Vol. 64: 299–309, 2011
doi: 10.3354/ame01529

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online September 20

Bacterial utilization of size-fractionated dissolved organic matter

Vishwas B. Khodse, Narayan B. Bhosle*

Marine Corrosion and Material Research Division, National Institute of Oceanography (CSIR), Dona Paula, 403004, Goa, India

ABSTRACT: Dissolved organic matter (DOM) is an important source of organic carbon for sustaining the growth of heterotrophic bacteria. We investigated the bacterial utilization of high-molecular-weight (HMW; >30 kDa to 0.22 µm) and low-molecular-weight (LMW; >10 to 30 kDa) fractions of DOM. DOM was collected from a station in Dona Paula Bay, on the west coast of India, and fractionated into HMW and LMW portions. Each size fraction was inoculated with a natural population of bacteria and incubated over a period of 15 d at room temperature (28 ± 2°C); during this period, sub-samples were removed and the following were measured: dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), free monosaccharide (MCHO), dissolved uronic acid (DURA), $\delta^{13}C$, bacterial abundance (BA), and bacterial production (BP). The LMW fraction was isotopically heavier ($\delta^{13}C$ = –23.7 to –21.7‰) than the HMW fraction ($\delta^{13}C$ = –27.0 to –26.2‰), and the initial TDCHO content of these fractions was 25 and 16%, respectively. The initial DURA content was similar in the LMW DOM (7.4% DOC) and the HMW DOM (7.0% DOC). BA and BP were consistently higher in the LMW DOM than in the HMW DOM. In 15 d incubation, greater proportions of DOC (35%), TDCHO (76%) and DURA (36%) were utilized in the LMW DOM than in the HMW DOM. This suggests that the LMW DOM was more biologically reactive, i.e. it was utilized more rapidly, than the HMW DOM. It appears that the bioreactivity and origin of the DOM strongly influenced its utilization by natural heterotrophic bacteria.

KEY WORDS: Dissolved organic matter · Size fractions · Carbohydrates · Polysaccharides · Bacteria · Bacterial production · Dona Paula Bay

INTRODUCTION

In the marine environment dissolved organic matter (DOM) stores a large amount of biologically reactive organic carbon that is equal to the amount of carbon dioxide (CO_2) in the earth's atmosphere (Hedges 1992). The sources (terrestrial or marine) and cycling of dissolved organic carbon (DOC) are of considerable importance in the global carbon cycle (Kirchman et al. 2009, Porcal et al. 2009). Phytoplankton primary production is the main source of DOC in marine environments, whereas allochthonous inputs can be important in freshwater systems (Gueguen et al. 2006). Heterotrophic bacteria are major consumers and remineralizers of DOC. In some ecosystems up to 40 to 60% of autochthonous primary production is cycled through bacteria (Hoch & Kirchman 1993). The availability of DOM to heterotrophic bacteria depends on factors such as biochemical composition, molecular size, inorganic nutrient concentrations, photochemical transformation, temperature, and the structure of the microbial community (Amon & Benner 1996, Carlson et al. 2004, Rosenstock et al. 2005, Abboudi et al. 2008).

Total dissolved carbohydrate (TDCHO) is one of the largest pools of bioreactive DOM in aquatic environments. TDCHO accounts for a significant fraction of the DOC pool in both oceanic waters (20 to 30%)

*Corresponding author. Email: bhosle@nio.org

(Pakulski & Benner 1992) and estuarine waters (9 to 60%) (Hung et al. 2001, Khodse et al. 2010). Heterotrophic bacteria use TDCHO as a source of carbon and energy (Hanisch et al. 1996, Amon et al. 2001). The ratio TDCHO-C:DOC is a useful proxy for assessing the potential bioreactivity of DOC — a relatively higher ratio indicating more bioreactive and less diagenetically altered material, and vice versa (Amon et al. 2001, Goldberg et al. 2009). Similarly, utilization of uronic acid by heterotrophic bacteria has been reported in waters of the Bay of Bengal (Khodse et al. 2007) and the Gulf of Mexico (Hung et al. 2003).

In a coastal environment such as Dona Paula Bay, DOM is derived from autochthonous production, from pore waters of sediments and from allochthonous sources. Dona Paula Bay receives allochthonous inputs from the Mandovi and Zuari estuaries (Wafar et al. 1997). Allochthonous sources can be distinguished from autochthonous sources by the carbon isotope ratio; the rationale for this is that marine DOM is isotopically heavier ($\delta^{13}C_{oc}$ ca. −22‰) than terrestrial DOM ($\delta^{13}C_{oc}$ ca. −27‰) (Engelhaupt & Bianchi 2001).

DOM can contain organic compounds that may differ in source (terrestrial or marine), in TDCHO content and in bioreactivity. Differences in the chemical composition of DOM fractions may influence their utilization by bacteria. Our objective was to compare the utilization of 2 fractions of DOM — i.e. a low-molecular-weight (LMW) fraction (>10 to 30 kDa) and a high-molecular-weight (HMW) fraction (>30 kDa to 0.22 µm) — by a natural population of bacteria. We asked two questions: (1) Are there differences in the chemical and biochemical composition of various polymeric fractions of the DOC pool? (2) If present, do these differences influence the utilization of DOM by natural populations of bacteria?

MATERIALS AND METHODS

Collection of water samples and size fractionation of DOM. Surface (~1 m) water (25 l) was collected using a Niskin sampler (5 l) during the monsoon season (September, 2007) from Dona Paula Bay (15° 27′ N, 73° 48′ E) on the west coast of India. Filtration of water samples and size fractionation of DOM were carried out following the procedure described earlier (Fernandes et al. 2007, Khodse et al. 2008). The various steps involved in size fractionation of the DOM are shown in Fig. 1. Briefly, the water sample was first filtered through a nylon screen (mesh size 200 µm) to remove large zooplankton, then filtered through a pre-ashed GF/F filter (47 mm, 0.7 µm, Whatman), and finally filtered through a polycarbonate filter (47 mm, 0.22 µm, Nuclepore). Subsequently, the sample was filtered through a cellulose membrane ultra-filter with a cut-off value of 30 kDa (#YM-30) using an Amicon Ultra-filtration system,

