Biogeosciences Discuss., 7, 787–822, 2010
www.biogeosciences-discuss.net/7/787/2010/

Biogeosciences Discussions

This discussion paper is/has been under review for the journal Biogeosciences (BG).
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Increased bacterial growth efficiency with environmental variability: results from DOC degradation by bacteria in pure culture experiments

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Received: 18 January 2010 – Accepted: 20 January 2010 – Published: 1 February 2010

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

This paper assesses how considering variation in DOC availability and cell maintenance in bacterial models affects Bacterial Growth Efficiency (BGE) estimations. For this purpose, we conducted two biodegradation experiments simultaneously. In experiment one, a given amount of substrate was added to the culture at the start of the experiment whilst in experiment two, the same amount of substrate was added, but using periodic pulses over the time course of the experiment. Three bacterial models, with different levels of complexity, (the Monod, Marr-Pirt and the dynamic energy budget (DEB) model), were used, and calibrated using the above experiments. BGE has been estimated using the experimental values obtained from discrete samples and from model generated data. Cell maintenance was derived experimentally, from respiration rate measurements. The results showed that the Monod model did not reproduce the experimental data accurately, whereas the Marr-Pirt and DEB models demonstrated a good level of reproducibility, probably because cell maintenance was built into their formula. Whatever estimation method was used, the BGE value was always higher in experiment two (the periodically pulsed substrate) as compared to the initially one-pulsed-substrate experiment. Moreover, BGE values estimated without considering cell maintenance (Monod model and empirical formula) were always smaller than BGE values obtained from models taking cell maintenance into account. Since BGE is commonly estimated using constant experimental systems and ignore maintenance, we conclude that these typical methods underestimate BGE values. On a larger scale, and for biogeochemical cycles, this would lead to the conclusion that, for a given DOC supply rate and a given DOC consumption rate, these BGE estimation methods overestimate the role of bacterioplankton as CO_2 producers.

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1 Introduction

Dissolved organic carbon (DOC) represents one of the largest active organic carbon reservoirs in the biosphere (Hedges, 1992; Amon and Benner, 1996). It is commonly assumed that numerous processes are responsible for DOC production such as its release by phytoplankton, egestion, excretion and sloppy feeding from grazers and cellular lysis generated by viruses (Nagata, 2000). Bacteria are considered to be the major consumers and remineralisers of DOC in the ocean (Pomeroy, 1974; Williams, 2000). According to bacterial reactivity, DOC is usually fractionated into three pools: the refractory (R-DOC), semi-labile (SL-DOC) and labile DOC (L-DOC) (Williams and Druffel, 1987; Carlson and Ducklow, 1995; Hansell et al., 1995). Bacterial activity is often measured using the bacterial growth efficiency (BGE). This is the proportion of DOC that is converted by bacteria into particulate organic matter (POC) that can be later consumed by higher trophic levels (Cajal-Medrano and Maske, 2005). The L-DOC component and BGE can be determined by measuring the bacterial DOC consumption in biological assays (Carlson and Ducklow, 1996; Cherrier et al., 1996; Sempéré et al., 1998; Carlson et al., 1999). Both DOC production and consumption occur in the natural environment through different processes, therefore any experimental design must endeavour to uncouple these two processes in order to study either one or the other. Consequently, such experiments are generally performed over 1–2 weeks either by isolating natural assemblages of bacteria from the primary producers and grazers by filtering in situ seawater samples, or by working with monospecific cultures.

It is generally assumed that the complementary proportion (1-BGE) corresponds to respiration and results in metabolic CO_2 release in the ambient medium. BGE is commonly used as a constant parameter in biogeochemical models (Baretta-Bekker et al., 1995; Blackburn et al., 1996; Anderson and Williams, 1998, 1999; Lancelot et al., 2002), which are subsequently used to investigate the carbon cycle (Anderson and Williams, 1998, 1999). According to this, the general mathematical definition of BGE is: $BGE = \Delta BB / -\Delta DOC$, where ΔBB is the bacterial biomass produced from the

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consumption of available ΔDOC. However, BGE is generally determined experimentally from bacterial production (BP) and respiration (BR) measurements, or from BP and bacterial carbon demand (BCD) according to the following formula: BGE=BP/BCD where BCD=BP+BR (del Giorgio and Cole, 1998; Sempéré et al., 1998; Rivkin and Legendre, 2001; Reinthaler and Herndl, 2005). BGE may also be estimated from mathematical models as it often consists of a model parameter (Eichinger et al., 2006) or is a function of the specific growth rate (Cajal-Medrano and Maske, 1999, 2005).

Although biological assays provide a large set of BGE values, they are difficult to extend to real ecosystems due to the wide range of methods used and the utilisation of conversion factors which also exhibit large variations (Cherrier et al., 1996; del Giorgio and Cole, 1998). Despite this, previous studies have indicated that BGE varies greatly depending on biological and physical factors (del Giorgio and Cole, 1998) such as : DOC chemical nature/molecular weight (Amon and Benner, 1996; Cherrier et al., 1996), elemental ratio (Goldman et al., 1987), distance of the study site from the seashore (La Ferla et al., 2005), season (Reinthaler and Herndl, 2005), temperature (Rivkin and Legendre, 2001), depth (Eichinger et al., 2006) and UV exposure of dissolved organic matter prior to incubation (Abboudi et al., 2007). Moreover, batch experiments in which DOC monotonously decreases according to its consumption by bacteria are certainly not representative of the real world.

To date, to the best of our knowledge, there has been no study focusing on the direct effects of DOC availability on BGE. Indeed, due to physical, chemical, and biological processes (Carlson and Ducklow, 1995; Carlson et al., 2004; La Ferla et al., 2005), and to the decoupling between DOC production and consumption (Hansell et al., 1995; Carlson et al., 2002), the DOC concentration fluctuates spatially and temporally in oceanic ecosystems. Thus, relatively weak temporal variations in the dynamics of the water column may have a great impact on the functioning of the pelagic system (González et al., 2002). This makes it crucial to study the response of microbial communities to intermittent or transient forms of reactive DOC (Cherrier and Bauer, 2004).

The first objective of this paper is to assess how the variation in DOC availability and supply affects BGE values using experimental and modelling approaches simultaneously. Most biogeochemical models use simplistic and empirical formulae to represent DOC consumption and bacterial growth, which are respectively described with Michaelis-Menten kinetics and Monod formulation. Thus they do not take into account cell maintenance, the importance of which has been highlighted in several studies (del Giorgio and Cole, 1998; Eichinger et al., 2009). The second aim of this paper is to highlight the cell maintenance process using biodegradation assays and varying DOC supplies, in order to study how this affects BGE estimates. For this purpose, we have used several bacterial growth models with different levels of complexity, some considering cell maintenance.

To meet these objectives, we performed two laboratory biodegradation experiments. In the first experiment, all the substrate was added at the beginning of the time series, whereas in the second experiment the same amount of substrate was divided into several smaller pulses and added to the culture every 2 days. Since the total quantity of substrate is the same at the end of both experiments, the only difference is the substrate regime.

This paper is organised as follows: in the first section we present a detailed description of the experiments carried out to assess the influence of the DOC load on BGE, and the various methods used for its estimation, including empirical calculations and model calibration. The different processes included in each model and their mathematical descriptions are given. The second section compares the experimental dynamics obtained from both experiments, and presents the calibration and simulation of the models. It also compares the BGE values estimated from both experiments, as obtained with each method. Finally, the last section presents the conclusions and discusses their implication for BGE determined in aquatic ecosystems.

2 Material and methods

2.1 Main concepts

To prevent problems arising from the sensibility of DOC measurements, the lability of DOC and bacterial activity, we used a monospecific bacterial strain and a highly labile carbon substrate source. Thus, we assumed that the decrease in DOC concentration is directly related to bacterial growth. We also applied an intensive sampling regime and used DOC concentrations well above oceanic conditions; the total L-DOC concentration added to the cultures was 8 mM C whereas oceanic DOC concentrations generally range from 40 µM C to more than 200 µM C. In this study, we defined L-DOC as the substrate and thus the DOC that is consumed during the time course of the experiment (with a turnover time of a few hours, approximately) whereas R-DOC was considered to be the DOC remaining at the end of the experiments. This R-DOC could consist of SL-DOC or R-DOC for our bacterial strain.

