

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

Corresponding author*: Tel: +91-832-2450234; Fax: +91-832-2450602

E-mail: bhosle@nio.org

ABSTRACT: Dissolved organic matter (DOM) is an important source of organic carbon to sustain growth of heterotrophic bacteria. Bacterial utilization of high molecular weight (HMW) (> 30 kDa to 0.22 μ m), and low molecular weight (LMW) (>10 kDa to 30 kDa) DOM fractions were investigated. DOM was collected from a station in the Dona Paula Bay, west coast of India, and fractionated into HMW, and LMW size fractions. Each size fraction was inoculated with a natural bacterial population, and incubated over a period of 15 days at room temperature ($28 \pm 2^\circ\text{C}$). During the period of incubation, sub-samples were removed and dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), free monosaccharide (MCHO), dissolved uronic acid (DURA), $\delta^{13}\text{C}$, bacterial abundance (BA), and bacterial production (BP) were measured. The LMW fraction was isotopically heavier ($\delta^{13}\text{C} = -23.7$ to -21.7 ‰), than the HMW fraction ($\delta^{13}\text{C} = -27.0$ to -26.2 ‰), and the initial TDCHO content of these fractions were 25 % and 16 %, respectively. DURA content was similar in LMW (7.4 % DOC) and HMW DOM (7.0 % DOC). Bacterial abundance, and production were consistently higher in LMW DOM than HMW DOM. Greater proportions of DOC (35 %), TDCHO (76 %), and DURA (36 %) of the LMW fraction were utilized over the period of incubation than in HMW DOM. This suggests that LMW DOM was most biologically reactive, and hence it was more rapidly utilized than the HMW DOM. It appears that the bioreactivity and origin of dissolved organic matter 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 large amount of biologically reactive organic carbon that is equal to the amount of carbon dioxide (CO₂) 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, Procal 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 microbial community structure (Amon & Benner 1996, Carlson et al. 2004, Rosenstock et al. 2005, Abboudi et al. 2008).

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 (20 to 30 %) (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 to DOC is a useful proxy to assess the potential bioreactivity of DOC with relatively greater 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 and the Gulf of Mexico (Hung et al. 2003, Khodse et al. 2007).

In the coastal environment such as Dona Paula Bay, DOM is derived from autochthonous production, sediment pore waters and allochthonous sources. Dona Paula Bay receives allochthonous inputs from the Mandovi and Zuari estuaries (Wafer et al. 1997). Carbon isotope ratio is used to distinguish allochthonous and autochthonous sources. This is because marine DOM is isotopically heavier ($\delta^{13}\text{C}_{\text{oc}} \sim -22 \text{‰}$), than terrestrial DOM ($\delta^{13}\text{C}_{\text{oc}} \sim -27 \text{‰}$) (Engelhaupt & Bianchi 2001).

DOM can contain organic compounds that presumably may differ in source (terrestrial or marine), TDCHO content and bioreactivity. Differences in chemical composition of DOM fractions may influence their utilization by bacteria. Our objective was to compare the utilization of two fractions i.e. LMW (>10 kDa to 30 kDa), and HMW (>30 kDa to 0.22 μm) DOM by natural bacterial

population. The question we asked was whether there were differences in the chemical and biochemical composition of various polymeric fractions of the DOC pool, and whether these differences influenced their utilization by natural bacterial populations.

MATERIAL AND METHODS

Water sample collection 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 the Dona Paula Bay (15° 27' N, 73° 48' E), west coast of India. Filtration of water sample and size fractionation of DOM were carried out following the procedure described earlier (Fernandes et al. 2007, Khodse et al. 2008). Briefly, water sample was filtered through 200 µm nylon screen to remove large zooplankton, and then filtered through pre-ashed GF/F filter (47 mm, 0.7 µm, Whatman) followed by polycarbonate filter (47 mm, 0.22 µm, Nucleopore). Various steps involved in size fractionation of the DOM are shown in Fig. 1. The sample was filtered through an 30 kDa MW cut-off cellulose membrane ultra-filter (#YM-30) using a Amicon Ultra-filtration system equipped with a stirred cell until 40 ml remained. Concentrated sample retained by the 30 kDa ultra-filter was defined as the high molecular weight (HMW, >30 kDa to 0.22 µm) fraction of DOM. Filtrate from the 30 kDa ultra-filter was then filtered through 10 kDa MW cut-off ultra-filter (#YM-10) until 40 ml remained. Concentrated sample retained by the 10 kDa ultra-filter was defined as the low molecular weight (LMW, >10 kDa to 30 kDa) fraction of DOM. Both fractions were desalted using the stirred cell and UV-Milli-Q water that was 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 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, and 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 MgSO₄, 0.019 NH₄Cl, 0.2 CaCl₂, 0.70 K₂HPO₄, 0.3 KH₂PO₄, and 1 ml trace metal solution, pH 7.5 (Jain & Bhosle 2009) was used as a growth medium to assess degradation of different size fractions of DOM. Three flasks, each containing 400 ml of the BSS medium were sterilized at 120 °C and 15 lb pressure for 10 min. To one of the flasks, 40 ml of the HMW DOM (340 µM C final concentration), to another 40 ml LMW DOM (261 µM C final concentration), and to the third flask 40 ml of 0.22 µm filter sterilized BSS (34 µM C) was added and was treated as a control.