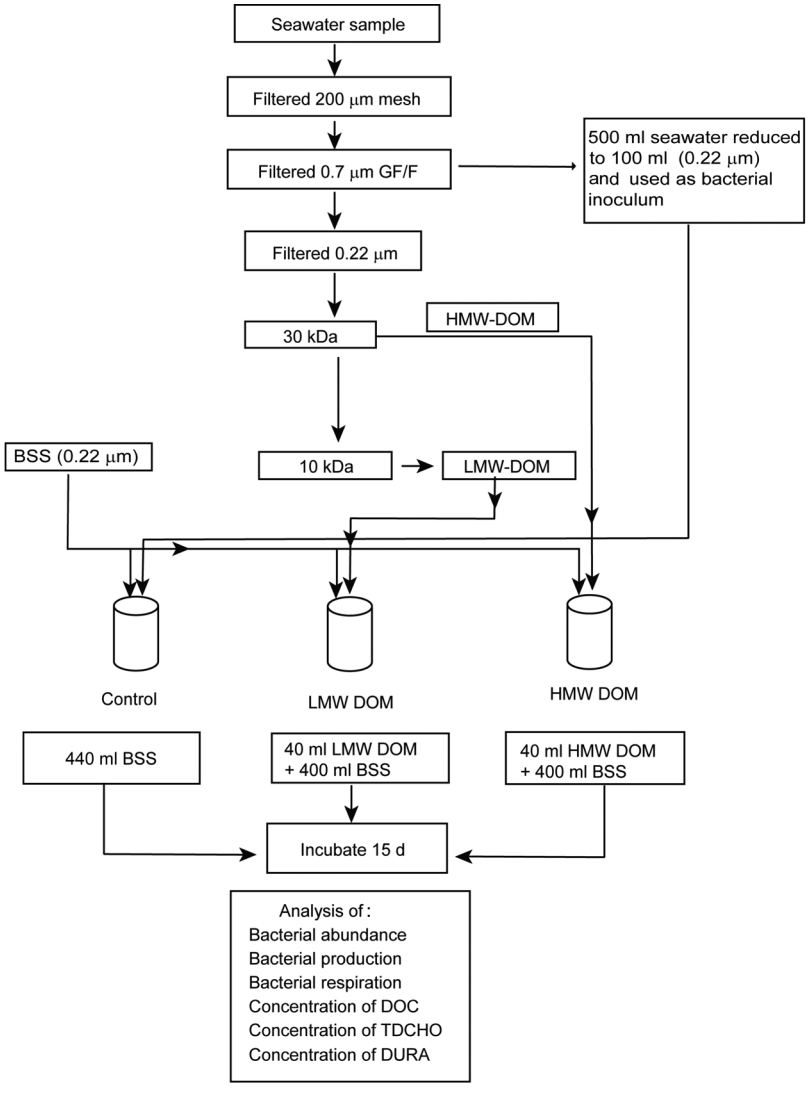

Fig. 1. The protocol used for fractionation of dissolved organic matter (DOM) and its degradation by bacteria. BSS = basal salt solution; DOC = dissolved organic carbon; LMW = low-molecular-weight; HMW = high-molecular-weight; TDCHO = total dissolved carbohydrates; DURA = dissolved uronic acids

equipped with a stirred cell, until 40 ml remained; the concentrated sample retained by this filter was defined as the HMW fraction of DOM. Filtrate from the ultra-filter was then filtered through another ultra-filter with a cut-off value of 10 kDa (#YM-10) until 40 ml remained; the concentrated sample retained by this filter was defined as the LMW fraction of DOM. Both fractions were desalted using the stirred cell and UV-Milli-Q water that had been passed through a 10 kDa ultra-filter. In general, the working pressure in the stirred cell of the ultra-filtration unit was maintained at ~30 psi using analytical reagent(AR)-grade nitrogen gas. Subsequently, the size-fractionated water samples were stored at −20°C until analysis.

Before use, the ultra-filters were cleaned with UV-Milli-Q water, 0.1 N NaOH, and then with UV-Milli-Q water; they were then rinsed 3 times with 100 ml of sample water. Teflon tubing was cleaned with 1 N HCl and rinsed several times with UV-Milli-Q water. Before use, all the glassware was cleaned with dichromic acid and rinsed several times with UV-Milli-Q water.

Growth medium. A basal salt solution (BSS), containing (g l^{-1}) 25.0 NaCl, 0.75 KCl, 7.0 $MgSO_4$, 0.019 NH_4Cl, 0.2 $CaCl_2$, 0.70 K_2HPO_4, 0.3 KH_2PO_4, and 1 ml trace metal solution, pH 7.5 (Jain & Bhosle 2009), supplemented with DOM was used as a growth medium to assess degradation of different size fractions of DOM. Three flasks, each containing 400 ml of BSS , were sterilized at 120°C and 15 psi pressure for 10 min. To one of the flasks we added 40 ml of the HMW DOM (340 µM C final conc.). To another flask we added 40 ml LMW DOM (261 µM C final conc.). To the third flask we added 40 ml of 0.22 µm filter-sterilized BSS (34 µM C); this was used as a control.

Preparation of the inoculum. In order to prepare the natural bacterial inoculum, 500 ml of surface water (~1 m) from Dona Paula Bay were first filtered through a GF/F glass filter (0.7 µm); the filtered water, containing a natural bacterial population, was concentrated to 100 ml by filtering through a polycarbonate filter (0.22 µm) (Amon & Benner 1996, Young et al. 2004, 2005). The water containing the natural bacterial population was used as a source of inoculum.

Utilization of the HMW and LMW DOM size fractions. A quantity of 5 ml of the concentrated (100 ml) bacterial suspension was used to inoculate a control flask, and flasks with HMW and LMW DOM prepared as above. Flasks were incubated in the dark at room temperature (28 ± 2°C) over a period of 15 d. All the flasks were shaken (100 rpm) using an orbital shaker. Sub-samples for bacterial and chemical analyses were removed from each flask at 0, 2, 4, 8, 10 and 15 d following inoculation. The samples were analysed for bacterial abundance (BA), bacterial production (BP), DOC, $\delta^{13}C$, TDCHO, monosaccharide (MCHO) and dissolved uronic acids (DURA).

Sample analyses. DOC was analyzed by the high-temperature (680°C) oxidation method using a Shimadzu TOC-5000 carbon analyzer. Sample values were corrected for the instrument blank (Benner & Strom 1993). Potassium hydrogen phthalate was used as a standard (50 to 400 µM C). Eight samples were analysed between injection of UV-Milli-Q water and the working standard solution. The analytical variation of this method was ±1.8%. The $\delta^{13}C_{oc}$ composition of total organic carbon (TOC) was performed with the Thermo Finnigan Flash 1112 elemental analyzer, linked with a Thermo Finnigan Delta V plus isotope ratio mass spectrometer. The coefficient of variation for $\delta^{13}C_{oc}$ was 0.2‰. All isotopic compositions are reported as per-mil (‰) relative to variation (δ) from the PDB standard.

$$\delta^{13}C_{oc} = \{(^{13}C/^{12}C)_{Sample}/(^{13}C/^{12}C)_{PDB} - 1\} \times 1000 \quad (1)$$

MCHO was determined without hydrolysis by the 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method (Myklestad et al. 1997). Briefly, 1 ml of filtered (0.22 µm pore size polycarbonate filter, Nuclepore) water sample was mixed in a test tube with 1 ml of potassium ferricyanide (0.7 mM) and kept in a boiling water bath for 10 min. Then, 1 ml of ferric chloride (2 mM) followed by 1 ml of TPTZ (2.5 mM) were added immediately and mixed well on a vortex-mixer. After 30 min, absorbance was measured at 595 nm.