2.2 Experimental design

The culture medium was composed of artificial seawater (Lyman and Fleming, 1940), containing vitamins, minerals and excess nutrients at the beginning of each experiment; KH_2PO_4 and NH_4Cl were provided at concentrations of 0.2 and 6.7 mM, respectively, and the pH was adjusted to 7.5. Pyruvate was selected as the carbon substrate and *Alteromonas infernus* as the bacterial strain (refer to Eichinger et al., 2009, for the explanations of these choices). Each medium was autoclaved for 20 min at 110 °C prior to inoculation. The cultures were incubated in a temperate room at 25 ± 1 °C, in the dark, and were continuously agitated. To prevent bacterial contamination, the material used to sample the batch cultures was sterilised by autoclaving 20 min at 110 °C, and all samples were handled under a laminar flow air hood. To prevent carbon contamination, all borosilicate glass materials used for the cultures and sample collection and storage were pre-combusted for 6 h at 450 °C.

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Two experiments were performed: one using a single substrate addition (B), and one using pulse additions of substrate (P). In the case of experiment P, the initial conditions were: (DOC)=1.6 mM C and bacterial density=6.10^6 cells cm^{-3} subsequently followed by pulse additions of DOC (1.6 mM C) every 48 h. The initial conditions set for experiment B were: (DOC)=8 mM C (equivalent to 5×1.6 mM C) and bacterial density=6.10^6 cells cm^{-3}. The initial conditions set for experiment P were chosen so that; (1) DOC decrease and bacterial growth were substantial, (2) the pulse period was long enough to allow sample collection between subsequent pulses, and (3) substrate DOC was apparently exhausted and bacteria were in the stationary phase at the end of the pulse period. This latter condition was necessary to observe cell maintenance when bacteria were in "starving" conditions.

The total volume of the culture enabled samplings to take place over 5 pulses. Both experiments were conducted in 5 litre pre-combusted borosilicate bottles filled with 4 (experiment P) or 3 (experiment B) litres of culture medium. Because of the large volumes needed for sampling experiment P, 3 replicate bottles were used and successively sampled. This setup made possible to use the same apparatus in both experiments. We checked reproducibility by carrying out the same experiment independently several times and checking the dynamics were always identical (data not shown).

2.3 Sampling

Sampling was always carried out in the same order to avoid bias from any time lag occurring between the different measurements. To prevent carbon contamination the first sub-sample was always dedicated to carbon measurements (DOC and POC). The sampling order was: (1) POC/DOC, (2) cell count by microscopy and flow cytometry analyses, and (3) oxygen consumption (respiration). To ensure reproducibility, samples were always further homogenised before sampling by gentle hand-mixing. To ensure the results were significant, the final volume of the batch cultures was always higher than 50% of the initial culture volume.

DOC and POC were separated using pre-combusted GF/F filters (0.7 µm nominal porosity). DOC was measured by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC 5000 Analyzer following the same protocol as Sohrin and Sempéré (2005). At time 0, the DOC measured was derived from the vitamins and pyruvate. The vitamin-DOC concentration was negligible compared to that of the pyruvate and estimated to account for only 3 and 0.6% of the initial DOC for the P and B experiments, respectively. In this study, we refer to POC as the C-bacterial biomass. Following filtration onto the GF/F filter, each filter was dried in an oven (30°) carefully stored in a desiccator in the dark and then analysed with a carbon analyser (Leco SC-144) following the same protocol as Sempéré et al. (2000).

O_2 consumption was determined by measuring O_2 concentration dynamics with the Oroboros-2k oxygraph (OROBOROS, Austria). This oxygraph provides the instrumental basis for temporal high-resolution respirometry due to a small time lag between two measurements (2 s). As recommended by the manufacturer the volume of the two thermoregulated chambers was set to 2 cm^3, and the stirrer speed to 750 rounds per minute. Respiration measurements were carried out at 25 °C, the same temperature as the cultures. Each day, the basal consumption of each polarographic oxygen sensor (POS) was determined using a sterile medium sample. This value was then subtracted from each O_2 consumption rate measured the same day. The POS were calibrated with 0 and 100% oxygen saturation. 0% oxygen saturation was achieved by adding anhydre sodium hydrosulfite (Na_2SO_3) in excess, in order to complex all the oxygen in the chambers. The 100% oxygen saturation was calibrated prior to each measurement by introducing 2 cm^3 of sterile culture medium to each chamber and keeping the stopper open to equilibrate the gas with the atmosphere. Once equilibrium had been reached, the 100% oxygen saturation was recorded. This medium was then replaced by 2 cm^3 of culture. The decreasing O_2 concentration was measured at each time point. O_2 consumption was calculated by applying a linear regression on the O_2 concentrations, where the slope corresponds to consumption. In order to get rid of mechanical effects induced by the movement of the stoppers when opening and closing the chambers, the

O_2 consumption estimation started 10 min after closing the stopper.

Bacterial density was estimated using microscopy counts to calculate specific bacterial activities (specific carbon content and O_2 consumption). Bacteria fixation was carried out by adding 100 mm^3 of a 20% tetraborated formol solution into 900 mm^3 of culture. A few mm^3 were taken out from this formol-mixture and added to filtered MilliQ water. The volume of the formol-mixture was adjusted according to the expected bacterial density, so that there were at least 30 bacteria per field under the microscope. Bacteria were stained with Diamidino-4′,6-phénylindol-2 Dichlorhydrate (DAPI) (2.5 µg cm^{-3} final concentration). The final mixture was filtered onto a 0.2 µm porosity dark polycarbonate membrane. The counts (in cell cm^{-3}) were realised with an epifluorescent microscope (Olympus BH2 or BX61, Olympus, USA) by analysing 30 fields per sample.

In some samples bacterial density was also analysed using flow cytometry for total counts, and in the DOC samples to check for any bacterial transfer through the GF/F filters. Bacterial fixation was carried out using 1.8 cm^3 sample and 0.2 cm^3 of a 20% para-formaldehyde (PFA) solution in 2 cm^3 cryotubes (Nalgene, USA). Samples were then stored in liquid nitrogen (−180 °C) until analysis. Before analysis the samples were gently thawed in a water bath at room temperature, stained with DAPI solution (2.5 µg cm^{-3} final concentration) and analysed by a MoFlo cell sorter (Dako, Dk).

2.4 BGE estimation

According to the general definition, BGE can be estimated from experimental data as BGE$=-\Delta$POC$/\Delta$DOC. ΔPOC was estimated from the difference between the maximum and initial POC values and ΔDOC from the difference between the total substrate amount introduced in the culture (8 mM C) and the remaining DOC amount at the end of the experiment.

