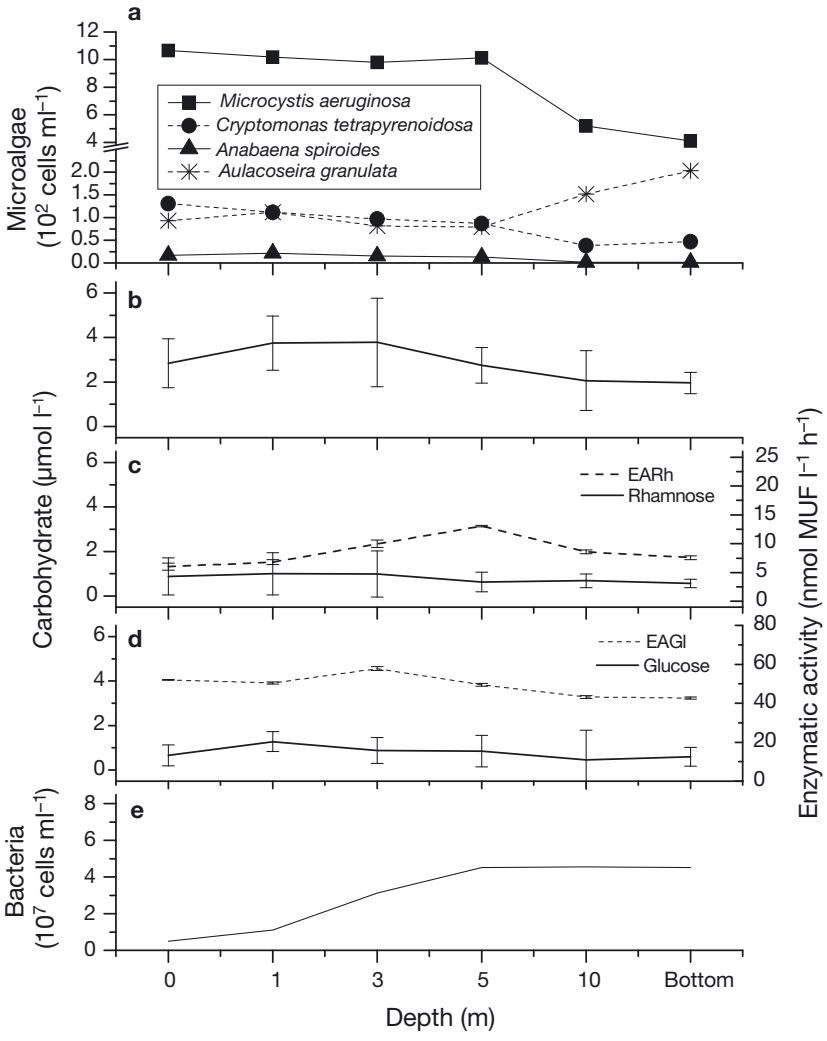

Fig. 2. *Anabaena spiroides*, *Aulacoseira granulata*, *Cryptomonas tetrapyrenoidosa* and *Microcystis aeruginosa*. (a) Depth variation of the 4 main phytoplanktonic species; (b) TDnC; (c) rhamnose concentration and EARh; (d) glucose concentration and EAGl; and (e) bacterial density. See Fig. 1 for acronyms

column (Figs. 1a & 2, Table 1). These 4 species frequently account for ~90% of total phytoplanktonic biomass in the reservoir (Jati 1998, Calijuri et al. 2002, Dellamano-Oliveira et al. 2007). Owing to the higher abundance of species in the euphotic zone, results are presented, when convenient, separately for the euphotic zone and the whole water column.

Concentrations of total dissolved neutral carbohydrates (TDnC) in the whole water column ranged from 0.01 to 22.55 µmol l^{-1} during the present study. Peaks of TDnC concentration happened at the beginning (April 2003) and end (September 2003) of the dry season (Fig. 1b, Table 2). However, the minima also happened during the dry season, in July and August 2003. Thus, TDnC concentrations did not show a well-defined seasonal pattern in the reservoir.

Maximum concentrations of TDnC coincided with the maximum density of the dominant phytoplankton measured in the present study, particularly *Microcystis aeruginosa* (Fig. 1a,b, Table 2). However, only for the euphotic zone was the concentration of TDnC linearly correlated with the total abundance of the 4 species (r_s = 0.554, p = 0.019, df = 16). When depth variation was considered, correlation is high, but not significant (r_s = 0.796, p = 0.058, df = 4). Analysis of the TDnC showed that 92% was in the form of polymeric carbohydrates and ~8% were free monosaccharides.

Monthly mean of rhamnose concentration ranged from 0.03 to 2.72 µmol l^{-1} during the study (Table 1); the highest monthly mean occurred in April 2003 (Fig. 1b). There was statistically significant correlation in time between the rhamnose concentration and abundance of *Microcystis aeruginosa* in the euphotic zone (r_s = 0.527, p = 0.025, df = 16). When the abundances of *Aulacoseira granulata*, *Cryptomonas tetrapyrenoidosa* and *Anabaena spiroides* were included, correlation with rhamnose was slightly better (r_s = 0.551, p = 0.018, df = 16) (Fig. 2). Rhamnose occurs at relatively high proportions in the EPS released by these 4 species (Colombo et al. 2004, Giroldo et al. 2005, Gouvêa et al. 2005, Vieira et al. 2008) (Table 3), which may explain this correlation. However, there was no statistically significant temporal correlation between the rhamnose concentration and total phytoplankton density, over the whole water column.

In Fig. 1, it can be seen that peaks in rhamnose concentration matched those of TDnC. In fact, during the whole period, rhamnose concentration was linearly correlated with TDnC concentration in both the euphotic zone (r_s = 0.897, p < 0.001, df = 16) and the whole water column (r_s = 0.77, p < 0.001, df = 16). This was also true of the depth variation (r = 0.899, p = 0.014, df = 4) (Fig. 2). Interestingly, the percentages of glucose and rhamnose in the TDnC composition

were almost constant from top to bottom of the water column (average 27.4 and 28.2%, respectively) (Fig. 3).