To determine TDCHO, a water sample (5 ml) was evaporated to dryness at 40°C using a rotary vacuum evaporator. The dried sample was treated with 1 ml of 12 M H_2SO_4 at room temperature for 2 h. The sample was diluted to 1.2 M using UV-Milli-Q water, transferred to a glass ampoule, flushed with nitrogen gas, and sealed. The sample was hydrolysed at 100°C for 3 h (Bhosle et al. 1998). The released MCHOs were analysed as above. Glucose was used as a standard; concentrations are expressed as glucose equivalent. The unit used is µM glucose, assuming that all monomers were hexoses. The unit µM C is obtained by multiplying µM values by 6, assuming 6 mol of carbon per mol of hexoses.

Polysaccharide (PCHO) concentrations were calculated by subtracting MCHO concentrations from the concentrations of TDCHO. The coefficient of variation of the method was 11%.

DURA was estimated following the method of Filisetti-Cozzi & Carpita (1991) as described in Hung & Santschi (2001). Glucuronic acid was used as a standard; concentrations are expressed as glucuronic acid equivalent. The unit used is µM. The unit µM C is obtained by multiplying µM values by 6, assuming 6 mol of carbon per mol of hexoses. The coefficient of variation of the method was 12%.

BA was estimated following staining with 4,6-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980). Briefly, a known volume of seawater (2 to 5 ml) was stained with DAPI (final conc. 0.01%) for 5 min and filtered onto a black Nuclepore polycarbonate filter (pore size 0.22 µm). Bacterial cells were counted in at least 25 randomly selected fields using a 100× objective and an epifluorescence microscope (Nikon). The average cell number per field was calculated and used to estimate total bacterial cells following the procedure described by Porter & Feig (1980). Bacterial biomass (BB) was calculated from bacterial cell numbers, assuming a bacterial carbon content of 15 fg C cell^{-1} (Caron et al. 1995).

BP was estimated from the incorporation rate of nucleoside ^{3}H-thymidine into bacterial DNA (Fuhrman & Azam 1982) as described by Bhaskar & Bhosle (2008). In brief, 10 ml of sample were added to a 20 ml scintillation vial. To this vial we then added 30 µl (15 nM) of the working solution of thymidine (specific activity = 12 000 mCi/mmole, Board of Radiation and Isotope Technology, Mumbai). Similarly, 10 ml of the sample was fixed with filtered formalin (4% final conc.) before the addition of ^{3}H-thymidine and treated as control. The sample and control vials were incubated in the dark for 60 min at room temperature (28°C). At the end of the incubation period the uptake of ^{3}H-thymidine was terminated by the addition of filtered formalin (4% final conc.). The sample was filtered at low pressure through a cellulose nitrate filter (pore size 0.22 µm, Millipore). The filter paper was then rinsed twice with 5% ice-cold trichloroacetic acid (TCA), followed by 3 rinses with ice-cold ethanol. When completely dry, the filter was transferred to a clean scintillation vial and completely dissolved in 2 ml ethyl acetate. Scintillation cocktail (4 ml) was added to each sample and allowed to stabilize overnight. The samples were counted using a scintillation counter (Model Wallac-1209). The thymidine uptake rate was converted to BP using a conversion factor (CF) of 2×10^{18} cells per mol of thymidine utilized (Riemann et al. 1987) and a bacterial carbon conversion factor of 15 fg C per bacterial cell (Caron et al. 1995).

Bacterial growth efficiency (BGE) in the different size fractions of DOM was calculated from bacterial C production (obtained from thymidine uptake) and DOC using the equation given below:

$$BGE = (BP/DOC) \times 100 \quad (2)$$

Values are given as mean ± SD.

RESULTS

Processing of organic matter

BA and the initial concentrations of DOC, TDCHO, PCHO, MCHO and DURA in the source seawater and the various size fractions are shown in Table 1. HMW DOM, LMW DOM, and very-low-molecular-weight (VLMW, <10 kDa) DOM accounted for 7.8, 6.1 and 63.7%, respectively, of the source water DOC. Mass balance calculations indicated that 78% of the DOC, 79% of TDCHO and 85% of DURA could be recovered from the source water using the ultrafiltration technique (Table 1). The observed recovery of the TDCHO compares well with results (68 to 87%, average = 80%) reported earlier (Khodse et al. 2008). This means that contamination or losses were minor during the ultra-filtration process.

Table 1. Dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), dissolved uronic acids (DURA), dissolved monosaccharides (MCHO) and dissolved polysaccharides (PCHO = TDCHO – MCHO) in the surface seawater of Dona Paula Bay on the western coast of India. The table also shows the recovery of DOC, TDCHO and DURA after ultrafiltration and dialysis. The bacterial abundance in the seawater was 6.9×10^{7} cells ml^{-1}. (–) = no data

	Before size fractionation (µM C)	After size fractionation (µM C) Fractions			Total (sum) after size fractionation (µM C)	Recovery (%)
		>30 kDa to 0.22 µm	>10 kDa to 30 kDa	<10 kDa		
DOC	271 ± 12	21.1 ± 0.6	16.4 ± 0.6	172.0 ± 10.6	210.0	78
TDCHO	45 ± 3	4.1 ± 0.6	6.2 ± 0.2	25.1 ± 1.7	35.4	79
DURA	18 ± 3	2.5 ± 0.1	2.4 ± 0.2	10.7 ± 0.4	15.6	85
MCHO	7 ± 1	–	–	6.6 ± 0.8	–	–
PCHO	37 ± 2	–	–	18.5 ± 0.9	–	–

Chemical characteristics of the DOM size fractions

There were apparent differences in the chemical composition of the HMW and LMW fractions of the DOM (Table 2). On Day 0, the LMW DOM fraction contained 261 µM C, 65.6 µM C and 19.4 µM C of DOC, TDCHO and DURA, respectively; similarly, on Day 0, the HMW DOM fraction contained 340 µM C, 51.7 µM C and 24.0 µM C of DOC, TDCHO and DURA, respectively (Table 2). Thus, the HMW DOM fraction had higher concentrations of DOC and DURA and lower concentrations of TDCHO compared with the LMW DOM fraction. The relative contribution of TDCHO-C to DOC was higher for the LMW DOM fraction as compared to the HMW DOM fraction (Fig. 2a). The amount of DOC accounted for by DURA-C in the HMW and LMW DOM fractions was similar (Fig. 2b). $\delta^{13}C$ values varied from −27.0 to −26.2‰ and from −23.7 to −21.7‰ for HMW and LMW DOM fractions, respectively (Table 2).