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

Utilization of the HMW and LMW DOM size fractions. A control flask and flasks with HMW and LMW DOM prepared as above were inoculated with 5 % of the natural bacterial inoculum prepared as above. Flasks were incubated in 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 then analysed for bacterial abundance (BA), bacterial production (BP), DOC, $\delta^{13}\text{C}$, 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 and Strom, 1993). Potassium hydrogen phthalate was used as a standard (50 μM C to 400 μM C). Eight samples were analysed between injection of UV-Milli-Q water and working standard solution. Analytical variation of this method was ± 1.8 %. $\delta^{13}\text{C}_{\text{oc}}$ composition of 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 $\delta^{13}\text{C}_{\text{oc}}$ was 0.2 ‰. All isotopic compositions are reported as, per-mil (‰) relative to variation (δ) from the PDB standard.

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

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, Nucleopore) water sample was mixed in a test tube with 1 ml of potassium ferricyanide (0.7 mM) and kept in 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 taken at 595 nm. In order to determine TDCHO, water sample (5 ml) was evaporated to dryness at 40 °C using a rotary vacuum evaporator. Dried sample was treated with

12 M H₂SO₄ at room temperature for 2 h. Sample was diluted to 1.2 M using UV-Milli-Q water, transferred to a glass ampoule, flushed with nitrogen gas, and sealed. 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, and 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 the DAPI (4,6 diamidino- 2 phenylindole) staining method (Porter & Feig 1980). Briefly, a known volume of seawater (2 to 5 ml) was stained with DAPI (final concentration 0.01%) for 5 min, and filtered onto 0.22 μm black Nuclepore polycarbonate filter. Bacterial cells were counted in at least 25 randomly selected fields using 100X objective, and an epifluorescence microscope (Nikon). 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 and assuming a bacterial carbon content of 15 fg C cell⁻¹ (Caron et al. 1995).

Bacterial production was estimated from the incorporation rate of nucleoside ³H-thymidine into bacterial DNA (Fuhrman & Azam 1982) as described by Bhaskar & Bhosle (2008). In brief, 10 ml sample was added into 20 ml scintillation vial. To this, 30 μl (15 nM) of the working solution of thymidine (Specific activity = 12000 mCi/mole, Board of Radiation and Isotope Technology, Mumbai) was added. Similarly, one set of the sample was fixed with filtered formalin (4 % final concentration) before the addition of ³H-thymidine and treated as control. Both sample and the control vials were incubated in dark for 60 min at room temperature. At the end of the incubation period ³H-thymidine uptake was terminated with the addition of filtered formalin (4 % final concentration). The sample was filtered at low pressure through 0.22 μm cellulose nitrate (Millipore company) filter. The filter paper was then rinsed two times with 5 % ice-cold trichloroacetic acid (TCA), followed by three rinses of ice-cold ethanol. After complete dryness, the filter was transferred to a clean scintillation vial and completely dissolved in 2 ml ethyl acetate. Four ml of

scintillation cocktail were 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 the conversion factor (CF) of 2×10^{18} cells per mole of thymidine utilized (Riemann et al. 1987) and bacterial carbon conversion factor of 15 fg C per bacterial cell was used (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

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

RESULTS

Organic matter processing

Bacterial abundance 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 (>30 kDa to 0.22 μM), LMW-DOM (>10 kDa to 30 kDa), 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 ultra filtration technique (Table 1). The observed recovery of the TDCHO compares well with those (68 to 87 %, average = 80 %) reported earlier (Khodse et al. 2008). This means contamination or losses were minor during the ultra-filtration process.

