

## ***Interactive comment on “Increased bacterial growth efficiency with environmental variability: results from DOC degradation by bacteria in pure culture experiments” by M. Eichinger et al.***

**M. Eichinger et al.**

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We would like to thank reviewer 2 for his relevant comments that allowed us improving our manuscript, specifically for a better understanding of model equations.

Reply to general comments :

In the methods section I would have liked to see a bit more on how the DOC and POC were measured including analytical details.

Reply: According to reviewer comments, we will add more analytical details about the DOC and POC measurements in the revised version of the manuscript:

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“DOC and POC were separated using pre-combusted GF/F filters (0.7  $\mu\text{m}$  nominal porosity). After collection, DOC samples were acidified to  $\text{pH} \approx 1$  with 85 % phosphoric acid and bubbled for 10 minutes with  $\text{CO}_2$ -free air to purge inorganic carbon. DOC was measured by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC 5000 Analyzer following the same protocol as Sohrin and Sempéré (2005). Three or four 100  $\text{mm}^3$  replicates of each sample were injected into the column heated at 680  $^\circ\text{C}$ . The coefficient of variation of DOC replicates was always smaller than 2 %. Quantification was performed by a four point-calibration curve with standards (from 0 to 2  $\text{mM C}$  for experiment P and from 0 to 8.5  $\text{mM C}$  for experiment B) prepared by diluting potassium hydrogen phthalate in Milli-Q water. 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. A variable amount of culture was filtered at each sampling time in order to get a reliable POC signal (mean value of 300  $\mu\text{g}$  of carbon on each filter). Following filtration onto the GF/F filter, each filter was dried in an oven (30 $^\circ$ ) carefully stored in a desiccator in the dark and then analysed with a carbon analyzer (Leco SC-144) following the same protocol as Sempéré et al. (2000). Calibration was performed with reference compound in the same order of magnitude than sample. The measurement uncertainty was between 3 and 8 % for these carbon concentrations.”

To my mind there are a lot of abbreviations in the manuscript, which is usually fine but I think abbreviations are easier to remember if they somehow reflect the original term. E.g. why was the abbreviation B chosen for the unpulsed experiment?

Reply: The abbreviation B was initially chosen because it consisted in a typical Batch experiment. To provide a better understanding to readers and according to reviewer comment, we will change this abbreviation to SA, which means experiment with a single addition of substrate.

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The abbreviations used for the different models are unreadable (page 797). Is this usual practice or is it possible to form the abbreviations in the equations a little more incisive?

Reply: We agree on the fact that equations may be difficult to read. We choose to use DEB notation, even for non-DEB models, for a better consistency in the manuscript but also with previously published articles dealing with DEB models. According to reviewer comment, we decided to completely change the abbreviations in the equations and choose abbreviations that fit more to biogeochemical studies. As an example, all maintenance rates will be written  $\text{maint}^*$ , where  $*$  stands for the corresponding state variable, instead of using the notation  $j^{**}$ . However, as the DEB model has been previously published and to provide a good understanding to readers, the table of parameters will give the original DEB notation in parallel, even if it is not used in the present study. We will also simplified the writing of the DEB model.

Why was oxygen consumption measured? I did not quite understand why this measurement was not used to calculate BGE?

Reply: Oxygen consumption was measured to highlight the maintenance process and also to have a "metabolic" indicator of bacterial response to substrate pulses. Oxygen consumption values were not used afterwards, neither for BGE estimation, nor for modelling purposes. However, oxygen consumption dynamic allowed putting forward the required assumptions for the use of the different models. In the discussion (P. 807 L. 20-22 in the original version), we explained that we did not use these measurements to calculate BGE because "our experiments clearly demonstrated that the BR value varies greatly during an experiment, being high during assimilation and low during starvation periods". Because of this variability, it is not conceivable to choose the most appropriate BR value to calculate the BGE of a given experiment. However, according to reviewer 1 comments, we noticed that oxygraph measurements only provide potential respiration rates ( $V_{\text{max}}$ ). We thus calculated BR from the equation of mass balance ( $\text{BR} = (\Delta\text{DOC} - \Delta\text{POC}) / \Delta t$  (where  $t$  is time)) over the whole experiments and obtained

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coherent BR values (lower than values obtained with oxygraph): BR is about 60% of  $\text{O}_2$  saturation for the pulse experiment and this calculation showed that the culture was  $\text{O}_2$  depleted after few hours for the experiment B. However, as experimental bottles were frequently opened and shaken for sampling and thus the cultures were frequently aerated, we may assume that oxygen limitation did never occurred (see reply to general comments of reviewer 1 for more details).

I think that BGE estimates from an artificial experiment as presented here are as good or bad as the in situ measurements. Most of the more recent field data suggest low BGEs (including all the probable biases of conversion factors etc.) and these data span quite some temporal and spatial scales. Most of these data have been acquired from oxygen consumption measurements and bacterial production estimates for good reasons: The needed analytical precision and accuracy of DOC measurements is usually not sufficient to measure decrease in a sensible time. Furthermore I think there are more papers on temporal and spatial dynamics in BGE estimates from the field than discussed (cited). Overall I would suggest to edit the discussion such that it is clear(er) to reader what potential insight can be gained from the conducted experiment and compare this better with reports from the field. I.e. the shortcomings of both approaches should be discussed in more detail and thus overall the discussion is not really satisfying.