During the present study, the ranges of monthly mean values for EARh in the whole water column and euphotic zone were, respectively, 0.4 to 19.2 and 0.8 to 19.2 nmol MUF l^{-1} h^{-1}. The highest values found for EARh were in August 2002, September 2003 (dry season) and November 2002 (rainy season) (Table 2). Thus, EARh did not show a well-defined seasonal pattern. The highest values of EARh ran in parallel with the maximum densities of phytoplankton, particularly *Microcystis aeruginosa*, as can be verified in Fig. 1. The variation of the EARh in time was positively correlated with the total density of the 4 dominant phytoplankton species, in both the euphotic zone (r_s = 0.510, p = 0.032, df = 16) and the whole water column (r_s = 0.52, p = 0.028, df = 16).

Table 1. *Anabaena spiroides*, *Aulacoseira granulata*, *Cryptomonas tetrapyrenoidosa* and *Microcystis aeruginosa*. Monthly abundance (cells m^{-1}, range and mean) of dominant species of phytoplankton (error < 5%) and bacterial density (error < 10%) in the euphotic zone (EZ) and whole water column (WWC), for August 2002 to January 2004

Month	Sample source	*M. aeruginosa* Range	Mean	*A. granulata* Range	Mean	*C. tetrapyrenoidosa* Range	Mean	*A. spiroides* Range	Mean	Bacteria (10^7 cells ml^{-1})
2002										
Aug	EZ	760–1131	932	129–136	153	14–359	139	36–97	64	15.0
	WWC	126–1131	627	80–1287	373	0–359	75	0–97	36	29.1
Sep	EZ	360–529	461	12–28	18	111–208	140	49–83	70	26.6
	WWC	101–529	334	11–51	24	11–416	144	5–94	53	36.8
Oct	EZ	437–708	554	11–14	11	28–162	84	12–21	16	11.7
	WWC	347–1159	608	6–180	38	21–162	76	7–21	12	37.3
Nov	EZ	548–638	588	229–462	343	7.0–39	25	0	0	8.7
	WWC	14–638	425	121–462	262	0–39	16	0	0	24.7
Dec	EZ	305–984	535	17–41	28	33–67	52	0	0	18.6
	WWC	167–984	496	17–94	47	13–67	44	0	0	29.0
2003										
Jan	EZ	0–24	19	0	0	16–42	25	0	0	22.5
	WWC	0–25	15	0	0	16–99	53	0	0	41.6
Feb	EZ	1128–2727	2169	0–42	14	0–285	124	0–21	7	20.7
	WWC	715–2727	1610	0–42	10	0–285	115	0–21	5	42.2
Mar	EZ	27 -1785	875	18–60	38	15–45	26	0	0	4.4
	WWC	27–1785	812	15–146	57	8.0–83	39	0	0	19.6
Apr	EZ	1539–4484	2829	6–9	8	34–78	61	11–45	25	26.0
	WWC	73–4484	1561	0–687	122	0–78	34	0–45	12	47.0
May	EZ	511–633	583	47–92	74	173–316	245	0	0	13.8
	WWC	171–688	508	47–397	131	7–316	171	0	0	24.2
Jun	EZ	7–1121	384	112–213	147	7.0–97	54	0–15	5	9.5
	WWC	7–1121	432	112–299	203	7–154	77	0–15	2	17.6
Jul	EZ	260–550	393	22–60	39	0–94	42	0–7	2	5.8
	WWC	188–857	427	22–178	68	0–143	58	0–7	1	10.5
Aug	EZ	165–278	206	323–403	369	0–26	16	0–8	3	25.8
	WWC	165–4757	1903	220–403	322	0–29	17	0–8	1	36.5
Sep	EZ	426–4139	1953	47–56	51	180–667	418	0–29	16	13.0
	WWC	289–6380	2563	13–153	68	13–667	278	0–59	22	8.2
Oct	EZ	211–2458	1074	0–61	29	51–416	227	0	0	9.2
	WWC	49–2458	609	0–157	53	0–416	117	0	0	16.8
Nov	EZ	517–1587	1116	0–65	31	156–198	184	49–130	87	20.9
	WWC	192–1587	805	0–65	42	0–198	109	0–130	61	54.1
Dec	EZ	602–937	834	54–104	78	14–15	14	0–15	10	15.0
	WWC	257–937	607	7–104	45	0–32	15	0 15	5	35.4
2004										
Jan	EZ	158 -514	373	143–472	274	83–97	90	0–14	5	16.6
	WWC	0–514	288	143–472	292	0–97	60	0–14	2	37.0

Table 2. Range of values, mean and SD for the concentration of carbohydrates (µmol l^{-1}): rhamnose, glucose and total dissolved neutral carbohydrates (TDnC); and for enzymatic activity (nmol MUF l^{-1} h^{-1}) on MUF-α-L-rhamnopyranoside (EARh) and on MUF-β-D-glucopyranoside (EAGl), during the study period, in the euphotic zone (EZ) and whole water column (WWC) for Aug 2002 to Jan 2004