Chemical characteristics of DOM size fractions

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

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

DOC concentrations decreased from 340 to 296 $\mu\text{M C}$, and from 261 to 171 $\mu\text{M C}$ in HMW and LMW DOM fractions, respectively over the period of incubation (Table 2). Bacterial utilization of HMW DOC varied from 3.5 to 12.9 % (average 9.6 ± 3.9 %) (Table 3). Similarly, bacterial utilization of LMW DOC varied from 15.3 to 34.5 % (average 26.1 ± 7.9 %) (Table 3). DOC utilization rates were higher for the LMW DOM than HMW DOM (Table 4). $\delta^{13}\text{C}$ values of HMW DOM did not vary much over the period of incubation (Table 2). In contrast, $\delta^{13}\text{C}$ values of LMW DOM decreased from -21.7 ‰ at d 0 to -23.7 ‰ at d 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 $\mu\text{M C}$ at d 0 to 27.7 $\mu\text{M C}$ at d 15, whereas the LMW-TDCHO decreased from 65.6 $\mu\text{M C}$ at day 0 to 15.8 $\mu\text{M C}$ at d 15 following inoculation (Table 2). Bacterial utilization of TDCHO of HMW DOM and 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 (ANOVA, $p < 0.01$) for the LMW DOM than the TDCHO of the HMW DOM (Table 4). PCHO concentrations in both fractions showed significant differences (ANOVA, $p < 0.01$). PCHOs of both LMW and HMW DOM were degraded by bacteria, and MCHOs were produced (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 at d 8 and at d 10 for the LMW and HMW DOM fractions, respectively following inoculation (Table 2). MCHO/TDCHO ratio increased with the incubation period and was higher in LMW fraction than HMW fraction (Fig. 2c).

Utilization of HMW and LMW DURA fractions

DURA concentrations in 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 $\mu\text{M C}$ in HMW and from 19.4 to 12.4 $\mu\text{M C}$ in LMW DOM. Bacterial degradation of DURA of HMW DOM and 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 rates for DURA were higher for LMW DOM than HMW DOM with the exception of one value (Table 4). DURA/TDCHO ratio increased with degradation of DOM and the ratio was relatively higher in

LMW fraction than HMW fraction implying preferential removal of TDCHO of LMW DOM (Fig. 2d). BA showed significant positive correlations with DURA/TDCHO ratio of LMW ($r = 0.949$, $p < 0.001$) and HMW ($r = 0.798$, $p < 0.02$) DOM fractions.

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 LMW-DOM than in HMW-DOM (Fig. 3a, b). When grown on HMW-DOM, BA gradually increased from 1.6×10^{10} cells L^{-1} at d 0 to 2.2×10^{10} cells L^{-1} at d 4 and then remained nearly same over the remaining period of incubation (Fig. 3a). For LMW-DOM, BA increased from 1.7×10^{10} cells L^{-1} at d 0 to 3.1×10^{10} cells L^{-1} at d 8 following inoculation, 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 LMW-DOM than 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 LMW DOM when DOC was used to calculate BGE (Table 3).

DISCUSSION

Source and bioreactivity of organic matter

Both LMW and HMW DOM fractions were utilized by natural bacterial populations. Bacteria utilized 35 % of LMW DOC, and 13 % of HMW DOC over the period of incubation. Considering the uncertainties in the DOC analysis, DOC loss can vary from 6.4 to 15.2 % (average 11.4 ± 3.2 %), and 0.3 to 13.8 % (average 7.6 ± 9.3 %) for the HMW DOC fraction. Similarly, DOC loss 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, it was evident that the LMW DOM was more bio-reactive, and was preferentially used by bacteria in this set of experiments. The observed differences in the relative bioreactivity of LMW DOM and HMW DOM may be related to its source, composition, and TDCHO content.