BGE has also been estimated by using three models with different levels of complexity: the Monod, Marr-Pirt and Dynamic Energy Budget (DEB) models. For each model, BGE was estimated as: BGE$=-dB_B/dL$, where B_B is the bacterial biomass

and L the substrate concentration (L-DOC) (Table 1). The DEB model takes into account one C-reserve compartment as well as two C-maintenance fluxes (see below); the Marr-Pirt model considers one C-maintenance flux but no reserve compartment; the Monod model comprises neither reserve nor maintenance. Descriptions of state variables and parameters are given in Table 1. The DEB model originates from the dynamic energy budget theory (Kooijman, 2000). It has been specifically developed for the conditions in experiment P and its construction has been extensively described in Eichinger et al. (2009). Briefly, the DEB theory assumes that bacterial biomass is composed of a reserve (M_E) and structure (M_V). Differential equations on the left part of (Eq. 1) correspond to a typical growth model for heterotrophic bacteria, whereas differential equations on the right part describe bacterial dynamics in starvation conditions. During growth, substrate is first assimilated into the reserve and then C-energy is allocated to growth. Maintenance is paid from the mobilized reserve if the flux is large enough (growth case). Otherwise, structure is used to pay the remaining part of the maintenance costs (starvation case), which causes size reduction of the cell (Tolla et al., 2007; Eichinger et al., 2009). Release of recalcitrant-to-degradation DOC (R) was associated with the use of structure for maintenance purpose (Eq. 1). For this model: $\mathrm{BGE}=-\frac{d}{dL}\left(M_E/y_{M_E M_V}+M_V\right)$. Contrary to the DEB model, the Marr-Pirt model (Marr et al., 1963; Pirt, 1965) assumes a direct transfer from assimilation to growth and includes one maintenance term only. As this model does not comprise any reserve compartment, maintenance is directly realised from biomass (Eq. 2). As in the DEB model, R is produced from the maintenance of the biomass. For this model, $\mathrm{BGE}=-dB_B/dL$. The Monod model (Monod, 1942) assumes that the absorbed substrate is directly and instantaneously transformed into biomass with a constant efficiency (Eq. 3) (Eichinger et al., 2006): $\mathrm{BGE}=-dB_B/dL$.

DEB model: (Eq. 1)

$$\frac{dL}{dt}=-\alpha L M_V$$

if $k_E M_E > j_{M_E M} M_V$

$$\begin{cases} \frac{dM_E}{dt} = y_{M_E L}\alpha L M_V - j_{M_E M} M_V \\ \qquad\qquad - y_{M_E M_V} \frac{k_E M_E - j_{M_E M} M_V}{M_E + y_{M_E M_V} M_V} M_V \\ \frac{dM_V}{dt} = \frac{k_E M_E - j_{M_E M} M_V}{M_E + y_{M_E M_V} M_V} M_V \\ \frac{dR}{dt} = 0 \end{cases}$$

if $k_E M_E < j_{M_E M} M_V$

$$\begin{cases} \frac{dM_E}{dt} = y_{M_E L}\alpha L M_V - k_E M_E \\ \qquad\qquad - \frac{j_{M_E M} M_V - k_E M_E}{M_E + j_{M_E M}/j_{M_V M} M_V} M_E \\ \frac{dM_V}{dt} = - \frac{j_{M_E M} M_V - k_E M_E}{M_E + j_{M_E M}/j_{M_V M} M_V} M_V \\ \frac{dR}{dt} = y_{R M_V} \frac{j_{M_E M} M_V - k_E M_E}{M_E + j_{M_E M}/j_{M_V M} M_V} M_V \end{cases}$$

Marr-Pirt model: (Eq. 2)

$$\frac{dL}{dt} = -\alpha L B_B$$

$$\frac{dB_B}{dt} = y_{B_B L}\alpha L B_B - j_{B_B M} B_B$$

$$\frac{dR}{dt} = y_{R B_B} j_{B_B M} B_B$$

Monod model: (Eq. 3)

$$\frac{dL}{dt} = -\alpha L B_B$$

$$\frac{dB_B}{dt} = y_{B_B L}\alpha L B_B$$

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Note that in this study we also report that bacteria are able to produce unusable DOC, referred to here as R-DOC. The Monod model does not permit any product formation therefore in order to compare parameters governing assimilation and growth on L-DOC between the three models, the DOC data were modified, for the utilisation of this model only (see below), to deal with just the labile fraction of DOC. In experiment B, apparent R-DOC concentrations seem constant (Fig. 1a). L-DOC values were thus estimated by offsetting the R-DOC values at the end of the experiment to the total DOC values. In experiment P, apparent R-DOC concentrations increased after each pulse and a linear regression on R-DOC values for all the experiment was applied (Fig. 1b). We calculated L-DOC values for the Monod model as L-DOC=DOC$-(0.0038t+0.1067)$, where DOC represents the measured DOC concentrations and t is time. This data modification does not influence BGE values as it is only based on L-DOC and bacterial biomass (POC) data.

3 Results

3.1 DOC and POC dynamics

Flow cytometry analyses revealed that the percentage of bacteria in DOC samples ranged from 0 to 14% in experiment B. Higher values were obtained during the exponential growth phase, whereas this percentage was close to 0% during the lag and the stationary phases (data not shown). This suggests that bacteria are larger during the non dividing period, and that cell division leads to bacteria shrinking, subsequently reaching the size limit of the filter retention in some cases. We thus corrected POC and DOC concentration values according to the fraction of bacteria crossing the filters. In experiment P, with the exception of two values of 11 and 13%, the percentage of bacteria in DOC samples were always lower than 4.5%. Unfortunately, some samples were not checked for bacterial density due to technical problems. Due to the low percentage found in analysed samples and to missing values, we decided not to correct

DOC and POC values for experiment P. Experimental dynamics of DOC and POC take into account the correction for experiment B (Fig. 1a) but not for experiment P (Fig. 1b).

DOC kinetics indicated an apparent remaining DOC during the time course of experiment P (Fig. 1b). Indeed, as represented by the dashed line in Fig. 1b, the baseline level for DOC concentration increased following each substrate addition. As hypothesised, bacteria always consumed the substrate added after each pulse, thus the increase in remaining DOC is not likely to be labile DOC for this strain. In this experiment we considered this "remaining-accumulating" DOC to be unconsumed DOC produced by the cultured bacteria and referred to it as R-DOC, over the scale of this study (refer to Eichinger et al. (2009) for additional details on this DOC production). On the contrary, no apparent R-DOC was produced over the time course of experiment B; however, there was a DOC concentration of about 1.9 mM C at the end of the experiment (Fig. 1a). As no substrate was added during this experiment, it is difficult to determine if this remaining DOC consisted of unconsumed substrate due to a limitation such as nutrients or O_2, or whether the R-DOC was produced during another period of the experiment. We assumed this remaining DOC consisted of R-DOC, as in experiment P.

Over the entire experiment, about 6.1 and 7 mM of DOC were consumed in the P and B experiments, respectively. However, due to the very rapid reactivity of bacteria towards DOC supply in experiment P and to the time lag necessary for DOC sampling following the additions, it is difficult to estimate the exact total concentration of DOC that was supplied in this experiment. It is therefore possible that the total concentration of consumed DOC is higher than that estimated for experiment P. POC concentrations were identical at time 0 (t_0) in both experiments. However, although the total concentration of substrate was the same in both experiments, the maximum POC concentrations differed. It reached a value of 1.8 and 1.1 mM C for experiments P and B, respectively. This suggests a higher productivity, of a factor of 1.6 for experiment P.

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3.2 Respiration dynamics

In both experiments, O_2 consumption increased rapidly a few hours after t_0 (Fig. 2). However, this increase was more than 3 times higher for experiment B: O_2 consumption peaked at 0.35 and 0.10 mM O_2 h^{-1} for experiments B and P, respectively (Fig. 2). This difference is assumed to be due to the higher initial substrate concentration (×5) in experiment B. The increase in respiration rate was slow in experiment B, which correlates to the DOC degradation period (Fig. 1a). O_2 consumption then stabilised with only small variations around 0.25 mM O_2 h^{-1}. Respiration rates followed the DOC dynamics in experiment P, increasing very rapidly following each substrate pulse, with higher values reaching 0.6 mM O_2 h^{-1} (Fig. 2). As for the DOC measurements, O_2 consumption increased very rapidly following substrate addition, and as a period of at least 30 min was required before O_2 measurements could be carried out, it is highly possible that O_2 consumption was underestimated. Only a few hours after substrate addition, respiration rates decreased linearly never dropping to zero but stabilising at a constant value until the next substrate pulse. This constant value increased after each pulse as bacterial density increased throughout the experiment and demonstrated the maintenance process which can not be considered to be negligible. We also estimated the total concentration of O_2 consumed during the experiments using a trapezoidal integration method between the data points. We obtained about 30 and 20 mM O_2 for the P and B experiments, respectively. From this estimation, experiment P was 1.5 times more productive than experiment B. Consequently, observed O_2 consumption dynamics indicated an instantaneous response of bacteria to substrate pulses, a maintenance process between two subsequent pulses and greater consumption in experiment P.