Month	Sample source	Rhamnose			Glucose			TDnC			EARh			EAGl		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
2002																
Aug	EZ	0.91–1.86	1.24	0.08	0.22–4.15	1.56	0.63	1.42–17.19	6.69	3.69	7.9–36.1	18.9	1.06	39.9–52.6	46.5	0.82
	WWC	0.17–1.86	0.83	0.06	0.18–4.15	0.94	0.45	1.07–17.19	4.31	2.61	0.2–36.1	15.7	0.76	39.9–74.2	51.4	0.58
Sep	EZ	1.01–1.86	1.39	0.58	1.39–1.79	1.63	0.57	4.30–5.66	5.14	0.79	2.5–7.3	5.1	0.77	32.8–35.8	33.9	0.83
	WWC	0.77–1.86	1.13	0.42	0.81–1.79	1.35	0.58	2.40–5.66	4.33	0.71	0.7–7.3	3.6	0.55	32.8–49.3	39.2	1.07
Oct	EZ	0.50–2.85	1.31	0.47	0.60–1.33	0.92	0.99	2.44–5.16	3.49	0.93	6.6–12.8	8.9	0.36	63.3–77.5	71.8	0.21
	WWC	0.50–2.85	1.00	0.41	0.60–1.33	1.00	0.83	2.44–6.37	4.14	0.77	4.9–12.8	7.7	0.60	60.0–77.5	68.5	0.26
Nov	EZ	0.62–3.80	1.75	1.56	0.63–1.21	0.89	0.12	3.45–5.49	4.16	1.90	9.1–13.1	11.5	0.86	34.9–54.0	42.7	0.29
	WWC	0.62–3.80	1.39	1.10	0.31–2.17	1.01	1.12	1.82–5.49	3.68	1.75	9.1–57.8	19.2	0.82	45.4–54.0	45.5	0.59
Dec	EZ	0.51–1.68	0.47	0.29	0.97–1.77	1.32	1.24	3.00–5.09	3.87	1.45	5.8–9.9	7.6	0.13	55.7–63.6	60.0	0.01
	WWC	0.51–1.68	0.63	0.21	0.97–1.77	1.25	0.98	2.30–5.46	3.66	1.09	5.1–9.9	7.4	0.18	55.7–77.3	64.7	0.02
2003																
Jan	EZ	0.45–0.52	0.47	0.25	0.21–1.07	0.57	0.13	1.46–1.75	1.61	0.45	2.1–8.1	5.3	0.02	32.0–45.2	40.7	0.82
	WWC	0.45–0.96	0.63	0.23	0.21–1.07	0.45	0.18	1.26–1.87	1.60	0.38	2.1–8.1	5.5	0.03	32.0–63.8	51.0	1.08
Feb	EZ	0.51–2.77	1.75	1.91	0.12–0.47	0.26	0.05	1.60–3.89	2.79	1.81	11.5–20.5	15.4	0.81	13.2–33.1	25.9	0.04
	WWC	0.27–2.77	0.92	1.43	0.12–0.47	0.25	0.51	1.60–3.89	2.38	1.45	0.0–37.9	14.0	0.58	13.2–33.1	26.7	0.07
Mar	EZ	0.65–1.64	1.23	0.52	0.35–1.26	0.82	0.28	2.27–3.89	3.11	0.78	0.0–10.5	5.6	0.02	26.9–74.6	55.6	0.81
	WWC	0.33–1.64	0.97	0.41	0.12–3.93	1.13	0.20	1.56–8.08	3.73	0.72	0.0–17.3	9.5	0.09	20.0–87.2	49.8	1.08
Apr	EZ	2.36–2.95	2.72	0.45	0.37–0.67	0.52	0.15	6.34 – 9.59	7.14	1.14	5.4–22.2	11.7	0.01	18.5–21.0	20.1	0.00
	WWC	1.13–2.95	1.96	0.43	0.25–0.67	0.42	0.13	2.35 – 9.59	6.56	0.82	5.4–24.6	15.3	0.01	7.6–21.0	16.2	0.45
May	EZ	0.06–0.12	0.08	0.15	0.00–0.02	0.01	0.00	0.20–0.33	0.24	0.16	2.2–8.8	6.2	0.01	43.2–51.3	46.7	0.42
	WWC	0.00–0.12	0.04	0.25	0.00–2.63	0.67	2.02	0.20–2.63	0.79	2.01	0.0–8.8	4.2	0.01	32.0–53.2	43.3	0.94
Jun	EZ	0.22–0.42	0.34	0.10	0.00	0.00	0.00	0.59–0.91	0.70	0.56	0.8–0.9	0.8	0.02	45.3–83.9	64.7	0.04
	WWC	0.18–0.42	0.31	0.10	0.00 – 0.14	0.002	0.03	0.44–0.91	0.62	0.43	0.0 – 3.0	1.1	0.02	30.5–83.9	54.2	0.04
Jul	EZ	0.00–0.07	0.04	0.06	0.04–0.20	0.14	0.09	0.23–0.35	0.27	0.24	0.1–2.2	1.1	0.02	35.4–40.6	38.2	0.04
	WWC	0.00–0.07	0.03	0.05	0.00–0.54	0.17	0.22	0.00–1.08	0.34	0.34	0.0–2.2	0.6	0.04	10.3–40.6	29.1	0.42
Aug	EZ	0.06–0.18	0.10	0.03	0.07–0.15	0.09	0.01	0.18–0.40	0.30	0.05	2.7–11.3	7.1	0.01	7.4–35.4	22.3	0.03
	WWC	0.00–0.21	0.09	0.09	0.07–0.18	0.11	0.07	0.18–0.67	0.35	0.12	2.5–12.4	6.8	0.01	7.5–45.9	24.9	0.03
Sep	EZ	0.82–2.24	1.67	2.34	1.83–2.61	2.61	0.91	4.86–22.55	12.67	2.62	12.7–17.4	14.5	0.01	60.4–76.2	70.6	0.00
	WWC	0.12–2.24	1.12	1.79	0.17–2.61	1.37	0.65	2.27–22.55	8.08	1.95	12.7–26.4	17.6	0.01	25.8–76.2	54.3	0.04
Oct	EZ	0.06–0.64	0.26	0.38	0.04–0.34	0.24	0.04	0.14–1.64	0.83	0.38	2.8–6.4	4.1	0.02	46.9–76.2	66.0	0.56
	WWC	0.06 -0.64	0.23	0.27	0.04–0.77	0.37	0.42	0.14–1.64	0.87	0.53	1.4–6.4	3.3	0.02	46.9–76.2	67.7	0.46
Nov	EZ	0.41–0.52	0.46	0.14	0.11–1.07	0.53	0.51	1.03–2.93	2.14	1.48	2.5–4.8	3.6	0.01	62.7–114.8	86.3	0.03
	WWC	0.07–0.52	0.35	0.10	0.11–1.50	0.70	1.00	1.03–2.93	1.89	1.36	0.2–27.1	7.3	0.01	34.2–114.8	71.4	0.02
Dec	EZ	0.21–1.56	0.72	0.64	0.28–0.45	0.37	0.17	1.56–2.35	1.84	0.53	7.5–9.7	8.3	1.59	98.2–116.9	108.7	0.10
	WWC	0.21–2.19	1.25	0.48	0.16–0.45	0.33	0.16	1.56–2.76	2.24	0.40	7.5–34.1	17.2	1.17	10.6–116.9	76.5	0.24
2004																
Jan	EZ	0.18–0.80	0.57	1.61	0.29–0.54	0.41	0.32	1.75–2.47	2.18	1.91	0.3–1.3	0.8	0.20	46.3–76.8	61.2	1.95
	WWC	0.18–0.80	0.45	1.14	0.26–0.78	0.43	0.29	1.08–2.47	1.78	1.37	0.0–1.3	0.4	0.21	42.5–76.8	54.7	1.40

