Bioresource Technology 100 (2009) 5721–5728

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The application of a plug-flow reactor to measure the biodegradable dissolved organic carbon (BDOC) in seawater

Cinzia De Vittor [a,*], Chiara Larato [a], Serena Fonda Umani [b]

[a] Dipartimento di Oceanografia Biologica, Istituto Nazionale di Oceanografia e Geofisica Sperimentale, Via A. Piccard 54, 34014 Trieste, Italy
[b] Dipartimento di Scienze della Vita, Università di Trieste, Via Valerio 28/1, 34127 Trieste, Italy

ARTICLE INFO

Article history:
Received 5 February 2009
Received in revised form 16 June 2009
Accepted 17 June 2009
Available online 23 July 2009

Keywords:
DOC
BDOC
Plug-flow bioreactor
Coastal seawater

ABSTRACT

Most of the ambient dissolved organic carbon (DOC) is refractory to microbial degradation; bacteria can consume a minor but variable part of the DOC pool over periods of hours and days. It is important to increase our knowledge of the dynamics of the biodegradable fraction of DOC (BDOC) to understand the global carbon budget.

Several methods for determining BDOC have been developed; however, the problem of most of them is the time (days/weeks) required for the colonization and/or determination.

In this paper, we describe the application to seawater of a plug-flow bioreactor to measure BDOC within 3–4 h. The bioreactor was built following Søndergaard and Worm [Søndergaard, M., Worm, J., 2001. Measurement of biodegradable dissolved organic carbon (BDOC) in lake water with a bioreactor. Water Res. 35, 2505-2513.] protocols for the measurement of BDOC in lake water. We analyzed BDOC on samples collected in the Gulf of Trieste during autumn–winter and summer 2003–2004. BDOC concentrations varied from 8 to 24 μM and represented from 10.3% to 25.5% of the total DOC. To evaluate the effectiveness of this method, we compared bioreactor BDOC measurement with data obtained from batch cultures. The results indicate that BDOC in coastal seawater can be measured rapidly and reliably with this bioreactor.

1. Introduction

The biodegradability of dissolved organic carbon (DOC) relies on both its molecular and chemical composition and the enzymatic activity of bacteria (e.g. Becquevort et al., 2002). The interactions between bacteria and DOC play a major role in the global carbon cycle (Amon et al., 2001). Numerous studies showed that bacterial production is carbon limited, even though DOC concentrations are high throughout the water column (Carlson and Ducklow, 1995; Kirchman, 1990). DOC is currently divided into three pools defined by biodegradability: labile (turnover time of days or less), semilabile (turnover time of seasonal time) and refractory (turnover time of centuries or more) (see e.g. review of Kirchman et al., 1993). The latter seems to be the result of microbial activity, particularly of non-specific or promiscuous enzymatic activities that occasionally produce fragments from macromolecules that escape recognition by bacterial enzymes (Ogawa et al., 2001). Production of organic carbon by phytoplankton is the most important source of DOC in marine environments. The average amount of carbon fixed photosynthetically in the world's oceans (excluding adjacent seas) has been estimated at between 130 gC m^2 y^{-1} and 500 gC m^2 y^{-1} with a steady-state input of DOC about 0.0067–0.25% of the photosynthetically fixed carbon (e.g. Dafner and Wangersky, 2002).

Although bacterial nutrient limitation could lead to accumulation of otherwise biodegradable DOC (BDOC), it is the resistance to microbial attack, i.e. semilabile nature that controls the seasonal and episodic accumulation of DOC in the marine system (Søndergaard et al., 2000). The large reservoir of DOC that accumulates in seawater is therefore believed to be largely unavailable to meet bacterial carbon demand (Repeta and Aluwihare, 2006). Consequently, in order to estimate the potentially accumulating DOC, it is of great importance to achieve a rapid technique for the measurement of BDOC and indirectly for the evaluation of refractory DOC. Although numerous investigations have reported on the quantity and composition of DOC in marine environment, fewer studies have addressed the biodegradable fraction (Volk et al., 1997). The problems with most of the several methods proposed for BDOC determination is the length of time (from days to weeks) required for the start up (colonization) and/or determination (Khan et al., 2003). The majority of BDOC measurements have been carried out in batch cultures with an incubation period of 10–30 days (Malej et al., 2003; Ogawa et al., 2001; Puddu et al., 2003; Søndergaard et al., 2000). Faster procedures have been developed by Lucena et al. (1990) and Frías et al. (1992) with the introduction of a bioreactor, which was further improved by Kaplan and

* Corresponding author. Tel.: +39 040 2249723; fax: +39 040 2249770.
E-mail address: cdevittor@ogs.trieste.it (C. De Vittor).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.06.056

Newbold (1995) who used a plug flow bioreactor for the measurement of BDOC in stream water. Søndergaard and Worm (2001) reported that BDOC in lakes and other fresh waters could be measured quickly and reliably with a bioreactor. An innovative procedure for BDOC determination was then proposed by Khan et al. (2003), who used entrapped microbial cells in order to reduce the time required for the colonization of the bioreactor.

Among the several proposed techniques for BDOC analysis, we chose to set up a bioreactor similar to that of Søndergaard and Worm (2001) usable with seawater samples. After colonization, in fact, this kind of reactor is easy to maintain functional for continuous monitoring of BDOC fluctuations.

The objective of our research was to apply, to seawater, a reliable and fast method to assess the BDOC.

In this paper we describe the performances of a bioreactor colonized and maintained with seawater and present a first account of the BDOC measured in coastal seawater by a plug-flow bioreactor. Comparison with three batch experiments was also carried out to test the performances of the bioreactor.