3.3 Specific activities

Specific O_2 consumption (fmol O_2 h^{-1} $bact^{-1}$) and specific organic-C content (fmol C $bact^{-1}$) were estimated by dividing O_2 consumption values and POC concentrations by their respective bacterial density at each sampling time (Fig. 3). Specific O_2 consumption rapidly increased at t_0 and then decreased in both experiments (Fig. 3a). In experiment B, the specific respiration rate gradually decreased during the time course of the experiment. Contrary to this, the specific respiration rate sharply increased after each substrate addition and decreased a few hours later in experiment P. Specific respiration rates dropped to a mean value of 0.2 fmol O_2 h^{-1} $bact^{-1}$ between each subsequent pulse (and after the second pulse), a value which is an approximation of the specific maintenance respiration rate.

Although the initial POC concentration and bacterial density should be identical in both experiments, the initial specific organic-C contents were different, 38 and 11 fmol C $bact^{-1}$ for experiments B and P, respectively (Fig. 3b). This could be due to the initial values of POC concentration and bacterial density being low and thus difficult to accurately measure. Nevertheless, the specific organic-C content rapidly decreased after the start of both experiments to about 3–5 fmol C $bact^{-1}$. The specific POC content then gradually decreased from approximatively 4 to 2 fmol C $bact^{-1}$ during the remaining time of experiment B. Contrary to this, specific POC content increased after each substrate pulse in experiment P and was relatively stable, 5 fmol C $bact^{-1}$ between two subsequent pulses. After 20 h, specific POC values were always higher in experiment P than in experiment B (Fig. 3b).

3.4 Model fitting

The DEB, Marr-Pirt and, Monod models were calibrated from the data sets obtained from each experiment. Parameter estimation was based on the minimisation of the sum of squared deviations of model predictions to data points, using the Nelder Mead's simplex method. To compare model outputs to DOC and POC measurements, we made

the following assumptions: (1) for the DEB model, DOC=$L+R$ and POC=M_E+M_V; (2) for the Marr-Pirt model, DOC=$L+R$ and POC=B_B; (3) for the Monod model, DOC=L and POC=B_B. Calibrations were carried out using the whole original data set for experiment P and the whole corrected data set for experiment B, as DOC and POC values were corrected for the bacteria that crossed the filters. The Monod model was calibrated with modified data sets from both experiments as we subtracted the estimated R-DOC concentrations from each DOC data point of each experiment. Estimated parameter values for each model are given in Table 2 for experiment B and in Table 3 for experiment P.

The DEB and Marr-Pirt models fitted the DOC and POC data producing a good fit for both experiments (Fig. 4a,b,d and e). However, the DEB model showed greater flexibility than the Marr-Pirt one. After data modification (represented by squares in Fig. 4c and f), the Monod model accurately fitted the DOC dynamics of both experiments, which can be explained by the fact that L-DOC uptake was governed by the same formulation in the three models. However, the Monod model did not produce a good fit for the POC data (Fig. 4c and f). We indeed prevented the problems associated with unconsumed DOC release as this process was not incorporated into the Monod model. However, the absence of the maintenance process did not enable the model to demonstrate the decrease in POC, which is clearly visible at the end of experiment B.

3.5 BGE estimations

BGE was directly estimated experimentally as BGE=$-\frac{\Delta POC}{\Delta DOC}$. BGE was 0.14 and 0.27 for experiments B and P, respectively (Table 4), suggesting that bacteria were twice as efficient when provided with regular pulsed DOC inputs than when provided with a single DOC addition. BGE was computed for each experiment and each model (see Material and Method, BGE estimation). Whatever the estimation method, BGE values were constant over both experiments (Fig. 5). As was the case in the empirical estimation, BGE values were higher in experiment P than in experiment B: values were 0.34 and 0.21 for the DEB model, 0.38 and 0.20 for the Marr-Pirt model, and 0.23 and 0.14

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for the Monod model. Growth efficiencies differed in the same range of magnitude for all estimations: BGE values were approximately 50% higher in experiment P compared to experiment B in both the experimental and Marr-Pirt estimations, and 60% for the Monod and DEB estimations.

BGE estimations were always higher using the methods taking into account maintenance (DEB and Marr-Pirt models) compared to the Monod model and empirical estimations. The analytical calculations of BGE for the three models gave the following results: for the DEB model, BGE=$\frac{y_{M_{\mathrm{E}}L}}{y_{M_{\mathrm{E}}M_{\mathrm{V}}}}-\frac{j_{M_{\mathrm{E}}M}}{y_{M_{\mathrm{E}}M_{\mathrm{V}}}\alpha L}$ (by taking only the growth model into account, otherwise we would obtain a negative BGE); for the Marr-Pirt model, BGE=$y_{B_{\mathrm{B}}L}-j_{B_{\mathrm{B}}\mathrm{M}}/\alpha L$; and for the Monod model, BGE=$y_{B_{\mathrm{B}}L}$ (Table 4). BGE for DEB and Marr-Pirt models depend on L, and should thus vary according to time. Finally, the parameter estimation showed that the variable parts of BGE (for Marr-Pirt and DEB models) were negligible: the constant values observed in Fig. 5 corresponded to the constant part of BGE (Table 4).

4 Discussion

4.1 Maintenance process and model choice for bacterial growth

Respiration rate measurements enabled cell maintenance to be studied during the stationary phase. The Monod model is not suitable for this purpose. Indeed, it considers that a proportion BGE from assimilated substrate is used for growth, and implicitly that the remaining proportion (1-BGE) is used for respiration. However, when considering the equations, L=0 would imply that the respiration rate (which amounts to (1−BGE)$\alpha L B_{\mathrm{B}}$) is also null. This result is inconsistent with our experimental results that clearly exhibited respiration rate values different from zero during starvation periods. The choice of a model is highly influenced by the available data. Indeed, if our experiments had been stopped after the growth phase and we had not measured the respiration rate, the Monod model would have reproduced the experimental data.

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Consequently, performing an experiment that continuously alternated between supply and depletion of substrate, coupled to respiration rate measurements, enabled us to reject the Monod model, when measuring bacterial growth in a fluctuating substrate supply, i.e. in fluctuating environments. In this study, the Marr-Pirt and DEB models were almost equivalent in terms of dynamics and BGE estimations. However, by increasing the complexity of the experiment by including other trophic levels for example, one could likely appreciate the relevance of mechanistic approach, as in the DEB theory. It has been demonstrated using a food chain with bacteria, fed on glucose, and a predator, that the Monod and Marr-Pirt models were not able to reproduce the experimental dynamics, whereas the DEB model was (Kooi and Kooijman, 1994). Consequently, experimental developments, as those presented in this study, are needed in order to assess bacterial processes and should be included in ecosystem models.

The calibration of the three models using data sets from both experiments showed that the Monod model was weak in reproducing the experimental dynamics. We had previously reached this conclusion by analysing the respiration rate measurements, but the maintenance process was also evident from decreasing POC concentrations during starvation periods, dynamics that can not be produced with the Monod model. However, the formulation of the maintenance process had little impact on model outputs in this study. Nevertheless, the DEB model is more flexible than the Marr-Pirt due to its mechanistic formulation model and can be validated in more diverse situations. For example, if we had conducted a pulse experiment with the initial conditions of experiment B and over a longer pulse period, the Marr-Pirt model may have been unable to reproduce the experimental dynamics as it does not comprise a component for cell maintenance from the reserve, component that would not be longer negligible. Indeed, the maintenance from the reserve was higher in experiment B than in experiment P (j_{EM} is negligibly small in experiment P whereas it equals $0.021\,h^{-1}$ in experiment B (Tables 2 and 3)). Additionally, the maintenance from the reserve was higher than maintenance from structure in experiment B (j_{EM} is more than three times higher than j_{VM}) (Table 2). Consequently, a mechanistic model like the DEB model may be useful

in conditions other than our pulse experiment, and may show dynamic differences with the Marr-Pirt model. Tolla et al. (2007) discussed the interest and efficiency of a mechanistic formulation of maintenance.