Table 3. *Anabaena spiroides*, *Aulacoseira granulata*, *Cryptomonas tetrapyrenoidosa* and *Microcystis aeruginosa*. Monosaccharide composition (% of total identified carbohydrate) determined by gas chromatography of trimethylsilylated derivatives of extracellular polysaccharide (EPS) released by *A. spiroides*, *A. granulata*, *C. tetrapyrenoidosa* and *M. aeruginosa*. –: not detected

Monosaccharide	*A. spiroides*	*A. granulata*	*C. tetrapyrenoidosa*[a]	*M. aeruginosa*
Arabinose	0.8	–	0–0	–
Fucose	5.6	7.8	24.3–8.6	9.9
Galactose	2.0	7.0	13.7–36.0	16.3
Glucose	29.3	0.7	3.5–0.5	13.5
Mannose	24.2	5.4	15.4–0.8	7.4
Rhamnose	21.8	31.3	9.0–0.8	18.4
Xylose	7.8	24.5	4.7–0.4	19.7
Galacturonic acid	1.8	6.2	0–4.5	8.9
Glucuronic acid	6.6	13.2	4.1–47.0	–
N-acetyl-galactosamine	–	3.2	8.6–0.3	–
N-acetyl-glucosamine	–	0.7	16.8–1.1	10.5
Source	Colombo et al. (2004)	Vieira et al. (2008)	Giroldo et al. (2005)	Gouvêa et al. (2005)

[a]EPS composed of 2 fractions

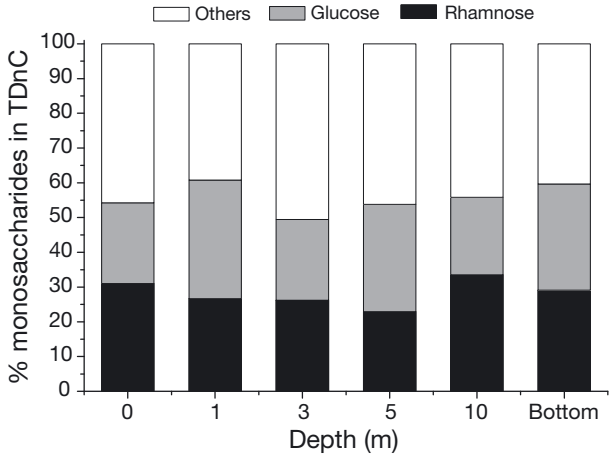

Fig. 3. Depth variation on the content (%) of rhamnose and glucose in total dissolved neutral carbohydrates (TDnC)

Throughout the study, the temporal variation of EARh showed good correlation with TDnC concentration, whether in the euphotic zone (r_s = 0.610, p = 0.009, df = 16) or the whole water column (r_s = 0.650, p = 0.003, df = 16) (Fig. 1b). It can also be seen in Fig. 2 that the depth variation of the EARh matched the TDnC concentration well, despite their decoupling at 5.0 m.

Temporal variation of EARh also showed a linear correlation with rhamnose, in both the euphotic zone (r = 0.670, p = 0.002, df = 16), and the whole column (r = 0.730, p < 0.001, df = 16). The higher peaks of EARh are almost in synchrony with the peaks of rhamnose concentration (Fig. 1b). When considering depth variation (Fig. 2), rhamnose concentration showed little variation in the euphotic zone, while EARh increased with depth, and both values decreased at depths below 5.0 m.

There was no statistically significant correlation between bacterial density and EARh or rhamnose (Fig. 1). However, when considering depth variation (Fig. 2), similarities in bacterial density and EARh may be noticed: values increased with depth in the euphotic zone and, beyond 5.0 m, EARh decreased while bacterial density was constant. It is also noticeable in Fig. 2 that reciprocal correlations exist between bacterial density and rhamnose concentration, and bacterial density and TDnC.

Monthly mean values for glucose concentration throughout the study ranged from 0 to 2.6 µmol l^{-1}, the highest peaks occurring during September 2003, in both the euphotic zone and the whole water column (Fig. 1c, Table 2). During the study, monthly means of β-D-glucosidase activity (EAGl) ranged from 16.2 to 108.7 nmol MUF l^{-1} h^{-1} in the whole water column and 20.1 to 108.7 nmol MUF l^{-1} h^{-1} in the euphotic zone (Table 2).

It is apparent in Fig. 1a,c that the highest peaks of glucose and phytoplankton matched well. However, no statistically significant correlations were found, in either the euphotic zone or the whole water column. Yet, Fig. 2 shows that both glucose concentration and phytoplankton abundance decreased in the aphotic zone.

Similar to the pattern for rhamnose, temporal variation of glucose concentration matched TDnC very closely, in both the euphotic zone and the whole water column (r_s = 0.858, p = 0.001, df = 16; and r_s = 0.732, p = 0.001, respectively). Although glucose monthly averages paralleled TDnC concentrations, EAGl showed no statistical correlation with TDnC, in either the euphotic zone or the whole water column. However, significant correlations were found in the case of depth variation (r = 0.839, p = 0.037, df = 4) (Fig. 4).

Visually, the highest peaks of EAGl matched those of phytoplankton (Fig. 1a,c), although no statistical correlation was found. However, EAGl variation with depth did show significant correlation (r = 0.831, p = 0.04, df = 4) with abundance of phytoplankton, especially *Microcystis aeruginosa* (Fig. 2). No significant correlation was found between bacterial density, EAGl and glucose.

Theoretical depolymerization time (Dt) of combined rhamnose (TDnC-rhamnose/EARh = hours) and glucose (TDnC-glucose/EAGl) was calculated and results are shown in Fig. 5. Dt for combined glucose oscillated during the study period (average = 6.1 h), roughly rising with *Microcystis aeruginosa*

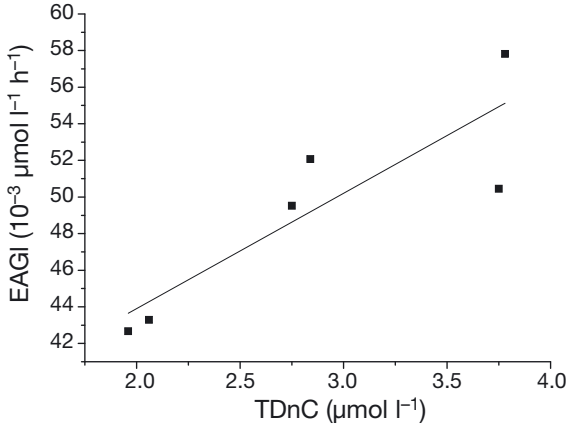

Fig. 4. Correlation between enzymatic activity on methylumbelliferyl (MUF)-β-D-glucopyranoside (EAGl) and total dissolved neutral carbohydrates (TDnC) throughout depth