Utilization of DOC and variation in $\delta^{13}C$ values

Over the period of incubation, DOC concentrations decreased from 340 to 296 µM C, and from 261 to 171 µM C in the HMW and LMW DOM fractions, respectively (Table 2). Bacterial utilization of HMW DOC varied from 3.5 to 12.9% (average 9.6 ± 3.9%); for LMW DOC it varied from 15.3 to 34.5% (average 26.1 ± 7.9%) (Table 3). DOC utilization rates were higher for the LMW DOM than for the HMW DOM (Table 4). $\delta^{13}C$ values of HMW DOM did not vary much over the period of incubation; in contrast, for LMW DOM they decreased from −21.7‰ on Day 0 to −23.7‰ on Day 8, and then increased again to their original value (−21.7‰) for the remaining period of incubation (Table 2).

Utilization of HMW and LMW TDCHO fractions

Over the period of incubation, TDCHO concentrations decreased in both size fractions of the DOM (Table 2). HMW-TDCHO decreased from 51.7 µM C on Day 0 to 27.7 µM C on Day 15, whereas LMW-TDCHO decreased from 65.6 µM C on Day 0 to 15.8 µM C on Day 15 (Table 2). Bacterial utilization of TDCHO in the HMW DOM and in the LMW DOM varied from 0.4 to 46.4% (average 21.5 ± 16.5%) and from 41.2 to 75.9% (average 65.2 ± 14.4%), respectively (Table 3). Bacterial utilization rates of TDCHO were significantly higher (analysis

Table 2. Changes in dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), free monosaccharides (MCHO), polysaccharides (PCHO), dissolved uronic acids (DURA) and the carbon isotopic signature ($\delta^{13}C$) in the high-molecular-weight (HMW) and low-molecular-weight (LMW) fractions over the incubation period (Inc.). Cont = control. No changes were detected in the controls for DURA and $\delta^{13}C$ (omitted)

Inc. (d)	DOC (µM C)			TDCHO (µM C)			MCHO (µM C)			PCHO (µM C)			DURA (µM C)			$\delta^{13}C$ (‰)	
	Cont	HMW	LMW	Cont	HMW	LMW	Cont	HMW	LMW	Cont	HMW	LMW	HMW	LMW		HMW	LMW
0	34 ± 3	340 ± 21	261 ± 28	1.2 ± 0.3	51.7 ± 2.7	65.6 ± 2.5	0.0	1.1 ± 0.3	1.5 ± 0.9	1.2 ± 0.1	50.6 ± 2.5	64.1 ± 1.4	24.0 ± 5.4	19.4 ± 1.7		−26.9	−21.7
2	36 ± 2	328 ± 10	221 ± 33	2.6 ± 0.4	51.5 ± 2.5	38.6 ± 8.8	0.0	1.7 ± 0.2	5.3 ± 0.7	2.6 ± 0.3	49.8 ± 2.3	33.2 ± 9.5	22.1 ± 5.3	19.3 ± 3.2		−26.5	−23.5
4	39 ± 4	313 ± 7	208 ± 12	3.4 ± 0.4	42.7 ± 2.1	24.3 ± 7.8	0.5 ± 0.1	3.4 ± 0.3	5.9 ± 0.2	2.9 ± 0.4	37.7 ± 2.4	18.4 ± 3.6	27.1 ± 3.6	17.7 ± 1.8		−26.3	−23.1
8	40 ± 3	302 ± 13	184 ± 12	2.7 ± 0.5	41.0 ± 3.7	18.6 ± 1.4	0.7 ± 0.1	3.3 ± 0.4	7.1 ± 1.0	2.0 ± 0.2	39.4 ± 3.2	11.5 ± 2.6	22.7 ± 0.9	16.5 ± 3.5		−26.3	−23.7
10	38 ± 6	298 ± 23	181 ± 27	2.6 ± 0.2	40.1 ± 0.7	15.8 ± 2.5	0.5 ± 0.2	3.9 ± 0.4	6.8 ± 0.8	2.1 ± 0.1	36.2 ± 1.3	9.0 ± 0.1	21.8 ± 3.0	14.0 ± 2.8		−26.2	−21.7
15	43 ± 5	296 ± 10	171 ± 18	1.4 ± 0.4	27.7 ± 3.7	16.7 ± 2.3	0.3 ± 0.1	3.7 ± 0.3	4.3 ± 0.2	1.1 ± 0.2	24.0 ± 4.1	12.4 ± 2.4	19.5 ± 4.9	12.4 ± 0.9		−27.0	−21.8