$\delta^{13}C$ values varied from -27.0 to -26.2 ‰, and from -23.7 to -21.7 ‰ for HMW and LMW DOM size fractions, respectively (Table 2). $\delta^{13}C$ values for DOM derived from terrestrial sources or

C3 plants varied from -28 to -26 ‰ (Raymond and Bauer 2001, Cai et al. 2008). These values are similar to those observed for HMW DOM of the present study, suggesting that it was derived from terrestrial sources. In contrast, $\delta^{13}\text{C}$ values of LMW DOM (-23.7 to -21.7 ‰) are in the range of values reported for marine plankton (Raymond and Bauer 2001, McCallister et al. 2004, 2006). Similarity in $\delta^{13}\text{C}$ values of LMW DOM and marine plankton suggest that it was derived from marine plankton. $\delta^{13}\text{C}$ values of LMW DOM decreased from -21.7 at d 0 to -23.7 at d 8 of the incubation period (Table 2). DOM is composed of heterogeneous mixture of organic compounds having different $\delta^{13}\text{C}$ values. Organic compounds such as polysaccharides and proteins are enriched in $\delta^{13}\text{C}$ than lipids (Marchand et al. 2005, Teece et al. 2007). Selective removal of PCHO may account for the observed depletion of $\delta^{13}\text{C}$ of the LMW DOM. This appears to be the case as 31 μM C of PCHOs were utilised by bacteria during the first 2 d period of incubation. Subsequently, enrichment in $\delta^{13}\text{C}$ at days 10 and 15 following inoculation was observed. Small changes in carbohydrates concentration during 8 to 15 d period of incubation, however strongly argue against their involvement in the enrichment of $\delta^{13}\text{C}$ values at days 10 and 15 following inoculation (Table 2). With data at hand, it is not possible to identify the factors involved in the enrichment of $\delta^{13}\text{C}$ at days 10 and 15 following inoculation.

The ratio TDCHO-C/DOC is a useful proxy to assess 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. TDCHO contribution to DOC decreased rapidly for LMW DOM (from 25 to 9 %), and slowly for the HMW DOM (16 to 9 %) over the period of incubation (Fig. 2a). The hypothesis that the HMW DOM is more terrestrial and therefore less labile was indicated by smaller changes in TDCHO. However, non-TDCHO-carbon (non-TDCHO-C = DOC - TDCHO-C) of both HMW DOC and LMW DOC was also utilised by bacteria, and the utilization was higher for the former (~45 to 98 %) than the latter (22 to 45 %).

Our results indicate that TDCHO's 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 (Burney

1986, Cherrier & Bauer 2004) and mesotrophic lake waters (Hanisch et al. 1996). This is also supported by higher MCHO/TDCHO ratio in LMW as compared to HMW DOM. The TDCHO utilization rates for the LMW DOM varied from 1.63 to 6.75 $\mu\text{g C l}^{-1} \text{h}^{-1}$ and were much higher than those recorded for HMW DOM (0.05 to 1.13 $\mu\text{g C l}^{-1} \text{h}^{-1}$). Our TDCHO utilization rates of LMW DOM also are higher than those recorded for other environments. For example, Burney (1986) reported that TDCHO utilization rate was 1.50 $\mu\text{g C l}^{-1} \text{h}^{-1}$ for the waters of the Atlantic. Jorgensen & Jensen (1994) observed a use rate of TDCHO of 2.75 $\mu\text{g C l}^{-1} \text{h}^{-1}$ for a mesotrophic Danish lake. TDCHO utilization rates varied seasonally (0.47 to 3.43 $\mu\text{g C l}^{-1} \text{h}^{-1}$) for mesotrophic lake Constance (Hanisch et al. 1996). Studies on the hydrolysis of polysaccharides using fluorogenic substrate analogs for glucosidase activity also indicate that TDCHOs are important substrate for growth of planktonic bacteria. Polysaccharides need to be hydrolysed to oligo- and monosaccharides before bacterial uptake. Chrost et al. (1989) observed good correlation between the concentrations of TDCHO and the activity of β -glucosidase during phytoplankton bloom in eutrophic lake Plußsee.