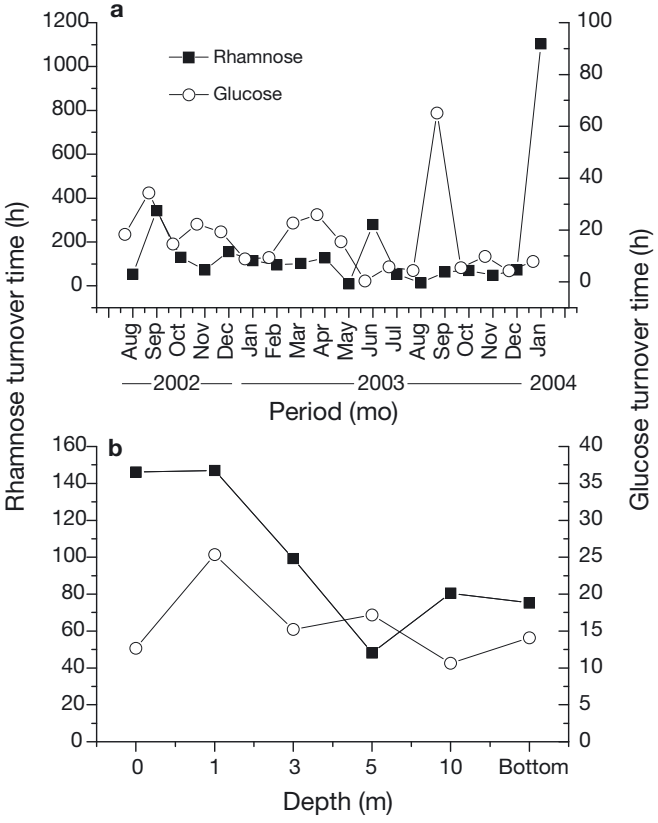

Fig. 5. (a) Temporal and (b) depth variation of the theoretical depolymerization time (Dt) of glucose and rhamnose

blooms, although no statistical correlation was found. However, significant correlation was found between glucose Dt and TDnC (r_s = 0.828, p < 0.001, df = 16) (Fig. 5a). The longest turnover time for glucose, on September 2003, reflects the increase in phytoplankton abundance, which was responsible for the rise in TDnC concentration. For combined rhamnose, correlation between Dt and phytoplankton was negative (r_s = –0.500, p = 0.038, df = 16). The longest rhamnose turnover time, on December 2003, was caused by very low EARh. Dt for combined rhamnose also showed oscillation, but reached much longer times than glucose (average = 165.5 h). When various depths were analyzed, Dt for combined glucose was almost constant at all depths (average = 15.9 h), while Dt for combined rhamnose decreased towards the bottom (Fig. 5b).

DISCUSSION

The hypereutrophic tropical environment of our study site—where inorganic nutrients are never limiting factors, the temperature is warm, and there is abundant phytoplankton biomass throughout the year—is clearly reflected in our results. Additionally, at least 7 large blooms (particularly of *Microcystis aeruginosa*) occurred in the period covered in the present study. These factors together provide an environment where substrates are continuously released by living cells or by their breakdown, producing different results for the kinetics of enzyme/substrate systems than are usually described for temperate environments.

The 4 species discussed in the present study are frequently responsible for >90% of the biomass produced by phytoplankton in the reservoir (Jati 1998, Calijuri et al. 2002, Dellamano-Oliveira et al. 2007) and were found throughout the study period, with a clear predominance of *Microcystis aeruginosa* during both the rainy and dry seasons. Given the ability of these organisms to produce rhamnose-rich EPS, the rather high content of rhamnose in TnDC found in the reservoir is not surprising. The EPS released by these species (Table 3) certainly played an important role in the origin of this substrate. It must be noted, however, that other polymeric compounds generated by algae may also provide sources of rhamnose in the environment. *Microcystis aeruginosa*, a major part of the phytoplankton biomass, is a Gram-negative bacterium, and cell degradation of these organisms may release outer membrane lipopolysaccharides (LPS), which may, depending on the strain, be composed of reasonably high concentrations of rhamnose (2.8 to 4.9%) and glucose (5.6 to 14.8%), including β-linked glucose (Martin et al. 1989, Seltmann & Holst 2002). EPS from heterotrophic bacteria is another possible source of rhamnose, which has already been studied in the bacterial population of Barra Bonita Reservoir, grown on glucose as unique carbon source (Panhota et al. 2007).

In the present study, rhamnose accounted for, on average, 28.0% of the total dissolved neutral monosaccharides, a value similar to the glucose content (~27.5%). Although the EPS released by most cyanobacteria are known to be glucose-rich polysaccharides (De Phillippis & Vicenzini 1998), rhamnose also occurs in large amounts and, in some cyanobacterial strains, it even supplants glucose, e.g. *Microcystis flos-aquae* C3-40, whose rhamnose content was twice that of glucose (Plude et al. 1991), some *Cyanothece* strains (De Philippis et al. 1998) and *Nostoc calcicola* (Singh & Das 2011).

As shown in Fig. 1, TDnC concentration matched phytoplankton abundance, and both enzymatic activities showed similar variations, reinforcing our assumption that TDnC largely consisted of rhamnose

and glucose-rich polymers released by dominant phytoplankton. Furthermore, glucose and rhamnose concentrations showed little variation during the study, changing only when large *Microcystis aeruginosa* blooms were formed, and, consequently, higher amounts of TDnC were released (April 2003 and September 2003).

In light of the good linear correlation found between TDnC and the enzymatic activities, it may be deduced that the highest peaks in EARh and EAGl were most certainly induced by rhamnose and glucose-rich TDnC (see Table 3). In fact, both enzymatic activity variations paralleled TDnC oscillations. Also, when plotted against depth, the curves of TDnC and activities of both enzymes are very similar: higher in the euphotic zone and, at greater depth, decreasing towards the bottom. Many studies have reported the induction of hydrolase activity, including β-D-glucosidase, by polymeric carbohydrates released by phytoplankton (Chróst et al. 1989, Chróst 1991, Vrba et al. 1992, Arnosti 2000, Caruso et al. 2005, Mudryk & Skórczewski 2006). Hashimoto et al. (1999, 2003) found that synthesis of α-L-rhamnosidase by soil-inhabiting *Bacillus* sp. was strongly induced by gellan, a rhamnose-rich extracellular polysaccharide released by the soil bacterium *Sphingomonas paucimobilis*. These data may support the idea that EPS induced the enzymatic activity in our study. However, no data are available on rhamnosidase activity induced by L-rhamnose-rich polysaccharides in freshwater systems.