2. Methods

2.1. Overview

The seawater used in all the experiments described was collected at different times from a coastal station (C1 – 45°42'03"N, 13°42'36"E) in the Gulf of Trieste (Northern Adriatic Sea). The main interest of the bioreactor method is its application to seawater samples characterized by variable concentrations of DOC and BDOC. Consequently, different sets of experiments were carried out. The accuracy, precision and response of the bioreactor to different DOC and BDOC concentrations were evaluated measuring replicate seawater samples during winter and summer. The investigated area, in fact, is characterized by a clear seasonality in DOC concentration (ranging between 50 and 194 μM) even though there are important fluctuations also on a short time scale (De Vittor et al., 2008). DOC accumulated over spring - summer totally disappeared from the water column in winter, suggesting that the Gulf of Trieste as the rest of the Northern Adriatic is able to bring back DOC concentrations at low levels despite the significant external and internal organic matter loads (De Vittor et al., 2008).

The response of the bioreactor to a rapid increase in BDOC concentration was tested with the addition of glucose to inlet seawater from station C1.

Other analyses were carried out on samples obtained from a mesocosm experiment in which a phytoplankton bloom was induced and high concentrations of labile DOC obtained.

In order to test the system-specificity and capacity of the reactor, the results obtained by three batch cultures characterized by different initial DOC concentrations were compared with the results from the bioreactor. In one of them, during each sampling for DOC analysis, the BDOC from the bioreactor was contextually measured with the aim to verify DOC versus BDOC relationship during the incubation time.

2.2. Sample collection and preparation

Field water samples for bioreactor BDOC analyses were collected at the surface from a coastal station (C1 – 45°42'03"N, 13°42'36"E) in the Gulf of Trieste (Northern Adriatic Sea) on November 10th, 11th, 18th, 20th, December 5th (2003), January 20th, February 11th and 25th, June 14th and 16th, July 1st and 2nd (2004).

During July–August 2003, a mesocosm experiment was set up to induce a phytoplankton bloom, with consequent labile DOC release, in order to test bioreactor performance in the presence of a high quantity of BDOC. Eighty litres of seawater were collected at station C1 and pre-screened through a 200-μm mesh to remove microplankton grazers. Pre-screened water of three 20 L traslucent polycarbonate bottles (Nalgene) was amended with $NaNO_3$ (final concentration 50 μM), $Na_2SiO_3 \cdot 9H_2O$ (50 μM), $FeCl_3$ (9.5 μM), $NaH_2PO_4 \cdot H_2O$, (4.5 μM) and vitamins (like in f/2 medium (Guillard and Ryther, 1962)). Each replicate was enriched with phytoplankton organisms collected at 10 m depth at the same station. The mesocosm bottles were incubated in field at the surface for 9 days, a phytoplankton bloom was observed and replicate samples were collected for bioreactor BDOC measurements.

2.3. BDOC analysis

2.3.1. Bioreactor method (setup, inoculation, and application)

The bioreactor was built following the recommendations of Søndergaard and Worm (2001) for the measurement of BDOC in lake water. The authors propose the use of chromatographic glass columns of 140 and 210 mL with the purpose of reducing the volume of the water sample needed for BDOC analysis. Previously, similar bioreactors were used for BDOC investigation in drinking water (Frías et al., 1995 Ribas et al., 1995) or in stream water (Kaplan and Newbold, 1995; Volk et al., 1997), but as far as we know, there is no data on the application of a plug-flow bioreactor for BDOC measurements to seawater. These bioreactors were filled with sintered porous glass beads (SIRAN Schott Glasswerke, Mainz, Germany), as inert support, to allow for the rapid colonization of bacteria.

Our bioreactor (Fig. 1) consists of: an Amersham Pharmacia Biotech modular system, an inert support of open-pored sintered glass spheres (SIRAN™ microcarriers – Jaeger Biotech Engineering

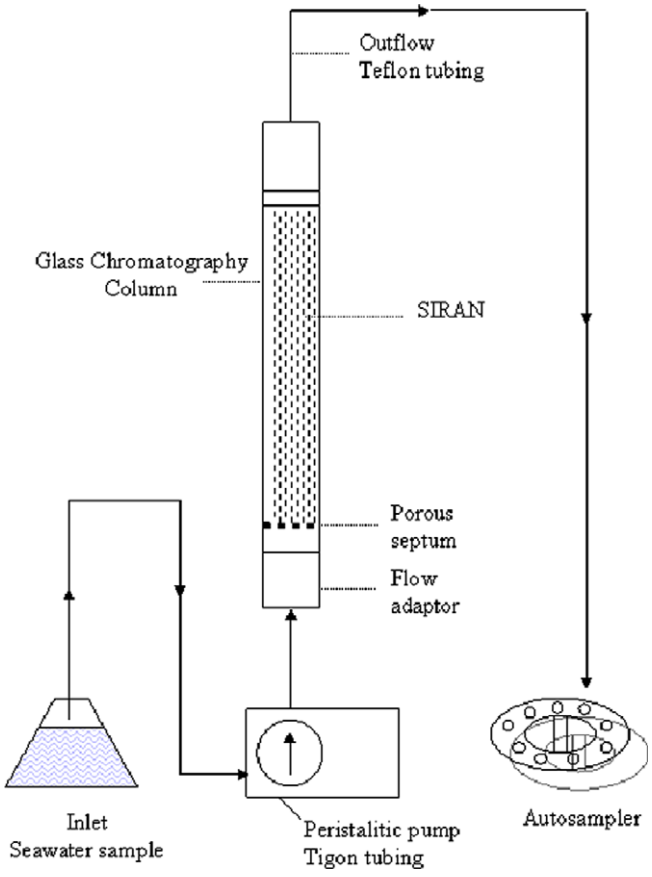

Fig. 1. Scheme of the "mature" bioreactor for BDOC determination.

Inc., 1–2 mm diameter, 60–300 μm pore dia.) which have a large surface area to volume ratio (90 m^2/L) and a programmable peristaltic pump (Autoclude – VELP SCIENTIFICA –VTL model) with a variable flow rate. The modular system is made up of a glass darkened chromatographic column (C26/40 – i.d. 26 mm, length 40 cm) and by a flow adaptor (AC26), which allows optimal bed heights for each application. With the adaptor, column volume can be changed from 122 to 202 mL. C-column inlet is set at the bottom and allows a homogeneous flux on the entire column section. The column was filled with acid-washed, muffled (4 h, 480 °C) glass spheres and connected to the pump with Teflon tubing, and the pump operated with a Tygon tube in the "push mode". An auto sampler was set up to collect samples of adequate volume (generally 10 mL) at the outlet.