DURAs are acidic polysaccharides and generally account for a small proportion of organic carbon. DURA concentration in both HMW and LMW fractions decreased over the period of incubation implying their utilization by heterotrophic bacteria. DURA concentration decreased with increasing sediment core depth in the Dabob Bay (Bergamaschi et al. 1999), and with increasing water column depth in the Bay of Bengal (Khodse et al. 2007). In both the studies, the decrease in DURA concentration was suggested to be due to heterotrophic bacterial uptake. The utilization of DURA was greater for LMW DOM than HMW DOM implying that the former was utilized faster (Table 3, 4). The increase in DURA/TDCHO ratio for HMW (0.46 to 0.76) and LMW (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 BA, and BP in LMW DOM than HMW DOM imply that the former DOM fraction was more bioreactive and preferentially utilized by natural marine bacterial population (Fig. 3a, b). The $\delta^{13}\text{C}$ signature suggests that the LMW DOM was originated from marine phytoplankton, whereas HMW DOM was of terrestrial origin. The higher BA and BP in LMW DOM than HMW DOM suggest that bacteria preferentially assimilated an isotopically enriched $\delta^{13}\text{C}$ organic component derived from $\delta^{13}\text{C}$ 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 and HMW DOM fractions may also result from differences in the heterotrophic bacterial species or groups developed 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 and Cole (1998) numerous factors can influence BGE. Greater BGE in HMW DOM than in LMW DOM suggests that bioreactivity and source of DOM did not influence BGE in the present study. Other factors including temperature, salinity, pressure, nutrient concentrations may not have influenced BGE because they were nearly similar for both LMW DOM and HMW DOM experiments. Presumably, differences in physiological condition of cells and taxonomic composition of bacteria in LMW DOM and HMW DOM may be responsible for the higher BGE in HMW DOM than in LMW DOM (del Giorgio and Cole, 1998).

Conclusion

Bacterial utilization of HMW DOM (>30 kDa to 0.22 μm) and LMW DOM (>10 kDa to 30 kDa) isolated from Dona Paula Bay, west coast of India was measured. $\delta^{13}\text{C}$ values suggest that HMW DOM fraction was mostly derived from terrestrial sources, whereas LMW DOM fraction was originated from marine phytoplankton. Concentrations of TDCHO and PCHO showed that PCHO served as active substrate. Non-TDCHO-carbon of HMW DOM (45 to 98 %) and LMW DOM (22 to 45 %) also was utilised by bacteria. Bacteria preferentially utilized DOC, TDCHO and DURA of the LMW DOM as compared to DOC, TDCHO and DURA of the HMW DOM. This conclusion was well supported by higher BA, BP, and greater decrease in DOC, TDCHO and DURA in LMW DOM. Our results showed that bacterial consumption of polymeric fractions of DOM of the Dona Paula Bay was strongly affected by the source and bioreactivity of DOM.

Acknowledgements. The authors are grateful to Dr. Satish R. Shetye, the Director National Institute of Oceanography for encouragement and facilities. The first author is grateful to CSIR, New Delhi for providing senior research fellowship to take up this research work. We also thank Dr. S.G.P. Matondkar for bacterial production measurements. 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, Pujol-Pay M, Oriol L, Sempere R, Charriere B, Joux F (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 sedimentary 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, Douglas TA (2008) Temporal variations in organic carbon species and fluxes from the Chena River, Alaska. *Limnol Oceanogr* 53:1408-1419
- Carlson CA, Giovannoni SJ, 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 northwestern Sargasso Sea. *Limnol Oceanogr* 49:1073-1083
- Caron DA, Dam HG, Kremer P, Lessard EJ, Madin LP, Malone TC, Napp JM, Peele ER, Roman MR, Youngbluth MJ (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
- Chorost 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
- Filisetti-Cozzi TMCC, Carpita NC (1991) Measurement of uronic acids without interference from neutral sugars. *Anal Biochem* 197:157-162
- 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 Northwestern Sargasso Sea. *Deep-Sea Res I* 56:672-685
- 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
- Gueguen C, Guo DL, Wang D, Tanaka N, Hung C-C (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 C-C, Santschi PH (2001) Spectrophotometric determination of total uronic acids in seawater using cation exchange separation and pre-concentration lyophilization. *Anal Chimica Acta* 427:111-117
- Hung C-C, 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 C-C, 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
- Jorgensen NOG, Jensen RE (1994) Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by PAD-HPLC. *FEMS Microb 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 Microbol* 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
- Meon B, Kirchman DL (2001) Dynamics and molecular composition of dissolved organic material during experimental phytoplankton blooms. *Mar Chem* 75:185-199
- 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 ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{14}\text{C}$) 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
- 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 Pollut 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, Bjoernsen PK, Newell S, Fallon R (1987) Calculation of cell production of coastal marine bacteria based on measured incorporation of ^3H thymidine. *Limnol Oceanogr* 32:471-476
- Rosenstock R, 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

Table 1. Dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), dissolved uronic acids (DURA), dissolved monosaccharides (MCHO) and dissolved polysaccharides (PCHO) and bacterial abundance in the surface seawater of the Dona Paula Bay; and recovery of DOC, TDCHO and DURA after ultrafiltration and dialysis.