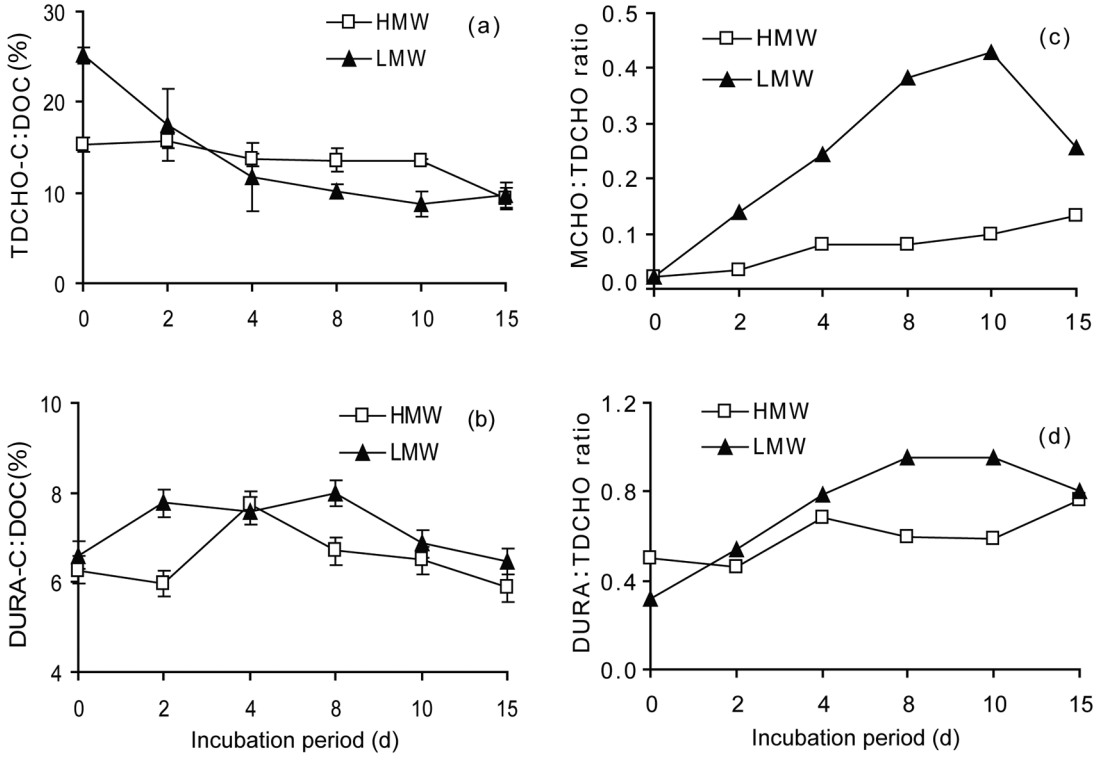

Fig. 2. Changes in (a) % TDCHO-C:DOC, (b) % DURA-C:DOC, (c) the MCHO:TDCHO ratio, and (d) the DURA:TDCHO ratio during the bacterial degradation of high-molecular-weight (HMW) and low-molecular-weight (LMW) dissolved organic matter (DOM). TDCHO = total dissolved carbohydrate; DURA = dissolved uronic acid; MCHO = free monosaccharide; DOC = dissolved organic carbon

Table 3. Range and average utilization (as % of initial concentration) of dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), dissolved uronic acids (DURA) in the high-molecular-weight (HMW) and low-molecular-weight (LMW) DOM fractions by a natural bacterial population. The table also shows bacterial growth efficiency (BGE) on HMW and LMW DOM fractions; BGE = BP/DOC × 100 (BGE was calculated from the DOC data in Table 2)

Parameters	HMW DOM			LMW DOM		
(%)	Range	Average	SD	Range	Average	SD
DOC	3.5–12.9	9.6	3.9	15.3–34.5	26.1	7.9
TDCHO	0.4–46.4	21.5	16.5	41.2–75.9	65.2	14.4
DURA	–12.9 to 18.8	5.7	11.3	0.5–36.1	17.6	14.3
BGE	4.3–9.1	5.7	1.9	1.2–6.8	4.3	2.5

of variance, ANOVA; $p < 0.01$) for the LMW DOM than for the HMW DOM (Table 4). PCHO concentrations in both fractions showed significant differences (ANOVA, $p < 0.01$). PCHOs in both LMW and HMW DOM were degraded by bacteria, producing MCHOs (Table 2). The decrease in concentrations of TDCHO or PCHO was associated with an increase in MCHO concentrations over the period of incubation. The production of MCHO was highest on Day 8 and on Day 10 for the LMW and HMW DOM fractions, respectively (Table 2). The MCHO:TDCHO ratio increased with the incubation period and was higher in the LMW fraction than in the HMW fraction (Fig. 2c).

Utilization of HMW and LMW DURA fractions

DURA concentrations in the LMW and HMW DOM fractions were not significantly different (ANOVA, $p > 0.05$) (Table 2). Over the period of incubation, DURA concentrations decreased from 24.0 to 19.5 µM C in the HMW DOM and from 19.4 to 12.4 µM C in the LMW DOM. Bacterial degradation of DURA in the HMW DOM and in the LMW DOM varied from –12.9 to 18.8% (average 5.7 ± 11.3%), and 0.5 to 36.1% (average 17.6 ± 14.3%), respectively (Table 3). Bacterial utilization

Table 4. Utilization rates (µg C l^{-1} h^{-1}) of dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO) and dissolved uronic acids (DURA) in the high-molecular-weight (HMW) and low-molecular-weight (LMW) DOM fractions over the period of incubation

Incubation period (d)	DOC		TDCHO		DURA	
	HMW	LMW	HMW	LMW	HMW	LMW
0	0.00	0.00	0.00	0.00	0.00	0.00
2	3.00	10.00	0.05	6.75	0.42	0.02
4	3.38	6.63	1.13	5.16	–0.34[a]	0.19
8	2.38	4.81	0.67	2.94	0.07	0.16
10	2.10	4.00	0.58	2.49	0.10	0.24
15	1.47	3.00	0.80	1.63	0.13	0.21

[a]Negative utilization rate was noted on Day 4 following incubation and was assumed to be due to DURA produced by microorganisms

rates for DURA were higher for LMW DOM than for HMW DOM, with the exception of 1 value (on Day 2; Table 4). The DURA:TDCHO ratio increased with degradation of DOM, and the ratio was relatively higher in the LMW fraction than in the HMW fraction, implying preferential removal of TDCHO in the LMW DOM (Fig. 2d). BA showed significant positive correlations with the DURA:TDCHO ratio of the LMW DOM ($r = 0.949$, $p < 0.001$) and of the HMW DOM ($r = 0.798$, $p < 0.02$).

BA and BP

BA and BP were strongly affected by the bioreactivity and the source of the DOM polymeric fractions (Fig. 3). Both BA and BP were higher in the LMW DOM than in the HMW DOM (Fig. 3a,b). With HMW DOM, BA gradually increased from 1.6×10^{10} cells l^{-1} on Day 0 to 2.2×10^{10} cells l^{-1} on Day 4 and then remained nearly the same over the remaining period of incubation (Fig. 3a). For the LMW DOM, BA increased from 1.7×10^{10} cells l^{-1} on Day 0 to 3.1×10^{10} cells l^{-1} on Day 8, and then decreased for the remaining period of incubation (Fig. 3a). BA showed significant inverse correlations with the concentrations of TDCHO of the HMW DOM ($r = -0.853$, $p < 0.01$) and LMW DOM ($r = -0.850$, $p < 0.01$).

As observed for other parameters, BP rates were consistently higher in the LMW DOM than in the HMW DOM (Fig. 3b). Bacterial carbon accounted for 5.78 to 9.37% of HMW DOC, and 7.96 to 21.26% of LMW DOC (Fig. 3c). In contrast, BGE was higher for HMW DOM than for LMW DOM when DOC was used to calculate BGE (Table 3).