The residence time of the bioreactor, with a flow rate of 1 ± 0.1 mL min^{-1}, was determined measuring the variation of conductivity in the outlet after having added to the inlet a solution of NaCl. Conductivity was measured by a conductometer WTW (model 191T).

Judging from the stabilisation of DOC utilization, microbial colonization was carried out in 32 days at a flow rate of 1 ± 0.1 mL min^{-1} with seawater from the station C1 filtered across 1 μm (Millipore FA, Fluoropore™ PTFE) membrane.

As reported by Søndergaard and Worm (2001), the longitudinal resource gradient and density of colonization was clearly visible with dense brown colouration at the inlet approaching the original Siran colour at the outlet. The maintenance of the bioreactor was achieved with the same water collected weekly and stored in acid-washed glass tanks (20 L) at sampling temperature (±2 °C).

For BDOC analyses, the bioreactor operated at room temperature, except during comparison with batch cultures, when the temperature was kept close (±2 °C) to that of the batches.

BDOC measurements were carried out as reported by Søndergaard and Worm (2001) obtaining the same level of detection. BDOC concentrations were calculated as differences of DOC concentration at the inlet and the outlet (refractory DOC – RDOC) of the bioreactor. In all the measurements described, the DOC was analyzed at the bioreactor inlet (four replicate samples) and in the water collected (after 2 h) at the outlet every 10 min to obtain 10 mL samples (20 replicate samples) for a total of 24 samples for each measurement.

To test the accuracy of BDOC bioreactor determination, replicate samples were collected at station C1 at different times and repeatedly analyzed (every 4 h) as described above.

Samples for DOC analyses were filtered through precombuste (4 h at 480 °C) and acidified (1 N HCl) Whatman GF/F glass fibre filters (0.7 μm nominal pore size). Filtration was performed using a glass syringe and a filter holder in order to prevent atmospheric contamination. The filtered samples were analyzed immediately after collection. Before the analysis, samples were acidified (pH < 2) with 6 N HCl solution and purged for 8 min using high-purity oxygen bubbling (150 mL min^{-1}).

DOC analyses were made with the HTCO (High Temperature Catalytic Oxidation) method using a commercial unit, the Shimadzu TOC 5000A with a quartz combustion column filled with 1.2% Pt on silica pillows with an approximate diameter of 3 mm (Cauwet, 1994). One hundred microlitres of sample were injected into the instrument port. Carbon concentrations were calculated by subtracting the system blanks and dividing by the slope of the calibration curve (Thomas et al., 1995). Standardization was carried out every day using potassium hydrogen phthalate. Each value was determined from a minimum of three injections, with a variation coefficient <2%.

2.3.2. Batch methods

The results of the three batch experiments were compared with those of the bioreactor in order to verify the system-specificity and capacity of the reactor with respect to different BDOC concentrations.

In the first two experiments, the batch cultures were incubated in the dark at 20 °C and samples for DOC analysis were regularly taken until DOC values reached a plateau. In detail, the first experiment was conducted from 5 August to 4 September 2003, the second from 6 to 28 August 2003. The DOC measured on the last day is considered as refractory and the differences between DOC at time zero and refractory DOC gives an estimation of BDOC. The bioreactor BDOC was measured only at time zero.

The third experiment was carried out from 20 January to 11 February 2004. An experimental culture was set up in a Nalgene bottle of 20 L volume, previously washed with HCl (1 N) and rinsed with ultrapure water (Millipore Milli-Q).

Bacterial cells were maintained in 10 L of f/2 medium (Guillard, 1975; Guillard and Ryther, 1962) prepared with artificial seawater at a salinity of 32. The medium was modified from the original recipe by omitting the EDTA in order to avoid additional sources of DOC. Labile organic carbon (glucose-final concentration 4 mg/L) was added to DOC-free artificial seawater before the bacterial enrichment.

Seawater for the bacterial inoculum was collected at the surface at the coastal station C1 and immediately pre-screened through a 200-μm mesh. Twelve litres of natural seawater were filtered through a 1 μm Teflon filter (Millipore) to remove grazers. The filtrate was then passed through several 0.2 Teflon filters (Millipore) to retain bacterial cells. Subsequently, filters were washed in a 400 mL medium and then resuspended in the medium to obtain the final bacteria concentration of 1.4×10^6 cell/mL.

The bottle was incubated in the dark at 14 °C for 21 days and sampled initially (d_0), on January 21st (d_1), on January 23rd (d_3) and on February 3rd (d_{15}) and on February 10th (d_{21}) at the same time (between 12:00 and 13:00) for DOC and BDOC analyses.

2.4. Investigation of method limitations

D(+)-Glucose was assumed as labile compound to verify the bioreactor efficiency. Three different experiments were carried out to evaluate the role of inorganic nutrients in bacterial degradative process in the bioreactor.

In the first experiment, a 37‰ NaCl solution was introduced at the inlet of the bioreactor; in the second one, glucose was added to NaCl solution (final DOC concentration 167 μM). In the third, the same quantity of glucose was added to filtered (Whatman GF/F 0.7 μm) seawater from the Gulf of Trieste (filtered seawater DOC (98 ± 1 μM) + glucose = final DOC concentration 272 μM). In all experiments, replicate DOC analyses were carried out as described.

In order to verify that the decrease in DOC concentration in the bioreactor was predominantly due to microbiological activities rather than absorption, several measurements were carried out before the bioreactor colonization (in abiotic conditions). Moreover, water containing sodium azide (N_3Na), a bacteriostatic agent, at concentration of 400 mg L^{-1}, was added to the mature bioreactor (Ribas et al., 1995) and, the DOC of the outflow was measured in triplicate (one sample every 10 min) after 2, 3, 5 and 7 h from the beginning of the experiment until the differences between inflow and outflow DOC were equal to or lower than 0 μM (Ribas et al., 1995).