4.2 Bacterial versatility to respond to a perturbation

By estimating specific activities of bacteria faced to a pulsed substrate supply, we highlighted the versatility of bacterial metabolism, which would be difficult to observe in steady-state conditions. O_2 concentration measurements revealed that respiration rates sharply increased as soon as substrate was introduced to the culture. This increase was so rapid that we probably underestimated O_2 consumption due to the time lag between substrate addition in the culture, sampling, and respiration rate measurements. This conclusion is certainly true for DOC measurements, because if bacterial respiration rates increased so rapidly, they obviously consumed DOC very quickly after substrate addition. We may introduce the notion of population synchronisation as bacteria are constrained by the presence or absence of food. This synchronisation may be due to several factors, such as the stress generated by the absence of substrate and the large energy requirement for maintenance. Consequently, when we added substrate to the culture, the bacteria became simultaneously active to assimilate and grow, and cells continued maintenance even when the substrate was totally exhausted. This metabolic flexibility is necessary to cope with the in situ heterogeneity of a largely oligotrophic and ever-changing environment, and may result from the uncoupling of anabolic and catabolic processes (del Giorgio and Cole, 1998). We believe that the situation is the same in natural seawater, as food is not continuously available (Hanegraaf and Kooi, 2002) and bacteria may have to face long periods of absence of one or more nutrients (Konopka, 1999) and short periods of high substrate availability. Thus, by carrying out batch biodegradation experiments using in situ samples, bacteria may be in one or another situation. It is important to note that because bioassays often eliminate the effects of DOC production processes, these incubations only assess the standing stock of L-DOC at a given time (Raymond and Bauer, 2000). The resulting BGE values

are obviously affected by the temporal variation of substrate availability and are finally not necessarily representative of the studied site. Therefore, extrapolating BGE values from incubations to the field may result in misrepresentation. Consequently, we have to be very cautious when comparing BGE from different study sites and periods, without considering the "history" of the water mass. In a study using daily sampling of DOC in the central equatorial Pacific Ocean, Carlson and Ducklow (1995) demonstrated that they sampled different water each day because of the upstream.

4.3 Higher BGE in a realistically perturbed environment

In this study, four constant BGE values (one empirical and three modelled BGE) differed, but they were always higher in the pulse experiment. It seems that bacteria were unable to efficiently grow when large amounts of substrate were present, whereas growth was stimulated when the same amount of substrate was added periodically. Results on higher BGE values in transient environments are consistent with the analysis of BGE values in diverse aquatic systems described in the literature. For instance, BGE values are higher in estuarine when compared to open ocean systems (del Giorgio and Cole, 1998). Indeed, estuarine systems are more influenced by episodic inputs of DOC compared to oceans. Raymond and Bauer (2000) reported a negative relationship between the L-DOC concentration and BGE in an estuarine system. This outcome could also mean that these higher BGE values resulted from a frequent input of L-DOC, but in a low concentration, which is unfortunately difficult to measure in situ. If considering oceanic systems, our results confirm those of Coffin et al. (1993) who reported a marked diel cycle, with BGE ranging from 37 to 72% and increasing during the day, presumably following inputs of algae derived organic substrates.

We also demonstrated that BGE values were higher when maintenance was taken into account in the calculation. BGE are generally estimated from steady-state experiments without considering maintenance. This would mean that these widely used methods underestimate BGE values. It is commonly assumed that the complementary proportion (1-BGE) represents the fraction of DOC that is released as metabolic

CO_2 in the medium. This would finally lead to the conclusion that most of the previous studies investigating BGE using batch experiments overestimate the role of bacteria as CO_2 producers. It is thus important to take into account the spatial and temporal variability of DOC when assessing and quantifying the role of bacteria in the oceanic carbon cycle. We need to find a more consistent method for investigating bacterial growth and utilisation of DOC in natural environments in order to correctly compare results from different study sites and periods. By using a daily sampling strategy in the Pacific Ocean, during two periods in the spring and autumn, Carslon and Ducklow (1995) demonstrated the presence of a high frequency variability of bulk DOC, which is driven by physical processes. By using the same sampling strategy and carrying out degradation experiments on each water sample, one could examine the effect of this variability on bacterial metabolism and BGE.

4.4 More consideration for estimating BGE

In this study, the coupling of the experimental and modelling work made possible to estimate BGE values using two methods: the empirical (by calculating ΔDOC and ΔPOC) and the modelling methods (by calibrating three models on the data sets). However, most studies estimate BGE values via the utilisation of indirect methods by considering that BCD=BP+BR contrary to BCD=ΔDOC. To estimate BR, experimentalists generally apply a linear regression on the O_2 dynamics and only consider the slope of this regression (del Giorgio and Cole, 1998; Eichinger et al., 2006). However, our experiments clearly demonstrated that the BR value varies greatly during an experiment, being high during assimilation and low during starvation periods. The non systematic linearity of BR, and its impact on BGE estimation, has already been demonstrated by performing continuous oxygen measurements with oxygen microprobes (Briand et al., 2004). BP is generally estimated from radiolabeled thymidine or leucine incorporation or by the difference between the final and initial bacterial abundances. However, these estimates rely on various conversion factors that have great uncertainties (Jahnke and Craven, 1995). We also demonstrated in this study that these factors may vary in time.

Indeed, we obtained values for the specific POC content, which are equivalent to the carbon content factor (CCF) commonly used to convert bacterial density into bacterial biomass, varying from 3 to 38 fmol C bact^{-1}. This result means that we may produce an error of factor 10 when estimating bacterial carbon from bacterial density. The specific POC content always increased after substrate assimilation, reflecting the capability of bacteria to store carbon. It then decreased during starvation periods, until a "threshold" value. This would mean that CCF should be adapted to the physiological state of bacteria, which depends on substrate availability.

5 Conclusions

Numerous studies have investigated how biological, chemical, and physical factors affect BGE values (del Giorgio and Cole, 1998). However, there have only been a few investigations dealing with daily and detailed seasonal variation of natural BGE (del Giorgio and Cole, 1998). To the best of our knowledge, this is the first study demonstrating that the temporal variation in substrate availability greatly influences BGE, which may be twice as high in pulsed experimental conditions. Our simulated temporal variation of the DOC supply can simulate various biological factors occurring in situ, such as intermittent DOC release from phytoplankton and zooplankton, and transient physical forcing, as in turbulent eddies. This spatio-temporal variability of DOC distribution in the field would make BGE highly variable and makes it difficult to apprehend in situ. More experiments are however required to confirm our results, for example measuring nutrient concentration and progressively incorporating more natural DOC sources. However, we should still consider the pulse load of substrate and other kinds of variable inputs. The outcome of this study is even more important knowing that model formulation and parameter estimation from experimental dynamics are often used in global models to investigate the oceanic carbon cycle.

Acknowledgements. The authors received financial support from the LMGEM research grant, from the French National Program for Coastal Environment (AMPLI Project) and Foundation TOTAL from the REMECCA program. Ph.D. scholarships for M.E. came from the French Ministry of Research and Education. We would like to thank the Service Central d'Analyse du CNRS for POC analyses and the Regional Flow Cytometry Platform for Microbiology (PRECYM) on the Oceanology Centre of Marseille. Authors are grateful to M. Garel and A. Robert for their help in the experimental conception and realisation, and to T. Bentley for editorial assistance.

The publication of this article is financed by CNRS-INSU.