Sea water composition	Before size fraction $\mu\text{M C}$	After size fractionation			Total (sum) after size fractionation $\mu\text{M C}$	Recovery %
		$\mu\text{M C}$ Fractions				
		>30 kDa to 0.22 μm	>10 kDa to 30 kDa	<10 kDa		
DOC	271 \pm 12	21.1 \pm 0.6	16.4 \pm 0.6	172.0 \pm 10.6	210.0	78
TDCHO	45 \pm 3	4.1 \pm 0.6	6.2 \pm 0.2	25.1 \pm 1.7	35.4	79
DURA	18 \pm 3	2.5 \pm 0.1	2.4 \pm 0.2	10.7 \pm 0.4	15.6	85
MCHO	7 \pm 1	-	-	6.6 \pm 0.8	-	-
PCHO ^a	37 \pm 2	-	-	18.5 \pm 0.9	-	-
Bacteria ($\times 10^7$ cells/ml)	6.9	-	-	-	-	-

a = TDCHO - MCHO; - = no data.

Table 2. Changes in dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), free monosaccharides (MCHO), polysaccharides (PCHO), dissolved uronic acids (DURA) and carbon isotopes ($\delta^{13}\text{C}$) of HMW and LMW fractions over the incubation period.

Incubation period (d)	DOC $\mu\text{M C}$			TDCHO $\mu\text{M C}$			MCHO $\mu\text{M C}$			PCHO $\mu\text{M C}$			DURA $\mu\text{M C}$			$\delta^{13}\text{C}$ (‰)		
	Cont	HMW	LMW	Cont	HMW	LMW	Cont	HMW	LMW	Cont	HMW	LMW	Cont	HMW	LMW	Cont	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

Cont = Control, - = not detected

Table 3. The range and average utilization (as % of initial concentration) of dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO), dissolved uronic acids (DURA) of HMW and LMW DOM fractions by natural bacterial population and bacterial growth efficiency (BGE) on HMW and LMW DOM fractions.

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 – 18.8	5.7	11.3	0.5 -36.1	17.6	14.3
BGE ^a %	4.3 – 9.1	5.7	1.9	1.2 – 6.8	4.3	2.5

BGE^a = BP/DOC × 100. BGE was calculated from the DOC data given in Table 2.

Table 4. Utilization rates ($\mu\text{g C l}^{-1} \text{ h}^{-1}$) of dissolved organic carbon (DOC), total dissolved carbohydrate (TDCHO) and dissolved uronic acids (DURA) of HMW and 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*	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