3. Results

3.1. Bioreactor performance

With a 1 mL min^{-1} flow rate, the bioreactor hydraulic residence time was of 2 h as revealed by the conductivity values measured at

the outlet of the bioreactor. The conductivity reached a constant value after 2.05 h, which indicates that the hydraulic characteristics of the packed-bed bioreactor are consistent with a plug flow reactor.

The measurements before the bioreactor colonization showed the absence of abiotic DOC removal: no significant differences were detected between inlet and outlet DOC concentrations (CV < 3%).

The results of the addition of sodium azide to the bioreactor are shown in Fig. 2 and are similar to Kaplan and Newbold (1995) findings. DOC concentration at the inlet was 110 ± 0.3 μM and BDOC concentrations before the sodium azide addition reached the value of 30 ± 1.7 μM. Triplicate samples (one every 10 min) collected after 2, 3, 5 and 7 h from the introduction of sodium azide showed a gradual decrease in BDOC concentrations. After 7 h, the reactor did not measure any BDOC, in fact, the DOC at the outlet was slightly higher than at the inlet.

The maintenance of the bioreactor is a function of the characteristics of the water that flows through. Microbial community in the bioreactor cannot maintain itself (even for short periods) without a suitable supply of nutrients essential for its metabolism. For example, the introduction of synthetic seawater without a nutrient supply, leads to an immediate DOC release. This was verified by adding a 35‰ NaCl solution at the inlet of the reactor and continuously monitoring the DOC variations at the outlet until all the solution had passed through and for a further 2 days (every hour) after the reintroduction of filtered (0.1 μm) seawater. After 2 h (first samples) from NaCl solution introduction, DOC concentrations at the bioreactor outlet were higher than at the inlet and constantly increased until all the solution had passed through. The reintroduction of filtered seawater brought about the re-establishment of the original condition after about 1 day.

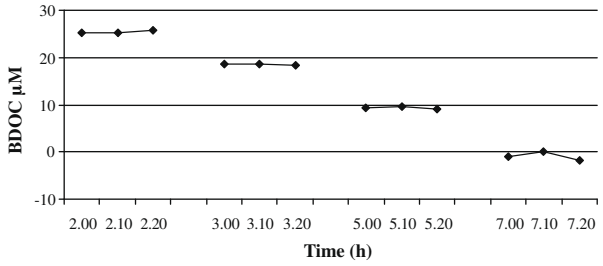

Fig. 2. Evolution of BDOC in the bioreactor after the introduction of sodium azide (initial DOC 110 ± 0.3 μM; initial BDOC 30 ± 1.7 μM).

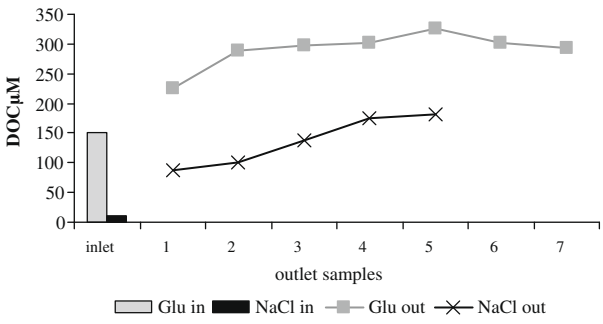

Fig. 3. DOC release in absence of nutrient supply. Histogram: DOC concentration in NaCl and NaCl + glucose solutions at the inlet (NaCl in, Glu in) and outlet (NaCl out, Glu out) of the bioreactor; lines: temporal DOC variation in the outlet samples of the NaCl and NaCl + glucose solutions (NaCl out, Glu out, respectively).

A further experiment, conducted adding D(+)glucose to the NaCl solution (final concentration 152 μM C), yielded analogous results with comparable DOC increment at the outlet (Fig. 3).

The degradative efficiency of the mature bioreactor, tested by adding glucose to filtered (GF/F 0.7 μm) seawater (and therefore in presence of inorganic nutrients) was elevated. The added glucose (final DOC concentration 272 μM) was almost entirely metabolized in the order of 96.6% showing that the reactor is capable of reacting immediately to an increase in BDOC.

The bioreactor residence time (2 h) gives stable BDOC values in about 3–4 h from the introduction of the sample being analyzed (with known initial DOC concentration) at the inlet. Between six and ten DOC samples need to be collected at the outlet to achieve a good BDOC estimation. By analyzing 20 outlet samples (10 mL each = 10 min each) in all BDOC measurements carried out during the present study, we established that after three samples, DOC concentration kept stable (CV < 3%).

The replicate samples of DOC and BDOC showed a dispersion between 1.5% and 3% allowing us to determine differences among samples of 8–12 μM with a confidence level of 95%.

Data obtained from replicate samples, repeatedly analyzed every 3 h, are reported in Table 1 and confirm the repeatability of BDOC bioreactor measurements.

3.2. BDOC variability in coastal seawater samples

Analyses on samples collected in the Gulf of Trieste (station C1) in November and December 2003 and January, February, June and July 2004, showed a significant variation both in BDOC concentrations and in the degradation percentage even when comparable DOC values were measured (Figs. 4 and 5). In detail: between November 10th and 11th DOC concentrations changed from 103 ± 0.2 μM to 106 ± 1 μM, BDOC varied from 11 ± 0.6 μM to 20 ± 2 μM showing a degradation of 10.9% and 19.0%, respectively. DOC concentrations progressively decreased from 98 ± 0.9 μM to 81 ± 1 μM in November 18th and 20th, respectively; in these samples the percentage was comparable (14.9–13.8%) therefore the BDOC proportionally decreased.