References

Abboudi, M., Jeffrey, W. H., Ghiglione, J.-F., Oriol, L., Sempéré, R., Charrière, B., and Joux, F.: 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(2), 344–357, doi:10.1007/s00248–007-9280-8, 2007.

Amon, R. M. W. and Benner, R.: Bacterial utilization of different size classes of dissolved organic matter, Limnol. Oceanogr., 41(1), 41–51, 1996.

Anderson, T. R. and Williams, P. J. L. B.: Modelling the seasonal cycle of dissolved organic carbon at station E-1 in the English Channel, Estuar. Coast. Shelf S., 46(1), 93–109, 1998.

Anderson, T. R. and Williams, P. J. L. B.: A one-dimensional model of dissolved organic carbon cycling in the water column incorporating combined biological-photochemical decomposition, Global Biogeochem. Cy., 13(2), 337–349, 1999.

Baretta-Bekker, J. G., Baretta, J. W., and Rasmussen, E. K.: The microbial food web in the European Regional Seas Ecosystem Model, Neth. J. Sea Res., 33(3/4), 363–379, 1995.
Blackburn, N., Zweifel, U. L., and Hagstrom, A.: Cycling of marine dissolved organic matter II., A model analysis, Aquat. Microb. Ecol., 11(1), 79–90, 1996.
Briand, E., Pringault, O., Jacquet, S., and Torréton, P.: The use of oxygen microprobes to measure bacterial respiration for determining bacterioplankton growth efficiency, Limnol. Oceanogr.-Meth., 2, 406–416, 2004.
Cajal-Medrano, R. and Maske, H.: Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton – revisiting the Pirt model, Aquat. Microb. Ecol., 19(2), 119–128, 1999.
Cajal-Medrano, R. and Maske, H.: Growth efficiency and respiration at different growth rates in glucose-limited chemostats with natural marine bacteria populations, Aquat. Microb. Ecol., 38(2), 125–133, 2005.
Carlson, C. A. and Ducklow, H. W.: Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean, 1992: daily and finescale vertical variations, Deep-Sea Res. Pt. II, 42, 639–656, 1995.
Carlson, C. A. and Ducklow, H. W.: Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea, Aquat. Microb. Ecol., 10(1), 69–85, 1996.
Carlson, C. A., Bates, N. R., Ducklow, H. W., and Hansell, D. A.: Estimation of bacterial respiration and growth efficiency in the Ross Sea, Antarctica, Aquat. Microb. Ecol., 19(3), 229–244, 1999.
Carlson, C. A., Giovannoni, S. J., Hansell, D. A., Goldberg, S. J., Parsons, R., Otero, M. P., Vergin, K., and Wheeler, B. R.: Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea, Aquat. Microb. Ecol., 30(1), 19–36, 2002.
Carlson, C. A., Giovannoni, S. J., Hansell, D. A., Goldberg, S. J., Parsons, R., and Vergin, K.: Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea, Limnol. Oceanogr., 49(4), 1073–1083, 2004.
Cherrier, J., Bauer, J. E., and Druffel, E. R. M.: Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern north Pacific surface waters, Mar. Ecol.-Prog. Ser., 139(1–3), 267–279, 1996.
Cherrier, J. and Bauer, J. E.: Bacterial utilization of transient plankton-derived dissolved organic

carbon and nitrogen inputs in surface ocean waters, Aquat. Microb. Ecol., 35(3), 229–241, 2004.
Coffin, R. B., Connolly, J. P., and Harris, P. S.: Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization, Mar. Ecol.-Prog. Ser., 101, 9–22, 1993.
del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu. Rev. Ecol. Syst., 29, 503–541, 1998.
Eichinger, M., Poggiale, J. C., Van Wambeke, F., Lefèvre, D., and Sempéré, R.: Modelling DOC assimilation and bacterial growth efficiency in biodegradation experiments: a case study in the Northeast Atlantic Ocean, Aquat. Microb. Ecol., 43(2), 139–151, 2006.
Eichinger, M., Kooijman, S. A. L. M., Sempéré, R., Lefèvre, D., Grégori, G., Charrière, B., and Poggiale, J. C.: DOC consumption and release by marine bacteria in pulsed-substrate environment: from experiments to modelling, Aquat. Microb. Ecol., 56(1), 41–54, 2009.
Goldman, J. C., Caron, D. A., and Dennett, M. R.: Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio, Limnol. Oceanogr., 32(6), 1239–1252, 1987.
González, N., Anadón, R., and Marañón, E.: Large-scale variability of planktonic net community metabolism in the Atlantic Ocean: importance of temporal changes in oligotrophic subtropical waters, Mar. Ecol.-Prog. Ser., 233, 21–30, 2002.
Hanegraaf, P. P. F. and Kooi, B. W.: The dynamics of a tri-trophic food chain with two-component populations from a biochemical perspective, Ecol. Model., 152(1), 47–64, 2002.
Hansell, D. A., Bates, N. R., and Gundersen, K.: Mineralization of dissolved organic carbon in the Sargasso Sea, Mar. Chem., 51(3), 201–212, 1995.
Hedges, J. I.: Global biogeochemical cycles: progress and problems, Mar. Chem., 39, 67–93, 1992.
Jahnke, R. A. and Craven, D. B.: Quantifying the role of heterotrophic bacteria in the carbon cycle – a need for respiration rate measurements, Limnol. Oceanogr., 40(2), 436–441, 1995.
Konopka, A.: Theoretical analysis of the starvation response under substrate pulses, Microb. Ecol., 38(4), 321–329, 1999.
Kooi, B. W. and Kooijman, S. A. L. M.: The transient-behaviour of food-chains in chemostats, J. Theor. Biol., 170(1), 87–94, 1994.
Kooijman, S. A. L. M.: Dynamic energy and mass budgets in biological systems, 2nd ed., Cambridge University Press, Cambridge, 424 pp., 2000.
La Ferla, R., Azzaro, F., Azzaro, M., Caruso, G., Decembrini, F., Leonardi, M., Maimone, G.,

Monticelli, L. S., Raffa, F., Santinelli, C., Zaccone, R., and Ribera d'Alcalá, M.: Microbial contribution to carbon biogeochemistry in the Central Mediterranean Sea: variability of activities and biomass, J. Marine Syst., 57, 146–166, 2005.
Lancelot, C., Staneva, J., Van Eeckhout, D., Beckers, J. M., and Stanev, E.: Modelling the Danube-influenced north-western continental shelf of the Black Sea. II: Ecosystem response to changes in nutrient delivery by the Danube River after its damming in 1972, Estuar. Coast. Shelf S., 54(3), 473–499, 2002.
Lyman J. and Fleming R.: Composition of sea water, J. Mar. Res., 3, 134–146, 1940.
Marr, A. G., Nilson, E. H., and Clark, D. J.: The maintenance requirement of *Escherichia coli*, Ann. NY Acad. Sci., 102, 536–548, 1963.
Monod, J.: Recherches sur la croissance des cultures bactériennes, Ph.D. thesis, University of Paris, Paris, France, 1942.
Nagata, T.: Production mechanisms of dissolved organic matter, in: Microbial Ecology of the Oceans, edited by: Kirchman, D. L., Wiley-Liss, New York, 121–152, 2000.
Pirt, S. J.: The maintenance energy of bacteria in growing cultures, P. Roy. Soc. Lond. B Bio., 163, 224–231, 1965.
Pomeroy, L. R.: The ocean's food web, a changing paradigm, Bioscience, 24, 499–504, 1974.
Raymond, P. A. and Bauer, J. E.: Bacterial consumption of DOC during transport through a temperate estuary, Aquat. Microb. Ecol., 22, 1–12, 2000.
Reinthaler, T. and Herndl, G. J.: Seasonal dynamics of bacterial growth efficiencies in relation to phytoplankton in the southern North Sea, Aquat. Microb. Ecol., 39(1), 7–16, 2005.
Rivkin, R. B. and Legendre, L.: Biogenic carbon cycling in the upper ocean: effects of microbial respiration, Science, 291(5512), 2398–2400, 2001.
Sempéré, R., Van Wambeke, F., Azourmanian, H., Chambaut, A. L., Ferrière, L., and Bianchi, M.: On the use of batch systems to determine DOC bacterial lability and bacterial growth efficiency in seawater samples, in: Integrated Marine System Analysis, edited by: Bayens, J., Dehairs, F., and Goyens, L., European Network for integrated Marine System Analysis, Brussels, 233–238, 1998.
Sempéré, R., Yoro, S. C., Van Wambeke, F., and Charrière, B.: Microbial decomposition of large organic particles in the northwestern Mediterranean Sea: an experimental approach, Mar. Ecol.-Prog. Ser., 198, 61–72, 2000.
Sohrin, R. and Sempéré, R.: Seasonal variation in total organic carbon in the Northeast Atlantic in 2000–2001, J. Geophys. Res.-Oceans, 110, C10S90, doi:10.1029/2004JC002731, 2005.