*= DURA production

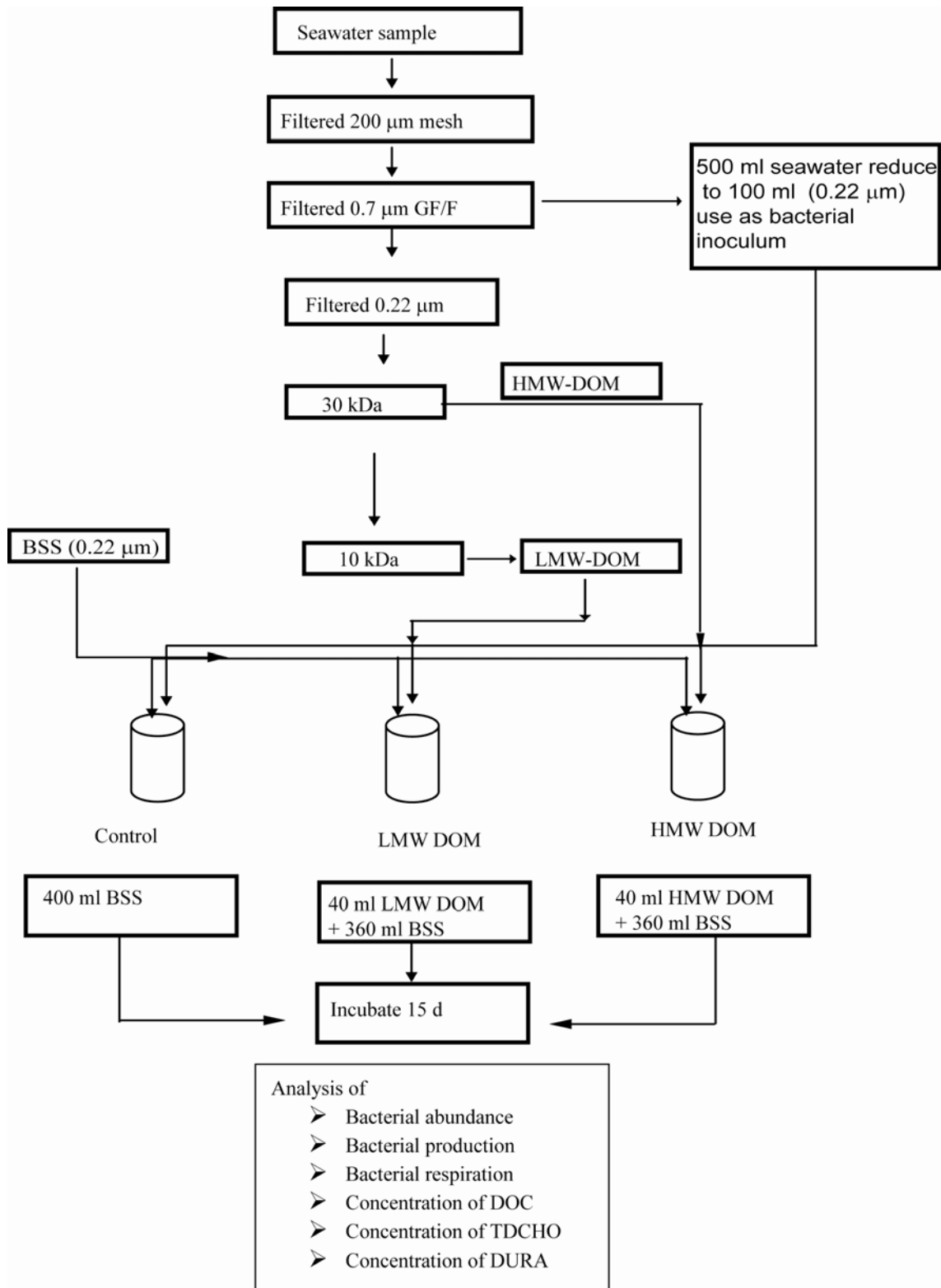


Fig. 1. Protocol for DOM size fractions and its degradation by bacteria

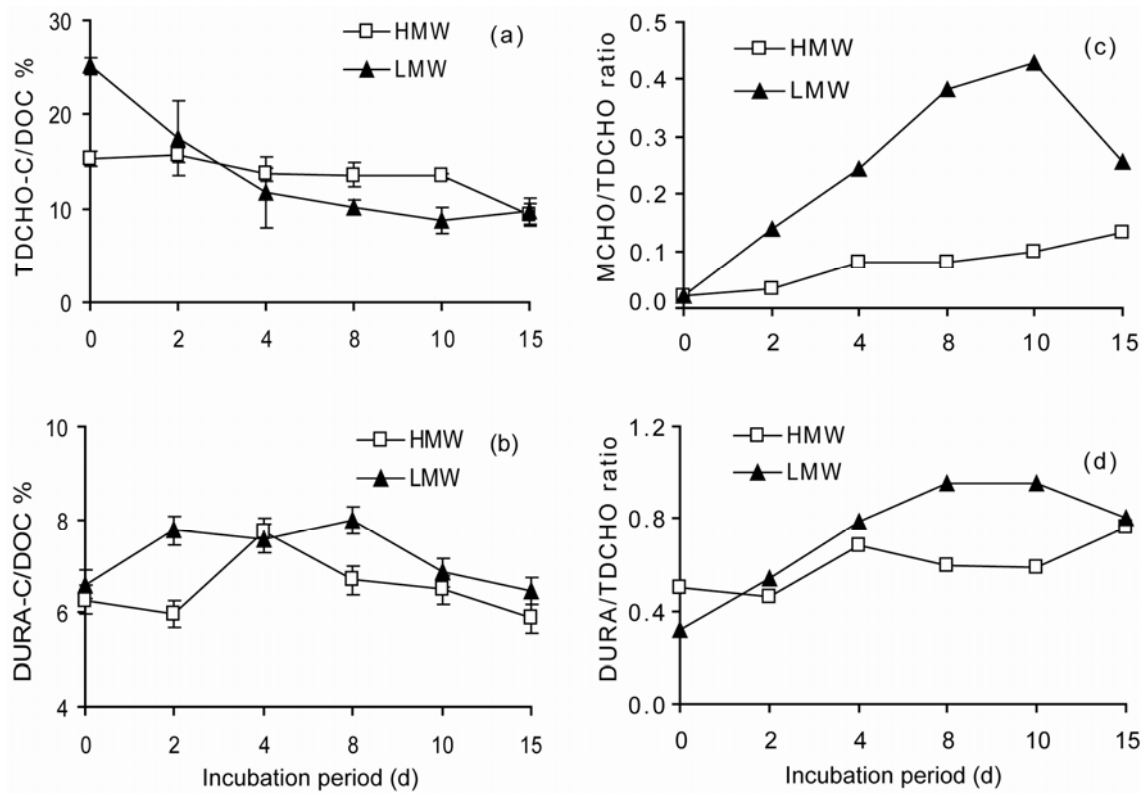


Fig. 2. Changes in % TDCHO-C/DOC (a), % DURA-C/DOC (b), MCHO/TDCHO ratio (c), and DURA/TDCHO ratio (d) during the bacterial degradation of HMW and LMW DOM. TDCHO: total dissolved carbohydrate; DURA: dissolved uronic acid; MCHO: free monosaccharide; DOC: dissolved organic carbon

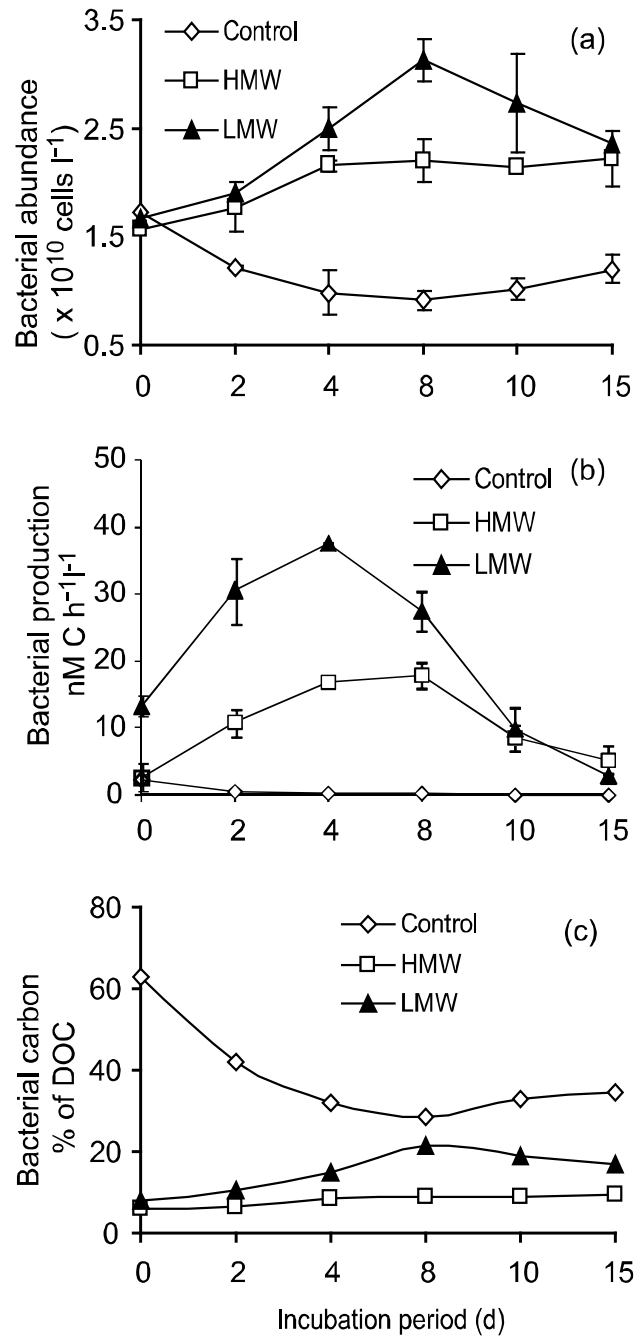


Fig. 3. Changes in bacterial abundance (a), bacterial production (b), and changes in bacterial carbon as a % of DOC (c) during HMW and LMW DOM degradation by natural bacterial population