In December and January, in spite of the similar DOC concentration (84 ± 0.7 μM and 90 ± 1.7 μM, respectively), BDOC increased,

Table 1
Data obtained from replicate samples, repeatedly analyzed every 3 h. Average and standard deviation among five successive measurements of outlet DOC concentrations for every replicate, and average and CV% among all analyzed outlet DOC samples, for each experimentation, are shown.

Date		DOC in	DOC out	DOC out	DOC out	DOC out
			Replicate 1	Replicate 2	Replicate 3	All data average and CV%
05 December 2003	Average	84	61	60	61	61
	Standard dev.	0.7	1.8	2.1	2.2	2.7%
20 January 2004	Average	90	72	71	73	72
	Standard dev.	2.5	3.0	1.8	0.9	2.9%
16 June 2004	Average	120	82	82	83	82
	Standard dev.	0.1	5.1	4.2	5.1	4.1%
02 July 2004	Average	127	85	87	88	87
	Standard dev.	0.4	0.6	1.8	0.4	0.02

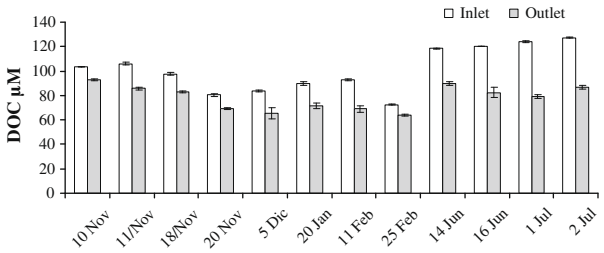

Fig. 4. Concentration of DOC field samples at the inlet and outlet of the bioreactor during winter 2003 and summer 2004.

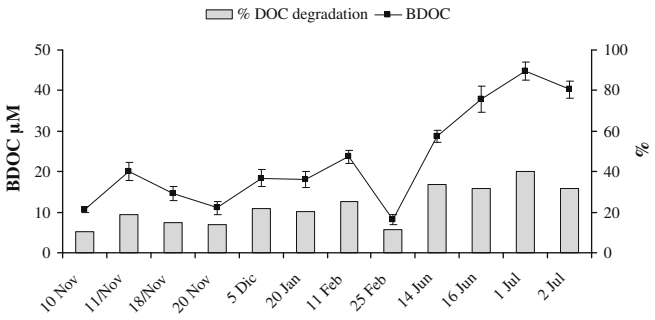

Fig. 5. Percentage of DOC degradation and BDOC values in the field samples during winter 2003 and summer 2004.

reaching a value of 18 ± 2 µM (with DOC degradability percentage of 22.0% and 20.0%, respectively). In February 11th BDOC concentration reached a value of 24 ± 1.6 µM (DOC 93 ± 0.9 µM) with a degradation percentage of 25.5%, while in February 25th the lowest DOC concentration (72 ± 0.5 µM) which corresponds to the minimum BDOC value measured (8 ± 1.3 µM) was detected.

During June and July 2004, BDOC values increased reaching concentrations varying between 29 ± 1.5 µM (DOC 118 ± 0.2 µM) and 45 ± 2.2 µM (124 ± 0.7 µM) with degradation percentage from 24.3% to 36.1%.

Summing up, DOC concentration varied from 72 ± 0.5 µM to 127 ± 0.4 µM, BDOC from 8 ± 1.3 µM to 45 ± 2.2 µM and the percentage of degradation from 10.3% to 36.1%. Significant relationship among concentrations of DOC at the inlet and outlet of the bioreactor (DOC *vs* RDOC; $r = 0.77$, $p < 0.01$) and between DOC and BDOC values (DOC *vs* BDOC; $r = 0.86$, $p < 0.01$) were observed.

3.2.1. Mesocosm experiments

From the mesocosm experiment conducted in July–August 2003, a final average DOC concentration of 211 ± 6 µM (initial DOC concentration was 123 ± 2 µM with a BDOC value of 20 ± 1 µM) was obtained. The value of BDOC was of 72 ± 4 µM with a degradation percentage of 33.94%. As a consequence, the estimated refractory DOC (RDOC = DOC – BDOC) was 140 ± 2 µM, therefore higher than the initial DOC concentration. Reintroduction of resulting sample (RDOC) in the bioreactor showed no further degradation.

3.3. Site-specificity of bioreactor (bioreactor versus batch cultures)

In the first batch experiment (6 August–4 September 2003), DOC values ranged from 91 ± 3 µM to 72 ± 2 µM (data from four replicate samples) with a BDOC value of 18 µM. This value corresponds to a degradation percentage of 20.1%. BDOC concentration, evaluated by the bioreactor at time zero, was of 33 µM, which corresponded to a degradation percentage of 35.9%, i.e. 15.8% higher than BDOC estimated from batch.

In the experiment conducted from 6 August to 28 August 2003, DOC concentrations varied from 229 ± 2 µM to 129 ± 1 µM with a BDOC value of 100 µM which corresponded to a degradation percentage of 43.9%. BDOC from the bioreactor was of 136 µM, with a degradation percentage of 59.2%, i.e. 15.4% higher than BDOC from the batch.

In the 21 day incubation experiment (winter 2004), in which we analyzed BDOC by bioreactor during each sampling for DOC, DOC concentrations, in the batch, varied from 280 ± 1 µM to 115 ± 1 µM. The time courses of DOC mineralization in the batch culture are exemplified by samples collected on days 0, 1, 3, 15 and 21. From day 0 to day 1, no DOC decomposition was detected; after this time, which was probably necessary for bacteria adaptation, DOC degradation was very high until the third day of experimentation. Definitively low DOC consumptions were successively detected (Fig. 6). At the end of the experiment, 58.87% of initial DOC was consumed.