Tolla, C., Kooijman, S. A. L. M., and Poggiale, J. C.: A kinetic inhibition mechanism for maintenance, J. Theor. Biol., 244(4), 576–587, 2007.
Williams, P. J. L. B.: Heterotrophic bacteria and the dynamics of dissolved organic material, in: Microbial Ecology of the Oceans, edited by: Kirchman, D. L., Wiley-Liss, New York, 153–199, 2000.
Williams, P. M. and Druffel, E. R. M.: Radiocarbon in dissolved organic-matter in the central north Pacific-Ocean, Nature, 330(6145), 246–248, 1987.

Table 1. Symbols, units and descriptions of notations, state variables and parameters used in each model.

Symbol	Unit	Description	Models		
			Monod	Marr-Pirt	DEB
General notations					
j_{*1*2}	h^{-1}	Specific flux of state variable $_{*1}$ associated with process $_{*2}$	x	x	x
y_{*1*2}	–	Yield coefficient (efficiency) of state variable $_{*2}$ on state variable $_{*1}$	x	x	x
Processes					
A		Assimilation	x	x	x
M		Maintenance	x	x	x
State variables					
L	mM C	Substrate (L-DOC) concentration	x	x	x
M_E	mM C	Reserve concentration			x
M_V	mM C	Structure concentration			x
B_B	mM C	Bacterial biomass	x	x	
R	mM C	R-DOC (non-labile DOC) concentration		x	x
Parameters					
j_{LAm}	h^{-1}	Maximum specific substrate utilisation rate	x	x	x
K	mM C	Half-saturation constant	x	x	x
$\alpha = j_{LAm}/K$	mM C^{-1} h^{-1}	Ratio between j_{LAm} and K	x	x	x
k_E	h^{-1}	Reserve turnover rate			x
y_{M_EL}	–	Yield coefficient from L-DOC to reserve			x
y_{B_BL}		Yield coefficient from L-DOC to biomass	x	x	
$y_{M_EM_V}$	–	Yield coefficient from structure to reserve			x
y_{RM_V}	–	Yield coefficient from structure to R-DOC			x
y_{RB_B}	–	Yield coefficient from biomass to R-DOC		x	
j_{M_EM}	h^{-1}	Maintenance flux from reserve			x
j_{B_BM}	h^{-1}	Maintenance flux from biomass		x	
j_{M_VM}	h^{-1}	Maintenance flux from structure			x

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Table 2. Parameter values for each of the three models for experiment B. POC and DOC data were corrected for bacteria passing through the filters. Parameters were estimated by the minimisation of the sum of squared deviations of model predictions to data points. As the Monod model cannot produce any release of non-labile material, DOC concentrations were modified by subtracting the R-DOC concentration at the end of the experiment from each DOC data point.

Parameters	Monod	Marr-Pirt	DEB
α	0.167	0.104	0.212
k_{E}	–	–	0.201
$y_{M_{\mathrm{E}}L}$		–	0.211
$y_{B_{\mathrm{B}}L}$	0.142	0.203	–
$y_{M_{\mathrm{E}}M_{\mathrm{V}}}$	–	–	1.000
$y_{RM_{\mathrm{V}}}$	–	–	1.000
$y_{RB_{\mathrm{B}}}$	–	1.000	–
$j_{M_{\mathrm{E}}M}$	–	–	0.021
$j_{B_{\mathrm{B}}M}$	–	0.012	–
$j_{M_{\mathrm{V}}M}$	–	–	0.006

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Table 3. Parameter values for each of the three models for experiment P. Parameters were estimated by the minimisation of the sum of squared deviations of model predictions to data points. As the Monod model cannot produce any release of non-labile material, DOC data were modified to account for only the labile part of DOC by using the following equation: L-DOC=DOC−(0.0038t+0.1067). This does not affect parameter and BGE estimations.

Parameters	Monod	Marr-Pirt	DEB
α	0.364	0.347	0.484
k_{E}	–	–	0.603
$y_{M_{\mathrm{E}}L}$	–	–	0.500
$y_{B_{\mathrm{B}}L}$	0.234	0.382	–
$y_{M_{\mathrm{E}}M_{\mathrm{V}}}$	–	–	1.492
$y_{RM_{\mathrm{V}}}$	–	–	1.000
$y_{RB_{\mathrm{B}}}$	–	0.855	–
$j_{M_{\mathrm{E}}M}$	–	–	0.000
$j_{B_{\mathrm{B}}M}$	–	0.004	–
$j_{M_{\mathrm{V}}M}$	–	–	0.008

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Table 4. Summary of the different formulae used to estimate BGE, directly from data points (experimental) or with three models (Monod, Marr-Pirt and DEB models) for the pulse (P) and batch (B) experiments, and their corresponding values. The right side of the analytical formulae has been calculated with equations of each model (Eq. 1–3). The last column represents parameters of each model to which BGE values corresponds analytically (see Fig. 5).

Method of BGE estimation	Analytical BGE formula	P experiment	B experiment	Parameter equivalent with BGE value
Experimental	$\frac{\Delta POC}{-\Delta DOC}$	0.27	0.14	
Monod model	$\frac{dB_B}{-dL} = y_{B_BL}$	0.23	0.14	y_{B_BL}
Marr-Pirt model	$\frac{dB_B}{-dL} = y_{B_BL} - \frac{j_{B_BM}}{\alpha L}$	0.38	0.20	y_{B_BL}
DEB model	$\frac{d\left(M_E/y_{EV}+M_V\right)}{-dL} = \frac{y_{M_EL}}{y_{M_EM_V}} - \frac{j_{M_EM}}{y_{M_EM_V}\alpha L}$	0.34	0.21	$\frac{y_{M_EL}}{y_{M_EM_V}}$

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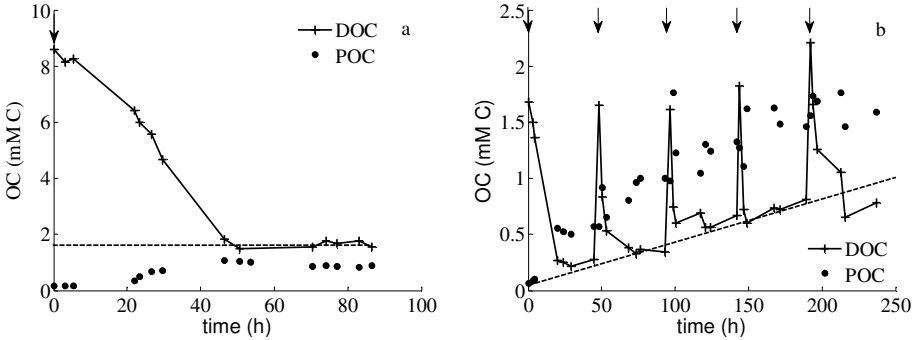

Fig. 1. Concentrations of dissolved and particulate organic carbon (DOC and POC, in mM C) measured in **(a)** the B and **(b)** the P experiments. Arrows represent time when substrate was added to the cultures. DOC represents the substrate (pyruvate) plus all other DOC forms produced during the experiment, and POC represents the bacterial biomass. DOC dynamics are visualized by lines connecting the data points. Dashed lines=DOC accumulation throughout the experiment. OC is organic carbon.