Samples analyzed in the glycolytic assay were filtered through a 70 µm nylon mesh; therefore, it was assumed that practically all the enzymatic activity acting on MUF-α-L-rhamnopyranoside and on MUF-β-D-glucopyranoside was measured: free dissolved enzymes, those associated with free living producers and those adsorbed on particles (Hoppe 1983, Chróst 1991, Wetzel 1991, Arnosti 2003). We assume that the enzymatic activity in the reservoir, detected by MUF-α-L-rhamnopyranoside hydrolysis, was mainly due to α-L-rhamnosidases, enzymes that catalyze the hydrolysis of rhamnosides, releasing L-rhamnose from substrates. According to Köster et al. (1997), MUF-substrates showed relatively few nonspecific reactions when screened against a variety of commercial enzymes. Also, Hashimoto et al. (1999, 2003) reported that α-L-rhamnosidase produced by a soil bacterium was specific to para-nitrophenyl-α-L-rhamnopyranoside and unable to hydrolyze other para-nitrophenyl sugars. Nevertheless, the occurrence of various rhamnosidases cannot be discarded. Substrates with β-linked glucose, like cellobiose, are well known to induce β-D-glucosidase activity. However, no information has been found on the occurrence of β- or α-linked glucose in the TDnC and/or EPS produced by the 4 dominant species in this study.

The average EARh for the whole study in the water column was low (8.1 nmol MUF l^{-1} h^{-1}) when compared to EAGl (51.4 nmol MUF l^{-1} h^{-1}) over the same period. β-D-glucosidase is generally considered one of the most active glycosyl hydrolases found in freshwater habitats (Chróst 1989, Arnosti 2003, Mudryk & Skórczewski 2006). The range of EAGl was higher than those found in the eutrophic freshwaters studied by Chróst (1989), Vrba et al. (1992) and Kisand & Tammert (2000). However, no comparative data have been found for EARh. The α-L-rhamnosidase and β-D-glucosidase measured could be produced by several organisms, such as fungi (Monti et al. 2004), protozoa (Vrba et al. 1992) and microalgae (Burczyk & Loos 1995). The focus of the present study was not, however, on the determination of possible sources of α-L-rhamnosidases. Moreover, bacteria are still considered the main exoenzyme releasers, and their production is strongly correlated with the influx of polymeric organic matter into the environment (Hoppe 1983, Chróst 1991).

In Barra Bonita Reservoir, no statistically significant correlation between EARh, EAGl and total bacterial density was found during the study period. This poor correlation of bacterial numbers with enzyme activity is not uncommon and has already been reported (Hoppe et al. 2002, Arnosti 2003). In the present case, it may have been caused by the monthly sampling, which may not have detected sufficient variation in bacterial density and enzyme activity to reveal the underlying correlation between these 2 parameters. However, since blooms produce a high diversity of substrates, another likely reason is that only fractions of the bacterial community, or other organisms, were responsible for the production of α-L-rhamnosidase. Bacteria capable of hydrolyzing MUF-rhamnopyranoside are not necessarily the same as those responsible for degrading the entire rhamnose-rich EPS or other substrates (Arnosti 2003), since only the final step of monomer cleavage by exohydrolase activity was measured with MUF sugars (Hoppe et al. 2002). Hashimoto et al. (1999, 2003) also found that α-L-rhamnosidase of a soil bacterium worked only on a disaccharide (rhamnosyl-glucose), a gellan degradation product, formed after successive reactions catalyzed by gellan lyases, to release rhamnose as a final product, leading to the conclusion that α-L-rhamnosidase acted as the final enzyme in the complete depolymerization of gellan.

The dynamic relation between bacterial density and TDnC suggests that the latter, which includes the EPS released by phytoplankton, was employed as substrate by bacterial populations. The analysis of the variation of TDnC concentration and bacterial density throughout depth (Fig. 2) was more successful than analysis of temporal variation in showing this dynamic relation in a hypereutrophic polymictic tropical environment. In such environments, although highly assimilated by the bacterial community, the substrates are still found constantly at high concentrations in the whole column, because substrates are continuously released, by either excretion or cell breakdown of phytoplankton. Nevertheless, higher concentration of bacteria at the bottom is caused not only by TDnC but by other substrates released by the cell breakdown that presumably occurs under the euphotic zone. Despite the polymictic character of the reservoir, phytoplankton species were concentrated in the euphotic zone, probably by means of gas vesicles on *Cyanobacteria* and flagella of *Cryptomonas tetrapyrenoidosa.* Thus, this zone is responsible for the continuous production and higher release of material (EPS and other). Reduced concentrations of TDnC below 3.0 m were caused not only by bacterial utilization, but also by lower production rates (lower phytoplankton density) and probably by dilution due to turbulence. Rhamnose and glucose percentages in TDnC were mostly constant from the surface to the bottom of the water column (Fig. 3), suggesting that TDnC at various depths is similar to that produced at surface. Other sources of rhamnose and glucose could be also present, raising their content in TDnC, explaining the higher values found in the water column when compared with the EPS of the 4 dominant phytoplankton species (see Table 3). Lipopolysaccharides from the outer membrane of cyanobacteria might have been an important source of these sugars.

Despite the similar concentrations of both glucose and rhamnose, the theoretical depolymerization time for combined glucose content in TDnC was 6.7 times shorter than that for combined rhamnose. The EAGl was 6 times higher than EARh, which could be explained by stronger affinity of β-D-glucosidase for glucose-rich TDnC or by higher enzyme production. Free glucose concentration was always low (0 to 0.08 µmol l^{-1}), which, as reported by Chróst et al. (1989), might induce the production and increase the EAGl. Glucose mono- and disaccharides are swiftly assimilated by large bacterial populations, like that in Barra Bonita (order of 10^7 to 10^8 cells) (Chróst 1989, Jørgensen & Jensen 1994, Kisand & Tammert 2000). Rhamnose, in contrast, is usually assimilated at slower rates, as has already been observed in bacterial degradation of EPS produced by a diatom (*Thalassiosira duostra*) (Giroldo et al. 2003). This information, combined with the high theoretical depolymerization time found for rhamnose, corroborates previous observations that α-L-rhamnosidase is more active in the final steps of complete depolymerization of polysaccharides (Hashimoto et al. 1999, 2003).