Looking at the cumulative DOC mineralization (BDOC consumed), it appears that the greatest quantity of DOC (137 µM, equivalent to 83% of total mineralized DOC) was consumed within the first 3 days. This fraction probably corresponded to the more labile BDOC fraction, constituted by glucose, while the subsequent decrement, from 3rd to 15th days, was due to mineralization of the less bioavailable semilabile fraction (Søndergaard et al., 2000). However, BDOC pool was dominated by labile fraction as expected as a consequence of glucose addition. A further DOC decrease on the 21st day suggests that perhaps during the incubation time it the plateau had not been reached, that is to say, the BDOC was not quantitatively degraded.

BDOC concentrations, estimated by the bioreactor, were extremely high during the first 2 days of experimentation, with values of 217 ± 3 µM and 212 ± 3 µM at t_0 and after 24 h, respectively. The concentrations drastically decreased from the third day reaching a minimum at the end of the experiment when, however, a BDOC concentration of 30 ± 4 µM was still detected.

Refractory DOC (RDOC), measured at the outlet of the bioreactor, remained almost constant up to day 15 (68 ± 5 µM) then increased by 25.9% at day 21 reaching the value of 85 ± 3 µM.

DOC and BDOC concentrations were positively correlated during the time course of the experiments ($r = 0.996$, $p < 0.001$).

DOC degradability measured in the bioreactor was similar at t0 and day 1 (77.7% and 76.3%, respectively) and it was 18.8% higher than that estimated by batch. This value is slightly higher than that found in the other two experiments and support the hypothesis that perhaps DOC had not reached a plateau during the time course of the experiment. Nevertheless, the difference is really slight and allows us to suppose that the last measured DOC concentration was fairly close to the plateau.

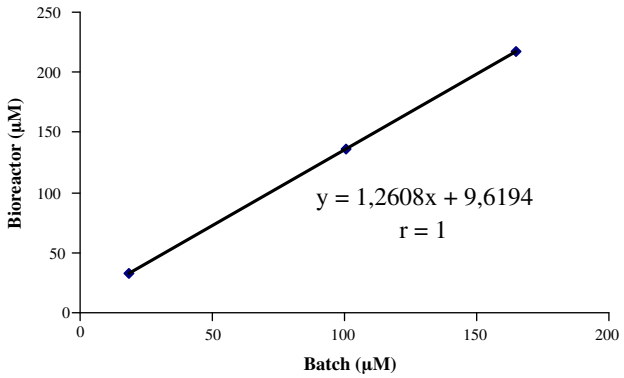

Fig. 6. Relationship between measurements of BDOC in bioreactor and batch cultures.

Percentage of degradation decreased to 48% during the two successive samplings and reached the minimum (26%) at day 21, confirming that the most labile DOC fraction was quickly consumed, while semilabile DOC needed some weeks.

As already mentioned, we are not sure whether during the last incubation experiment DOC had reached a plateau, nevertheless the comparison between BDOC measured in the bioreactor and in batch incubations showed a highly significant positive relationship (Fig. 6, BioreactorBDOC = 1.2608 batchBDOC + 9.6194, $r = 1$) even if we have only three experiments to go by.

4. Discussion and conclusion

Our results showed that plug-flow bioreactors can be used as a rapid alternative method to assess the ambient concentrations of DOC available for biological decomposition in marine ecosystems as previously found for lake waters (Søndergaard and Worm, 2001). One major advantage is that measurement of one sample can be performed in about 3–4 h instead of the 20–30 days needed for batch experiments. This can be achieved because of the high density of indigenous microorganisms colonizing the bioreactor (enhanced by the SIRAN support which allows for high concentrations of cells of microorganisms due to the large surface area to volume ratio of the porous glass carrier), which are exposed to a relatively low flow of organic matter.

The hypothesis that the decrease in the organic matter concentration while the water passes through the bioreactor column is due to microbiological activity rather than to the adsorption, is shown by the comparison with abiotic conditions. Measurements carried out before the bioreactor colonization, suggested the absence of any abiotic removal: DOC values at the inlet and outlet were statistically indistinguishable (among all samples measured CV < 4%). These results are in good agreement Ribas et al. (1995), who, in their experiments, reported that during the first days of circulation, before the bacterial colonization of the support, there were no significant differences between the DOC of the inflow and the outflow water.

Moreover, the role of microbiological processes in DOC removal is confirmed by the fact that during the first days of colonisations no differences were detected among inlet and outlet DOC concentrations. This conclusion is also supported by sodium azide data.

To consider the colonization effective, the bioreactor, in which at the beginning a sterile inert support is present, is continuously fluxed with filtered water as long as BDOC values are similar to those obtained by means of incubation experiments (Ribas et al., 1991).

Our colonized bioreactor developed a "mature" microbiological community able to metabolize fluctuating and elevated concentrations of BDOC, as shown in the experiments of glucose metabolism (≅170 μM) and during analysis of samples with concentrations ranging from 8 to 47 μM. The continuous once-through flowing of the sample in the bioreactor guarantees a unidirectional supply of carbon, inorganic nutrients and energy to the microbial community colonizing the inert support. As reported for BDOC analysis in streamwater (Volk et al., 1997), presumably the most labile molecules would be consumed firstly, and more recalcitrant molecules (semilabile DOC) would be metabolized over progressively longer residence times (distance) within the reactor. The resulting longitudinal gradients of diminishing DOC quantity and quality within the reactor, which, as described also by Søndergaard and Worm (2001), was clearly visible by the different colouration of the inert support, generate multiple niches and select diverse communities of bacteria capable of metabolizing DOC of different quality (labile and semilabile) (Volk et al., 1997). This specificity makes the direct analysis of BDOC possible and avoids the problems associated with indirect estimation of DOC uptake by means of parameters with large variability such as respiration (O_2 consumption or CO_2 production) or bacteria biomass and production which requires carbon conversion factors. Moreover, time needed for BDOC measurement is drastically reduced compared with batch cultures. Even though the use of bacterial abundance and biovolume and the conversion to biomass is widely applied, it is, in fact a difficult and uncertain procedure (Søndergaard and Middelboe, 1995). In addition, the two approaches of determination are conceptually different. While the BDOC can be considered the portion of DOC that can be mineralized by heterotrophic microorganisms, the conversion to biomass represents the measurement of only that portion of DOC, which can be converted to biomass, i.e. the assimilable organic carbon (AOC) (Frías et al., 1995).