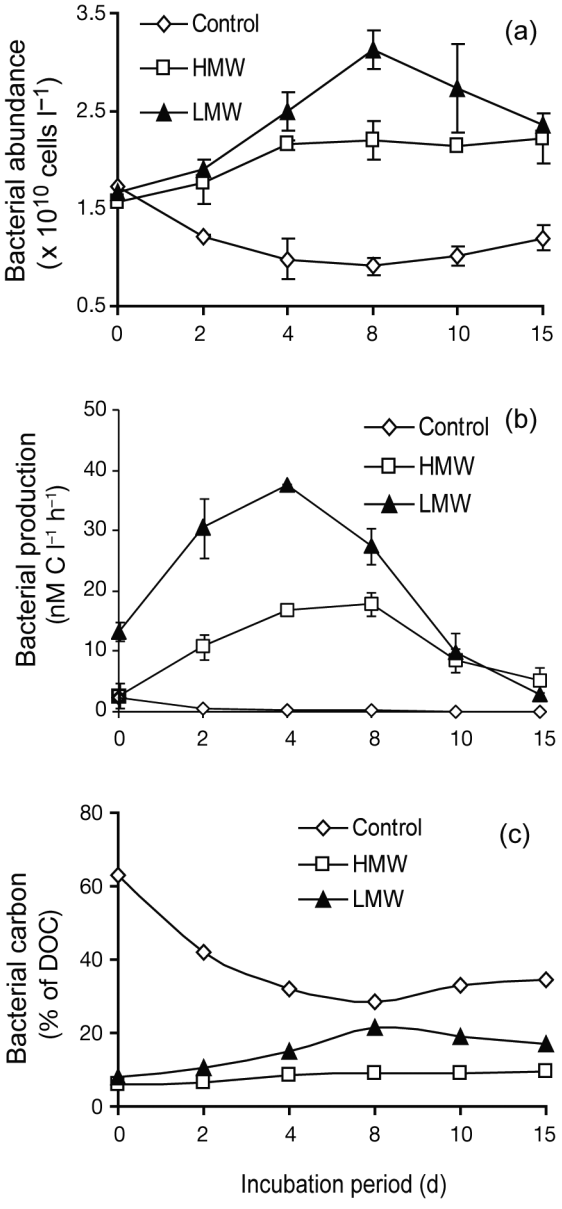

Fig. 3. Changes in (a) bacterial abundance, (b) bacterial production, and (c) bacterial carbon (as % of dissolved organic carbon [DOC]), during the degradation of high-molecular-weight (HMW) and low-molecular-weight (LMW) dissolved organic matter (DOM) by a natural bacterial population

DISCUSSION

Source and bioreactivity of organic matter

Both the LMW and HMW fractions of DOM were utilized by natural bacterial populations. Bacteria utilized 35% of LMW DOC, and 13% of HMW DOC over the period of incubation. Considering the uncer-

tainties in the DOC analysis, the loss in DOC can vary from 6.4 to 15.2% (average 11.4 ± 3.2%) and from 0.3 to 13.8% (average 7.6 ± 9.3%) for the HMW DOC fraction; similarly, it can vary from 12.1 to 34.6 (average 26.2 ± 8.9%) and from 15.9 to 34.3% (average 25.9% ± 8.4%) for the LMW DOC (Table 2). Irrespective of the uncertainties involved in DOC analysis (calculated from values given in Table 2), it was evident that the LMW DOM was more bioreactive, and was used preferentially by bacteria in this set of experiments. The observed differences in the relative bioreactivity of LMW DOM and HMW DOM may be related to source, composition, and content of TDCHO.

$\delta^{13}C$ values varied from −27.0 to −26.2‰, and from −23.7 to −21.7‰ for the HMW and LMW DOM size fractions, respectively (Table 2). $\delta^{13}C$ values for DOM derived from terrestrial sources or C3 plants vary from −28 to −26‰ (Raymond & Bauer 2001, Cai et al. 2008); these values are similar to those observed for HMW DOM in the present study, suggesting that the HMW DOM was derived from terrestrial sources. In contrast, $\delta^{13}C$ values of LMW DOM are in the range of values reported for marine plankton (Raymond & Bauer 2001, McCallister et al. 2004, 2006); the similarity in the $\delta^{13}C$ values for LMW DOM and marine plankton suggest that the LMW DOM was derived from marine plankton. The $\delta^{13}C$ values of LMW DOM decreased from −21.7 on Day 0 to −23.7 on Day 8 of the incubation period (Table 2). DOM is composed of a heterogeneous mixture of organic compounds having different $\delta^{13}C$ values. Organic compounds such as polysaccharides and proteins are more enriched in $\delta^{13}C$ than are lipids (Marchand et al. 2005, Teece & Fogel 2007). Selective removal of PCHO may account for the observed depletion of $\delta^{13}C$ in the LMW DOM. This appears to be the case, as 31 µM C of PCHOs were utilised by bacteria during the first 2 d of incubation. Subsequently, we observed enrichment in $\delta^{13}C$ on Days 10 and 15. Small changes in carbohydrate concentration during the 8 to 15 d period of incubation, however, strongly argue against their role in the enrichment of $\delta^{13}C$ values on Days 10 and 15 (Table 2). With the present data, it is not possible to identify the factors involved in the enrichment of $\delta^{13}C$ on Days 10 and 15.

The ratio TDCHO-C:DOC is a useful proxy for assessing the potential bioreactivity of DOM (Cowie & Hedges 1994, Goldberg et al. 2009, Kaiser & Benner 2009). Freshly produced DOM is rich in bioreactive components such as neutral carbohydrates (Cowie & Hedges 1994, Meon & Kirchman 2001, Kaiser & Benner 2009). The fraction of DOC as carbohydrates was higher (25%) for LMW DOM and lower for HMW DOM (16%) at the start of the experiment (Fig. 2a). The removal of TDCHO was higher (~76%) for LMW DOM and lower (46%) for HMW DOM. The contribution of TDCHO to DOC decreased rapidly for LMW DOM (from 25 to 9%), and slowly for the HMW DOM (from 16 to 9%) over the period of incubation (Fig. 2a). The hypothesis that the HMW DOM was more terrestrial, and therefore less labile, was supported by the smaller changes in TDCHO. However, non-TDCHO carbon (non-TDCHO-C = DOC – TDCHO-C) of both HMW DOC and LMW DOC was also utilized by bacteria, and the amount utilized was higher for the former (~45 to 98%) than for the latter (22 to 45%).