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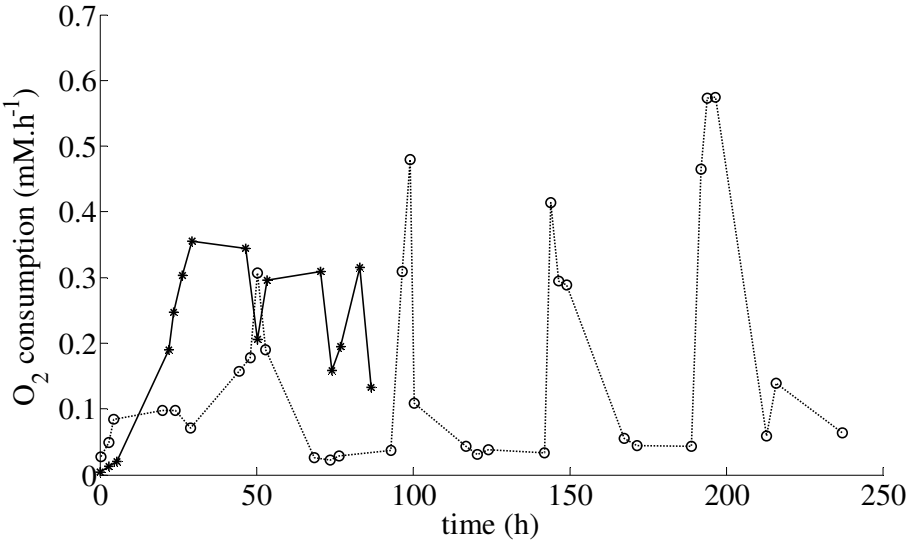

Fig. 2. Dynamics of O_2 consumption (mM h^{-1}) as calculated from the decrease in O_2 concentration at each sampling time for the B (*) and the P (∘) experiment. Data points are connected by solid and dashed lines for the B and P experiment, respectively.

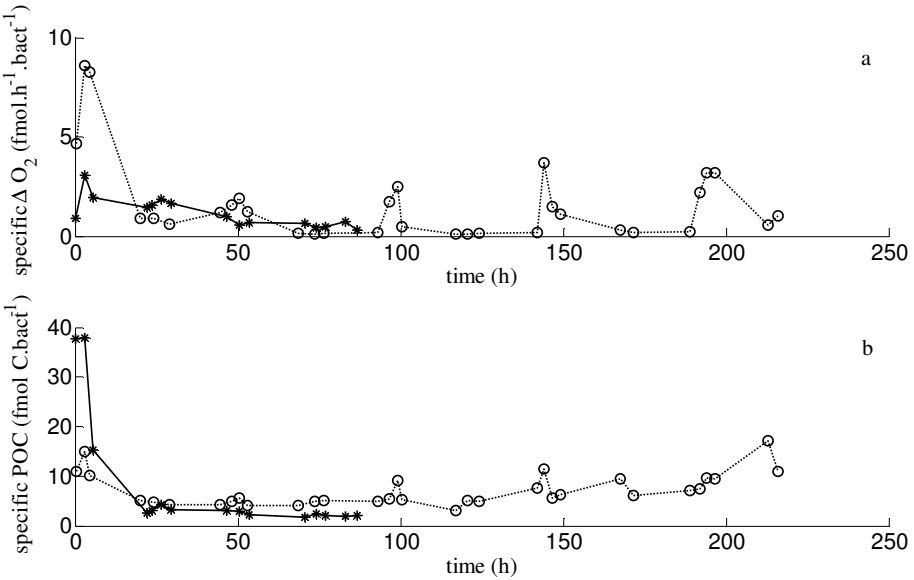

Fig. 3. Dynamics of **(a)** specific O_2 consumption (fmol h^{-1} $bact^{-1}$) estimated as the ratio between O_2 consumption and bacterial density and **(b)** specific POC (fmol C $bact^{-1}$) estimated as the ratio between POC concentration and bacterial density for the B (*) and the P (∘) experiments. Data points are connected by solid and dashed lines for the B and P experiment, respectively.

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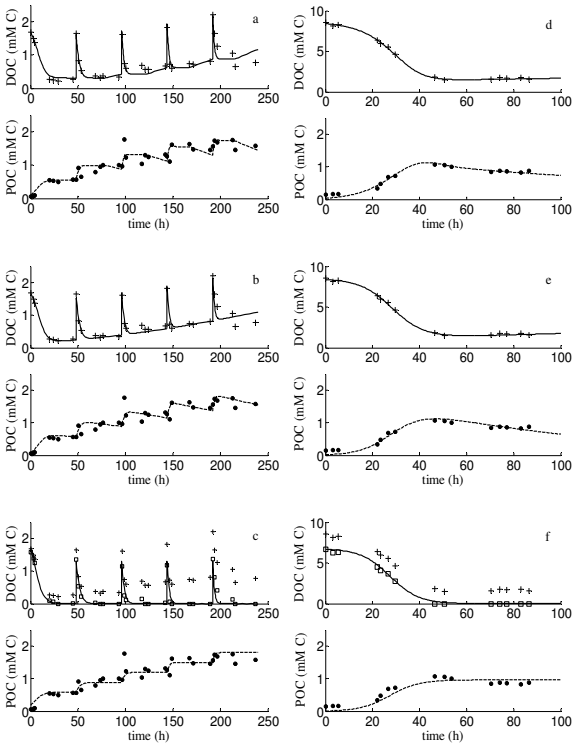

Fig. 4. Simulations of DOC (solid lines, mM C) and POC (dashed lines, mM C) concentrations for **(a)** the DEB, **(b)** Marr-Pirt and **(c)** Monod models for experiment P and for **(d)** the DEB, **(e)** Marr-Pirt and **(f)** Monod models for experiment B. Model simulations are compared to experimental measurements of DOC (+) and POC (•). Parameters of each model and for each experiment are provided in Tables 2 and 3. The Monod model was calibrated on and compared to modified DOC data to deal only with the labile part of DOC. This modified data set is represented by squares in Fig. 4c and f.

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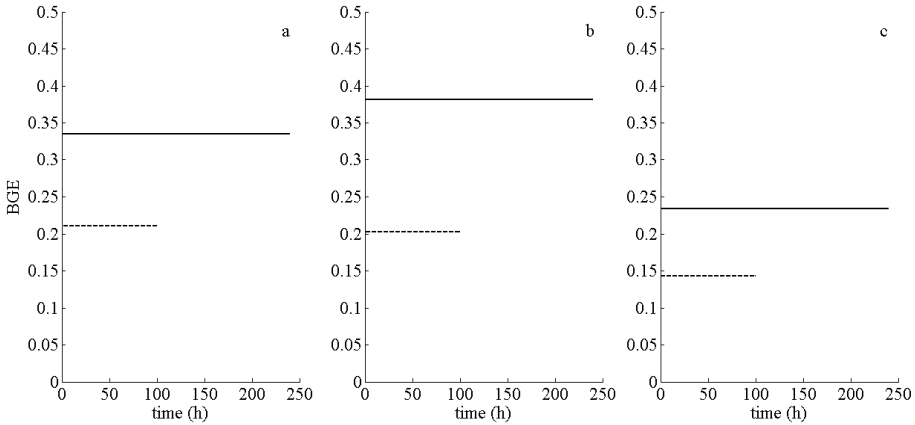

Fig. 5. Dynamics of BGE for the P (solid line) and the B (dashed line) experiments estimated with **(a)** the DEB, **(b)** Marr-Pirt and **(c)** Monod models. BGE were estimated as the ratio between the variation of the bacterial biomass and the variation of DOC. Their formula are summarised in Table 4.

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