The bioreactor assembled in the present study, follows the design proposed by Kaplan and Newbold (1995) and by Søndergaard and Worm (2001).

Even if there are several studies on BDOC distribution in seawater, the majority used batch culture experimentation to estimate BDOC (Malej et al., 2003; Ogawa et al., 2001; Puddu et al., 2003; Søndergaard et al., 2000; Søndergaard and Middelboe, 1995; Zweifel et al., 1993). Nowadays, a strict, worldwide accepted definition of BDOC has not yet been achieved; anyway the majority of decomposition experiments take from 5 to 30 days and the BDOC value corresponds to the difference between the initial DOC concentration and the asymptotic DOC value achieved during incubation time. The long time needed to obtain a BDOC value means that a great number of analyses cannot be carried out. Moreover, experimental conditions reproduced in culture could prevent the development of complex bacterial communities, with different decompositions capabilities and, therefore, prejudice BDOC estimation (Søndergaard and Worm, 2001).

The efficacy of the bioreactor utilization in "instantaneous" BDOC measurement has been demonstrated for drinking waters, lake, streamwaters (Frías et al., 1995; Kaplan and Newbold, 1995; Lucena et al., 1990; Ribas et al., 1991; Ribas et al. 1995; Søndergaard and Worm, 2001; Volk et al., 1997) and in water treatment in the semiconductor industry (Shibata et al., 1987); whereas, to the best of our knowledge, no similar application for seawaters has been reported in literature.

BDOC concentrations, in the samples collected from November 2003 to February 2004, ranged from 8 ± 1.3 μM to 24 ± 1.6 μM with degradation varying between 10.3% and 25.5%. Winter data are comparable to the values reported by Søndergaard and Middelboe (1995) which estimated that labile DOC, in marine environment, represents on average the 19 ± 12% of DOC with a median value of 14%. This data is slightly higher than the values (9%) measured by Becquevort et al. (2002) in an area of the Black sea influenced by the Danube.

In the samples collected during summer 2004 and in those obtained by the mesocosm experiment carried out during August 2003 (DOC production), the degradation percentage was generally higher reaching, in July, the value of 36.12%. The increased degradation percentage in field samples could be related to the higher summer DOC production characterizing the Gulf of Trieste, while in the mesocosm it is probably due to the higher quantity of labile DOC produced by phytoplanktonic bloom induced by the addition of phosphorous and other nutrients. Even if these values are higher with respect to the data reported by Søndergaard and Middelboe (1995), they are lower than the values obtained by Puddu et al. (2003) in similar mesocosm experiments, where from 48% to 65% of DOC accumulated during bloom was biodegradable. The differences in DOC utilization can be mostly ascribed to the variability of its composition (Chróst and Faust, 1983).

The lower percentages of degradation, observed in the seawater samples collected from November 2003 to February 2004, how-

ever, are probably due also to the lower DOC concentration, typical of the winter period (De Vittor et al. 2008). In our field samples, we found a positive relationship between DOC and BDOC values, despite the complexity of the marine ecosystem of the Gulf of Trieste, where the dynamics of the organic matter reflect the variety of the physical, chemical and biological processes, mainly due to the seasonal variations of the Isonzo River inputs (De Vittor et al. 2008).

This is in accordance with Middelboe et al. (1992) and with Søndergaard and Borch (1992), who reported that biodegradable DOC was positively related to total DOC, in coastal waters and lakes, respectively.

A positive relationship between the measurements of BDOC in the bioreactors and in batch cultures was pointed out. Comparing the results, it appears that the bioreactor consumes about 15% more DOC than the batch cultures (up to 19% in the mesocosm experiment of winter 2004). The calculated regression line showed a positive intercept, supporting the argument that the batch method underestimates the BDOC.

These results are in agreement with Søndergaard and Worm (2001) findings, which reported a positive relationship between measurements of BDOC in the bioreactors and in batch cultures incubated for 28 days and that, the reactor used about 20% more DOC than the batch cultures.

In agreement with Kaplan et al. (1996), they suggest that this could be explained by a broad metabolic capability in the reactors resulted from the development of less competitive but more specialized bacteria species along the bioreactor gradient.

Samples collected from batch culture during the time course of the experiment and analyzed simultaneously for DOC and for BDOC (by the bioreactor), showed the progressive decrease in the degradation percentage coupled with an increase in refractory DOC (RDOC) which shifted from 63 to 85 μM, that is from 22% to 74%. This suggests a progressive transformation of BDOC into RDOC in the batch during the incubation time. The concentration of BDOC, measured on day 21 (26% of DOC), suggests that the cumulative degradation of DOC during the 21 days of experimentation perhaps did not consume all BDOC present, but also supports the argument that the batch method underestimates the BDOC.

In this study, all analyses were carried out on seawater samples collected at the same location from which the seawater, used for colonization and maintenance of the bioreactor, was collected. To analyze water from different sources, the acclimation of the reactor to a specific organic source and site could be necessary (Kaplan et al., 1996). Nevertheless, Søndergaard and Worm (2001) analyzing BDOC in natural water from three locations not used for colonization and maintenance reported that acclimation period was not generally necessary.

The aim of this work, however, was to set up a functional system for the monitoring of BDOC in a specific environment (e.g. Gulf of Trieste) and after the initial colonization and acclimation phase, the bioreactor was operative and was used continuously during the time course of experimentation. It is a good precaution to change the filtered water for the maintenance periodically in order to allow the gradual acclimation of the reactor to the natural seasonal variation of quality and quantity of DOC characterizing the studied ecosystem.

To conclude, the bioreactor set up in the present study can monitor BDOC concentrations in the marine environment with measurements that can be accomplished within few hours instead of 20–30 days required in the incubation experiments. Results reported here constitute the first data of BDOC in the Gulf of Trieste obtained by a plug-flow bioreactor.