Our results indicate that TDCHOs are important substrates for the growth of planktonic heterotrophic bacteria, which is in agreement with previous studies (Hanisch et al. 1996, Kaiser & Benner 2009) (Table 3). As the bacterial cell numbers increased, TDCHO concentrations decreased and MCHO concentrations increased. Similar observations have been recorded for marine waters (Burney 1986, Cherrier & Bauer 2004) and mesotrophic lake waters (Hanisch et al. 1996). This is also supported by the higher MCHO: TDCHO ratio in LMW DOM as compared to HMW DOM. The TDCHO utilization rates for the LMW DOM varied from 1.63 to 6.75 µg C l^{-1} h^{-1} and were much higher than those recorded for HMW DOM (0.05 to 1.13 µg C l^{-1} h^{-1}). Our TDCHO utilization rates in LMW DOM are also higher than those recorded for other environments. For example, Burney (1986) reported that the TDCHO utilization rate was 1.50 µg C l^{-1} h^{-1} for the waters of the Atlantic. Jørgensen & Jensen (1994) observed a use rate of TDCHO of 2.75 µg C l^{-1} h^{-1} for a mesotrophic Danish lake. TDCHO utilization rates varied seasonally (0.47 to 3.43 µg C l^{-1} h^{-1}) for the mesotrophic Lake Constance, Germany (Hanisch et al. 1996). Studies on the hydrolysis of polysaccharides using fluorogenic substrate analogs for glucosidase activity also indicate that TDCHOs are important substrates for the growth of planktonic bacteria. Polysaccharides need to be hydrolysed to oligo- and monosaccharides before they can be taken up by bacteria. Chróst et al. (1989) observed a good correlation between the concentrations of TDCHO and the activity of β-glucosidase during a phytoplankton bloom in the eutrophic Lake Plußsee, Germany.

DURAs are acidic polysaccharides that generally account for a small proportion of organic carbon. The concentration of DURAs in both HMW and LMW fractions of DOM decreased over the period of incu-

bation, implying their utilization by heterotrophic bacteria. The concentration of DURAs decreased with increasing sediment core depth in Dabob Bay, Washington, USA (Bergamaschi et al. 1999), and with increasing water column depth in the Bay of Bengal (Khodse et al. 2007); in both of these studies it was suggested that the decrease in the concentration of DURA was due to uptake by heterotrophic bacteria. The utilization of DURA was greater for LMW DOM than for HMW DOM, implying that the former was utilized faster (Tables 3 & 4). The increase in the DURA:TDCHO ratio for HMW DOM (0.46 to 0.76) and LMW DOM (0.32 to 0.96) — as well as its significant positive correlations with BA for both LMW DOM ($r = 0.949$, $p < 0.001$) and HMW DOM ($r = 0.798$, $p < 0.02$) (Fig. 2d) — implies that TDCHO was preferentially utilized by bacteria, as compared to DURA (Hung et al. 2001, Khodse et al. 2007).

Effect of LMW and HMW DOM on BA and BP

The higher values of both BA and BP in LMW DOM, compared to HMW DOM, imply that LMW DOM was the more bioreactive fraction and was used preferentially by a natural marine bacterial population (Fig. 3a,b). The $\delta^{13}C$ signature suggests that the LMW DOM originated from marine phytoplankton, whereas the HMW DOM was of terrestrial origin. The higher values of BA and BP in the LMW DOM, compared with the HMW DOM, suggest that bacteria preferentially assimilated an isotopically enriched $\delta^{13}C$ organic component derived from $\delta^{13}C$-enriched phytoplankton. Although we have a small dataset, our results support the preferential selection of algal material by heterotrophic bacteria despite the quantitative abundance of allochthonous organic matter (McCallister et al. 2006). The observed variability in bacterial utilization of LMW DOM and HMW DOM may also result from differences in the heterotrophic bacterial species or groups that evolved during the period of incubation (Covert & Moran 2001, Cherrier & Bauer 2004).

BGE is an important factor in understanding bacterial influence on carbon flow in aquatic ecosystems. According to del Giorgio & Cole (1998) numerous factors can influence BGE. Greater BGE in HMW DOM than in LMW DOM suggests that bioreactivity, and the source of DOM, did not influence BGE in the present study. Other factors, including temperature, salinity, pressure and nutrient concentrations, may not have influenced BGE because they were nearly similar for both the LMW DOM and HMW DOM experiments. Presumably, differences in the physiological condition of cells, and in the taxonomic composition of bacteria in LMW DOM and HMW DOM, may be responsible for the higher BGE in HMW DOM (del Giorgio & Cole 1998).

CONCLUSION

We measured the utilization, by bacteria, of HMW DOM and LMW DOM isolated from Dona Paula Bay, on the west coast of India. The $\delta^{13}C$ values suggest that the HMW DOM fraction derived mainly from terrestrial sources, whereas LMW DOM originated from marine phytoplankton. Concentrations of TDCHO and PCHO showed that PCHO served as an active substrate. Non-TDCHO carbon in the HMW DOM (45 to 98%) and in the LMW DOM (22 to 45%) was also used by the bacteria. Bacteria preferentially used DOC, TDCHO and DURA in the LMW DOM as compared to their use in the HMW DOM. This conclusion was well supported by the higher BA and BP, and the greater decrease in DOC, TDCHO and DURA in LMW DOM. Our results showed that bacterial consumption of polymeric fractions of DOM from Dona Paula Bay was strongly affected by the source and bioreactivity of the DOM.

Acknowledgements. The authors are grateful to Dr. S. R. Shetye, Director, National Institute of Oceanography, Goa, India, for encouragement and facilities. The first author is grateful to CSIR, New Delhi, for providing a senior research fellowship to take up this research. We thank Dr. S. G. P. Matondkar for bacterial production measurements, and we are also grateful to Dr. C. A. Carlson, handling editor, and the three anonymous referees, for their helpful comments on the manuscript.

LITERATURE CITED

Abboudi M, Jeffrey WH, Ghiglione JF, Pujo-Pay M and others (2008) Effects of photochemical transformations of dissolved organic matter on bacterial metabolism and diversity in three contrasting coastal sites in the Northwestern Mediterranean Sea during summer. Microb Ecol 55:344–357

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

Amon RMW, Fitznar HP, Benner R (2001) Linkages among bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnol Oceanogr 46: 287–297

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

Bergamaschi BA, Walters JS, Hedges J (1999) Distributions of uronic acids and *O*-methyl sugars in sinking and sedi-

mentary particles in two coastal marine environments. Geochim Cosmochim Acta 63:413–425

Bhaskar PV, Bhosle NB (2008) Bacterial production, glucosidase activity and particle-associated carbohydrates in Dona Paula bay, west coast of India. Estuar Coast Shelf Sci 80:413–424

Bhosle NB, Bhaskar PV, Ramachandran S (1998) Abundance of dissolved polysaccharides in the oxygen-minimum layer of Northern Indian Ocean. Mar Chem 63:171–182

Burney CM (1986) Bacterial utilization of total *in situ* dissolved carbohydrate in offshore waters. Limnol Oceanogr 31:427–431

Cai Y, Guo L, Dougals TA (2008) Temporal variations in organic carbon species and fluxes from the Chena River, Alaska. Limnol Oceanogr 53:1408–1419

Carlson CA, Giovannoni S, Hansell DA, Goldberg SJ, Parsons R, Vergin K (2004) Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northern Sargasso Sea. Limnol Oceanogr 49:1073–1083

Caron DA, Dam HG, Kremer P, Lessard EJ and others (1995) The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda. Deep-Sea Res 42:943–972