Many factors explain the lack of correlation between glucose and EAGl, and between enzymes and bacteria throughout time and depth. First, we have no information about the amount of glucose released from the EPS by α-D-glucosidase or β-D-glucosidase. Also, since *Microcystis aeruginosa* produces huge amounts of biomass, its breakdown can release, besides LPS (lipopolysaccharide) from its outer membranes, large amounts of polymeric substrates (Kisand & Tammert 2000), mainly α-linked glucose (glycogen), which contributes to the increase in bacterial density, but does not stimulate EAGl. Furthermore, action of other enzymes besides β-D-glucosidase and α-D-glucosidase can release glucose from EPS. Some α-L-rhamnosidases acting during the terminal degradation of rhamnose-rich substrates, may also release, indirectly, glucose: if rhamnose is part of a rhamnosyl-glucose, α-L-rhamnosidases will release both monosaccharides (Hashimoto et al. 2003). This may also help explain why no correlation was found between the temporal variation of EAGl and glucose, contrary to what happened with EARh and rhamnose.

Production of rhamnose-rich EPS occurs during nearly the whole year in the hypereutrophic and tropical Barra Bonita Reservoir. Its high availability is related to blooms and/or the constant presence of phytoplankton-dominant species, especially *Microcystis aeruginosa*, and may be responsible for the high EARh and EAGl.

EPS availability may also interfere with the continuous supply of rhamnose available for utilization by microorganisms. However, its importance in the carbon flux and as an alternative metabolic pathway for microorganisms is still poorly documented in aquatic environments. Microorganisms are able to use pentoses and deoxyhexoses as their sole carbon source (Watanabe & Makino 2009). Thus, rhamnose released by cyanobacteria, phytoplanktonic microalgae and other organisms must also be taken into consideration. In fact, several bacteria possess an alternative pathway of L-rhamnose metabolism, which can be used in aerobic and anaerobic environments (Hacking et al. 1978, Baldomà & Aguilar 1987, Watanabe & Makino 2009).

Our results show that in a hypereutrophic environment, rhamnose- and glucose-rich EPS produced by phytoplankton are important sources of dissolved polymeric carbohydrates. The low glucose depolymerization time at all depths, combined with significant correlation between β-D-glucosidase, *Microcystis aeruginosa*, and TDnC at all depths, suggest the presence of β-linked glucose in EPS. Thus, in hypereutrophic and tropical environments, EPS from phytoplankton is suggested to be an important substrate for β-D-glucosidase activity, and a major source of glucose for the environment.

Also, our results suggest the occurrence of coupling between predominant phytoplankton species in Barra Bonita Reservoir (all producers of rhamnose and also glucose-rich EPS), α-L-rhamnosidase(s) activity and rhamnose, which must be a common occurrence in similar water bodies around the world. Finally, although no reports on the abundance of rhamnose in freshwater environments were found in the literature, this monosaccharide may play a major part in such environments.

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LITERATURE CITED

APHA (American Public Health Association) (1995) Standard methods for the examination of water and wastewater, 16th edn. APHA, Washington, DC

Arnosti C (2000) Substrate specificity in polysaccharide hydrolysis: contrasts between bottom water and sediments. Limnol Oceanogr 45:1112–1119

Arnosti C (2003) Microbial extracellular enzymes and their role in dissolved organic matter cycling. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego, CA, p 315–342

Baldomà L, Aguilar J (1987) Involvement of lactaldehyde dehydrogenase in several metabolic pathways of *Escherichia coli* K12. J Biol Chem 262:13991–13996

Bittar TB, Vieira AAH (2010) Transparent exopolymer particles formation from capsules of *Anabaena spiroides* (Cyanobacteria) in culture. J Phycol 46:243–247

Burczyk J, Loos E (1995) Cell wall-bound enzymatic activities in *Chlorella* and *Scenedesmus.* J Plant Physiol 146: 748–750

Calijuri MC, Dos Santos ACA, Jati S (2002) Temporal changes in the phytoplankton community structure in a tropical and eutrophic reservoir (Barra Bonita, SP, Brazil). J Plankton Res 24:617–634

Caruso G, Monticelli L, Azzaro F, Azzaro M and others (2005) Dynamics of extracellular enzymatic activities in a shallow Mediterranean ecosystem (Tindari ponds, Sicily). Mar Freshw Res 56:173–188

Choueri RB, Melão MGG, Lombardi AT, Vieira AAH (2007) Effects of cyanobacterium exopolysaccharides on life history of *Ceriodaphnia cornuta* SARS. J Plankton Res 29:339–345

Chróst RJ (1989) Characterization and significance of beta-glucosidase activity in lake water. Limnol Oceanogr 34: 660–672

Chróst RJ (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, Berlin, p 29–54

Chróst RJ, Krambeck HJ (1986) Fluorescence correction for measurements of enzyme activity in natural waters using methylumbelliferyl substrates. Arch Hydrobiol 106: 79–90

Chróst RJ, Velimirov B (1991) Measurement of enzyme kinetics in water samples: effect of freezing and soluble stabilizer. Mar Ecol Prog Ser 70:93–100

Chróst RJ, Münster U, Rai H, Albrecht D, Witzel PK, Overbeck J (1989) Photosynthetic production and exoenzymatic degradation of organic-matter in the euphotic zone of a eutrophic lake. J Plankton Res 11:223–242

Colombo V (2006) Polissacarídeos extracelulares e atividade enzimática das glicosidases no reservatório de Barra Bonita, SP, Brasil. PhD thesis, Universidade Federal de São Carlos

Colombo V, Vieira AAH, Moraes G (2004) Activity of glycosidases from freshwater heterotrophic microorganisms on the degradation of extracellular polysaccharide produced by *Anabaena spiroides* (Cyanobacteria). Braz J Microbiol 35:110–116

Cowie GL, Hedges JL (1984) Carbohydrates sources in a coastal marine environment. Geochim Cosmochim Acta 48:2075–2087

Dellamano-Oliveira MJ, Colombo-Corbi V, Vieira AAH (2007) Carboidratos dissolvidos do reservatório de Barra Bonita (Estado de São Paulo, Brasil) e sua relação com as algas fitoplanctônicas abundantes. Biota Neotrop 7: 59–66