The determination of BDOC in seawater by a dynamic method giving results in few hours is of particular concern to better understand the complexity of phenomena affecting DOC dynamics.

Acknowledgements

The study was carried out under the auspices of two projects (INTERREG 2 and 3, Italy–Slovenia) (July 1998–June 2006) funded by EU and Regional Administration.

References

Amon, R.M.W., Fitznar, H.-P., Benner, R., 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnol. Oceanogr. 46, 287–297.

Becquevort, S., Bouviera, T., Lancelot, C., Cauwet, G., Deliat, G., Egorov, V.N., Popovichev, V.N., 2002. The seasonal Modulation of organic matter utilization by bacteria in the Danube–Black Sea mixing zone. Estuar. Coast. Shelf Sci. 54, 337–354.

Carlson, C.A., Ducklow, H.W., 1995. Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean. Daily and fine scale vertical variations. Deep Sea Res. II 42, 639–656.

Cauwet, G., 1994. HTCO method for dissolved organic carbon analysis in influence of catalyst on blank estimation. Mar. Chem. 47, 55–64.

Chróst, R.J., Faust, M.A., 1983. Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. J. Plankton Res. 5, 477–493.

Dafner, E.V., Wangersky, P.J., 2002. A brief overview of modern directions in marine DOC studies part I. Methodological aspects. J. Environ. Monit. 4, 48–54.

De Vittor, C., Paoli, A., Fonda Umani, S., 2008. Dissolved organic carbon variability in a shallow coastal marine system (Gulf of Trieste, northern Adriatic Sea). Estuar. Coast. Shelf Sci. 78, 280–290.

Frías, J., Ribas, F., Lucena, F., 1992. A method for the measurement of biodegradable organic carbon in waters. Water Res. 26, 255–258.

Frías, J., Ribas, F., Lucena, F., 1995. Comparison of methods for the measurement of biodegradable organic carbon and assimilable organic carbon in water. Water Res. 29, 2785–2788.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanle, M.H. (Eds.), Culture of Marine Invertebrates Animals. Plenum Press, New York, USA, pp. 26–60.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustet, and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8, 229–239.

Khan, E., Babcock, R.W., Jongskul, S., Devadason, F.A., Tuprakay, S., 2003. Determination of biodegradable dissolved organic carbon using entrapper mixed microbial cells. Water Res. 37, 4981–4991.

Kaplan, L.A., Newbold, D., 1995. Measurement of streamwater biodegradable dissolved organic carbon with a plug-flow bioreactor. Water Res. 29, 2696–2706.

Kaplan, L.A., Ribas, F., Joret, J.C., Volk, C., Frias, J., Lucena, F., 1996. Measurement of Biodegradable Organic Matter with Biofilms Reactors. AWWA Research Foundation, pp. 69.

Kirchman, D.L., 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. Mar. Ecol. Prog. Ser. 62, 47–54.

Kirchman, D.L., Lancelot, C., Fasham, M., Legendre, L., Radach, G., Scott, M., 1993. Dissolved organic matter in biogeochemical models of the ocean. NATO-ASI Series. Proceedings of NATO Advanced workshop Towards a Model of Ocean Biogeochemical Processes 110, 200–225.

Lucena, F., Frias, J., Ribas, F., 1990. A new dynamic approach to the determination of biodegradable dissolved organic carbon in water. Environ. Technol. 12, 343–347.

Malej, A., Mozetič, P., Turk, V., Terzič, S., Ahel, M., Cauwet, G., 2003. Changes in particulate and dissolved organic matter in nutrient-enriched enclosures from an area influenced by mucilage: the northern Adriatic Sea. J. Plankton Res. 25, 949–966.

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

Ogawa, H., Amagai, Y., Koike, I., Kaise, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.

Puddu, A., Zoppini, A., Fazi, S., Rosati, M., Amalfitano, S., Magaletti, M., 2003. Bacterial uptake of DOM released from P-limited phytoplankton. FEMS Microbiol. Ecol. 46, 257–268.

Repeta, D.J., Aluwihare, L.I., 2006. Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: implication for organic carbon cycling. Limnol. Oceanogr. 51, 1045–1053.

Ribas, F., Frías, J., Lucena, F., 1991. A new dynamic method for the rapid determination of the biodegradable organic carbon in drinking water. J. Appl. Bacteriol. 71, 371–378.

Ribas, F., Frías, J., Ventura, F., Mohedano, L., Lucena, F., 1995. Assessment of biological activity and fate of organic compounds in a reactor for the measurement of biodegradable organic carbon in water. J. Appl. Bacteriol. 79, 558–568.

Shibata, M., Nakamura, K., Miyaji, Y., 1987. Biological decomposition of trace organic compounds. Water Sci. Technol. 19, 417–427.

Søndergaard, M., Borch, N.H., 1992. Decomposition of dissolved organic carbon (DOC) in lakes. Arch. Hydrobiol. Beih. Ergebn. Limnol. 37, 9–20.

Søndergaard, M., Middelboe, M., 1995. A cross-system analysis of labile dissolved organic carbon. Mar. Ecol. Progr. Ser. 118, 283–294.
Søndergaard, M., Worm, J., 2001. Measurement of biodegradable dissolved organic carbon (BDOC) in lake water with a bioreactor. Water Res. 35, 2505–2513.
Søndergaard, M., Borch, N.H., Riemann, B., 2000. Dynamics of biodegradable DOC produced by freshwater plankton communities. Aquat. Microb. Ecol. 10, 69–85.
Thomas, C., Cauwet, G., Minster, J.F., 1995. Dissolved organic carbon in the equatorial Atlantic Ocean. Mar. Chem. 49, 155–169.
Volk, C.J., Volk, C.B., Kaplan, L.A., 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. Limnol. Oceanogr. 42, 39–44.
Zweifel, U.L., Norrman, B., Hagstrom, A., 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar. Ecol. Prog. Ser. 101, 23–32.