Cherrier J, Bauer JE (2004) Bacterial utilization of transient plankton-derived dissolved organic carbon and nitrogen inputs in surface ocean waters. Aquat Microb Ecol 35: 229–241

Chróst RJ, Munster U, Rai H, Albrecht D, Witzel KP, 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

Covert JS, Moran MA (2001) Molecular characterization of estuarine bacterial communities that use high- and low-molecular-weight fractions of dissolved organic carbon. Aquat Microb Ecol 25:127–139

Cowie GL, Hedges JI (1994) Biochemical indicators of diagenetic alteration in natural organic-matter mixtures. Nature 369:304–307

del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. Annu Rev Ecol Syst 29:503–541

Engelhaupt E, Bianchi TS (2001) Sources and composition of high-molecular-weight dissolved organic carbon in a southern Louisiana tidal stream (Bayou Trepagnier). Limnol Oceanogr 46:917–926

Fernandes L, D'Souza F, Bhosle NB (2007) Carbohydrates in size-fractionated dissolved organic matter in a station of the Bay of Bengal. Indian J Mar Sci 36:193–198

Filisctti-Cozzi TMCC, Carpita NC (1991) Measurement of uronic acids without interference from neutral sugars. Anal Biochem 197:157–162

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109–120

Goldberg SJ, Carlson CA, Hansell DA, Nelson NB, Siegel DA (2009) Temporal dynamics of dissolved combined neutral sugars and the quality of dissolved organic matter in the Northern Sargasso Sea. Deep-Sea Res I 56:672–685

Gueguen C, Guo DL, Wang D, Tanaka N, Hung CC (2006) Chemical characteristics and origin of dissolved organic matter in the Yukon River. Biogeochemistry 77:139–155

Hanisch H, Schweitzer B, Simon M (1996) Use of carbohydrates by planktonic bacteria in a mesotrophic lake. Microb Ecol 31:41–55

Hedges JI (1992) Global biogeochemical cycles: progress and problems. Mar Chem 39:67–93

Hoch MP, Kirchman DL (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Mar Ecol Prog Ser 98:283–295

Hung CC, Santschi PH (2001) Spectrophotometric determination of total uronic acids in seawater using cation exchange separation and pre-concentration lyophilization. Anal Chim Acta 427:111–117

Hung CC, Tang D, Warnken KW, Santschi PH (2001) Distributions of carbohydrates, including uronic acids, in estuarine waters of Galveston Bay. Mar Chem 73:305–318

Hung CC, Guo L, Santschi PH, Alvarado-Quiroz N, Haye J (2003) Distributions of carbohydrate species in the Gulf of Mexico. Mar Chem 81:119–135

Jain A, Bhosle NB (2009) Biochemical composition of the marine conditioning film: implications for bacterial adhesion. Biofouling 25:13–19

Jørgensen NOG, Jensen RE (1994) Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by PAD-HPLC. FEMS Microbiol Ecol 14: 79–94

Kaiser K, Benner R (2009) Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. Mar Chem 113:63–77

Khodse VB, Fernandes L, Gopalkrishna VV, Bhosle NB, Fernandes V, Matondkar SGP, Bhushan R (2007) Distribution and seasonal variation of concentrations of particulate carbohydrates and uronic acids in the northern Indian Ocean. Mar Chem 103:327–346

Khodse VB, Fernandes L, Bhosle NB, Sardessai S (2008) Carbohydrates, uronic acids and alkali-extractable carbohydrates in contrasting marine and estuarine sediments: distribution, size fractionation and partial chemical characterization. Org Geochem 39:265–283

Khodse VB, Bhosle NB, Prabhu Matondkar SG (2010) Distribution of dissolved carbohydrates and uronic acids in a tropical estuary, India. J Earth Syst Sci 119:519–530

Kirchman DL, Moran XAG, Ducklow H (2009) Microbial growth in the polar oceans — role of temperature and potential impact of climate change. Nat Rev Microbiol 7: 451–459

Marchand C, Disnar JR, Lallier-Verges E, Lottier N (2005) Early diagenesis of carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French Guiana). Geochim Cosmochim Acta 69:131–142

McCallister SL, Bauer JE, Cherrier J, Decklow HW (2004) Assessing the sources and ages of organic matter supporting estuarine bacterial production: a novel multiple isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{14}C$) approach. Limnol Oceanogr 49: 1687–1702

McCallister SL, Bauer JE, Canuel EA (2006) Bioreactivity of estuarine dissolved organic matter: a combined geochemical and microbiological approach. Limnol Oceanogr 51:94–100

Meon B, Kirchman DL (2001) Dynamics and molecular composition of dissolved organic material during experimental phytoplankton blooms. Mar Chem 75:185–199

Myklestad SM, Skanoy E, Hestmann S (1997) A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater. Mar Chem 56:279–286

Pakulski JD, Benner R (1992) An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Mar Chem 40: 143–160

Porcal P, Kopriynjak JF, Molot LA, Dillon PJ (2009) Humic substances—part7: the biogeochemistry of dissolved organic carbon and its interaction with climate change. Environ Sci Pollution Res 16:714–726

Porter JD, Feig Y (1980) The use of DAPI for identification and enumeration of bacteria and blue-green algae. Limnol Oceanogr 25:943–948

Raymond PA, Bauer JE (2001) Use of ^{14}C and ^{13}C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: a review and synthesis. Org Geochem 32:469–485

Riemann B, Bjøernsen PK, Newell S, Fallon R (1987) Calculation of cell production of coastal marine bacteria based on measured incorporation of ^{3}H thymidine. Limnol Oceanogr 32:471–476

Rosenstock B, Zwisler W, Simon M (2005) Bacterial consumption of humic and non-humic low- and high-molecular-weight DOM and the effect of solar irradiation on the turnover of labile DOM in the Southern ocean. Microb Ecol 50:90–101

Teece MA, Fogel ML (2007) Stable carbon isotope biogeochemistry of monosaccharides in aquatic organisms and terrestrial plants. Org Geochem 38:458–473

Wafar S, Untawale AG, Wafar MVM (1997) Litter fall and energy flux in a mangrove ecosystem. Estuar Coast Shelf Sci 44:111–124

Young KC, Maurice PA, Docherty KM, Bridgham SD (2004) Bacterial degradation of dissolved organic matter from two northern Michigan streams. Geomicrobiol J 21: 521–528

Young KC, Docherty KM, Maurice PA, Bridgham SD (2005) Degradation of surface seawater dissolved organic matter: influence of DOM chemical characteristics and microbial populations. Hydrobiologia 539:1–11

Editorial responsibility: Craig Carlson, Santa Barbara, California, USA

Submitted: February 16, 2010; Accepted: June 24, 2011
Proofs received from author(s): September 9, 2011