De Philippis R, Vicenzini M (1998) Exocellular polysaccharides from cyanobacteria and their applications. FEMS Microbiol Rev 22:151–175

De Philippis R, Margheri MC, Materassi R, Vincenzini M (1998) Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. Appl Environ Microbiol 64:1130–1132

Freire-Nordi CS, Vieira AAH (1996) Utilization of extracellular polysaccharides from *Ankistrodemus densus* Kors (Chrorophyceae) by heterotrophic bacteria. Rev Microbiol 27:27–32

Giroldo D, Vieira AAH, Paulsen BS (2003) The relative increase of deoxy sugars during microbial degradation of an extracellular polysaccharide released by a tropical freshwater *Thalassiosira* sp (Bacillariophyceae). J Phycol 39:1109–1115

Giroldo D, Vieira AAH, Paulsen BS (2005) Extracellular polysaccharides produced by a tropical cryptophyte as a carbon source for natural bacterial populations. Eur J Phycol 40:241–249

Gouvêa SP, Vieira AAH, Lombardi AT (2005) Copper and cadmium complexation by high molecular weight

materials of dominant microalgae and of water from an eutrophic reservoir. Chemosphere 60:1332–1339

Gremm TJ, Kaplan LA (1997) Dissolved carbohydrates in stream water determined by HPLC and pulsed amperometric detection. Limnol Oceanogr 42:385–393

Hacking AJ, Aguilar J, Lin ECC (1978) Evolution of propanediol utilization in *Escherichia coli*: mutant with improved substrate-scavenging power. J Bacteriol 136: 522–530

Hashimoto W, Nankai H, Sato N, Kawai S, Murata K (1999) Characterization of α-L-rhamnosidase from *Bacillus sp.* strain GL1 responsible for the complete depolymerization of gellan. Arch Biochem Biophys 368:56–60

Hashimoto W, Miyake O, Nankai H, Murata K (2003) Molecular identification of an α-L-rhamnosidase from *Bacillus sp.* strain GL1 as an enzyme involved in complete metabolism of gellan. Arch Biochem Biophys 415:235–244

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar Ecol Prog Ser 11: 299–308

Hoppe HG, Kim S, Gocke K (1988) Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. Appl Environ Microbiol 54:784–790

Hoppe HG, Arnosti C, Herndl GF (2002) Ecological significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology and applications. CRC Press, Boca Raton, FL, p 73–107

Jati S (1998) Estrutura e dinâmica da comunidade fitoplanctônica no reservatório de Barra Bonita (SP): uma análise em diferentes escalas de tempo. MSc thesis, Universidade de São Paulo

Jørgensen NOG, Jensen RE (1994) Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by PAD-HPLC. FEMS Microbiol Ecol 14: 79–93

Kisand V, Tammert H (2000) Bacterioplankton strategies for leucine and glucose uptake after a cyanobacterial bloom in an eutrophic shallow lake. Soil Biol Biochem 32: 1965–1972

Köster M, Dahlke S, Meyer-Reil LA (1997) Microbiological studies along a gradient of eutrophication in a shallow coastal inlet in the Southern Baltic Sea (Nordrügensche Bodden). Mar Ecol Prog Ser 152:27–39

Lombardi AT, Vieira AAH, Sartori AL (2002) Mucilaginous capsule adsorption and intracellular uptake of copper by *Kirchneriella aperta* (Chlorococcales). J Phycol 38: 332–337

Martin C, Codd GA, Siegelman HW, Weckesser J (1989) Lipopolysaccharides and polysaccharides of the cell envelope of toxic *Microcystis aeruginosa* strains. Arch Microbiol 152:90–94

Monti D, Pisvejcova A, Kren V, Lama M, Riva S (2004) Generation of an α-L-rhamnosidase library and its application for the selective derhamnosylation of natural products. Biotechnol Bioeng 87:763–771

Mudryk ZJ, Skórczewski P (2006) Enzymatic activity and degradation of organic macromolecules by neustonic and planktonic bacteria in an estuarine lake. Pol J Ecol 54:3–14

Panhota RS, Bianchini I Jr, Vieira AAH (2007) Glucose uptake and extracellular polysaccharide (EPS) produced by bacterioplankton from an eutrophic tropical reservoir (Barra Bonita, SP, Brazil). Hydrobiologia 583:223–230

Paulsen BS, Aslaksen T, Freire-Nordi CS, Vieira AAH (1998) Extracellular polysaccharides from *Ankistrodesmus densus* (Chlorophyceae). J Phycol 34:638–641

Plude JL, Parker DL, Schommer OJ, Timmerman RJ, Hagstrom SA, Joers JM, Hnasko R (1991) Chemical characterization of polysaccharide from the slime layer of the cyanobacterium *Microcystis flos-aquae* C3-40. Appl Environ Microbiol 57:1696–1700

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

Seltmann G, Holst O (2002) The bacterial cell wall. Springer-Verlag, Berlin

Singh S, Das S (2011) Screening, production, optimization and characterization of cyanobacterial polysaccharide. World J Microbiol Biotechnol 27:1971–1980

Tien CJ, Krivtsov V, Levado E, Sigee DC, White KN (2002) Occurrence of cell-associated mucilage and soluble extracellular polysaccharides in Rostherne Mere and their possible significance. Hydrobiologia 485:245–252

Vieira AAH, Ortolano PIC, Giroldo D, Dellamano-Oliveira MJ and others (2008) Role of hydrophobic extracellular polysaccharide of *Aulacoseira granulata* (Bacillariophyceae) in the formation of aggregates in a hypereutrophic reservoir. Limnol Oceanogr 53:1887–1899

Vrba J, Nedoma J, Šimek K, Seda J (1992) Microbial decomposition of polymer organic matter related to plankton development in a reservoir: activity of α-, β- glucosidase, and β-N-acetylglucosaminidase and uptake of N-acetylglucosamine. Arch Hydrobiol 126:193–211

Watanabe S, Makino K (2009) Novel modified version of nonphosphorylated sugar metabolism: an alternative L-rhamnose pathway of *Sphingomonas* sp. FEBS J 276: 1554–1567

Wetzel RG (1991) Extracellular enzymatic interactions: storage, redistribution, and interspecific communication. In: Chróst RJ (ed) Microbial enzymes in aquatic environments. Springer-Verlag, Berlin, p 6–28

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