Reply: We agree with the reviewer on the fact that this approach could not be applied for in situ investigations. As he/she said, the analytical precision and accuracy of DOC measurements are not sufficient. Moreover, we could not related POC measurements to bacterial biomass, as done in our study. We used high substrate concentration due to this DOC analytical limitation and only used one bacterial strain. We are aware that we worked in idealized situation, but this choice was made for the modelling purpose: without the appropriate measurements and accuracies, models could not be calibrated accurately, and then compared on the basis of their parameter values. Indeed, the aim of this manuscript was to compare two systems, one affected by frequent inputs of

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substrate and one with a single addition. Values of obtained BGE are representative of this specific strain consuming this particular substrate. It is certainly not comparable to BGE calculated from in situ sampling. However, main results from our investigations are transposable to any situation: (1) in a system affected by frequent input of DOC/substrate, BGE will be higher than in a “one dose” system. Consequently, the estimation of BGE in such transitory system with typical batch/incubation experiments would certainly be underestimated; (2) estimation of BGE with methods that did not take maintenance into account (as the typical estimation with  $BGE=BP/(BP+BR)$ ) also underestimated BGE values compared to methods that did (as the Marr-Pirt and DEB models here). Of course, such estimations are difficult to realise from in situ sampling due to the analytical limitations mentioned above, and we for instance can not propose a better way to estimate BGE from field. However, we think that these results need to require our attention and to be kept in mind when BGE values are used to extrapolate the role of bacteria in ecosystem and specifically when they are used in biogeochemical modelling purposes. According to reviewer comment, we will improve our discussion to include more papers on temporal and spatial dynamics in BGE estimates from the field and to make clear(er) to readers what potential insight can be gained from the conducted experiment and compare this better with reports from the field.

Specific comments:

Material and Methods:

Maybe I understood something wrong but was there only one experiment conducted?

Reply: No, two experiments were conducted: one typical batch experiments (B in the original manuscript), where all the substrate was introduced in the culture at the beginning of the experiment, and one pulse experiment (P), where the substrate was frequently pulsed in the culture (P. 793 L. 1-2 in the original version: “Two experiments were performed: one using a single substrate addition (B), and one using pulse additions of substrate (P)”).

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Page 795, line 7: DAPI is 4',6-diamidino-2-phenylindole

Reply: Indeed, we will correct this in the revised version of the manuscript.

Results:

Page 796, line 16 :recalcitrant-to-degradation DOC: It's not clear to me what that is. Please describe differently.

Reply: As stated in the “Main concepts” part (P. 792 L. 9-13 in the original manuscript), we divided the total DOC pool into two components: the L-DOC pool, which corresponds to the substrate and is rapidly consumed by the cultured bacteria, and the R-DOC considered being the DOC remaining at the end of the experiments. This R-DOC corresponds to the recalcitrant-to-degradation DOC. However, in the nomenclature generally used when dealing with DOC, this R-DOC could consist of SL-DOC or R-DOC for our bacterial strain. For a better understanding to readers, we will remove the term recalcitrant-to-degradation DOC and keep the term refractory DOC in the whole manuscript.

Page 801, line 15-16: Flowcytometry or epifluorescence microscopy is quite sensitive, thus I don't think that the accuracy is a problem here? For the POC measurements I cannot tell as the analytical basic information is missing in the methods section. In any case, I suggest to rephrase this sentence to: Probably due to the low POC concentrations at the onset of the experiment accurate measurements were difficult (or similar).

Reply: As mentioned above, we will add more analytical details about the DOC and POC measurements in the revised version of the manuscript. As stated in the “Experimental design” part (P. 793 L. 14-16), because of the large volumes needed for sampling experiment P, 3 replicate bottles were used and successively sampled (after having checked the reproducibility of the experiment). At  $t_0$ , each of these 3 bottles was sampled but only value from the first bottle was reported in the manuscript. We ob-

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tained specific POC values ranging from 11 to 20 fmolC.bact<sup>-1</sup>. Each culture medium was prepared the same way and cultures were inoculated from the same bacterial culture, with the same volume. We estimated the POC measurement uncertainty being between 3 and 8 % for these carbon concentrations. However, as these measurements originate from the same initial culture, this difference may be due either to the measurement uncertainty, which might be higher for low POC concentrations, or to the difficulty of performing accurate measurements at the onset of the experiment, due to low POC concentrations. The conclusion is thus the same when comparing initial values of experiments B and P. This will be better explained in the revised version of the manuscript.

Page 802, paragraph 3 and 4: Is there any value e.g. a coefficient of variation that could indicate to the reader the goodness of fit for the different models? A good or not so good fit are quite subjective terms.

Reply: We plotted simulated organic carbon (DOC and POC together for both experiments) against measured organic carbon for each model, and calculated the correlation coefficient for each relationship  $y=a.x$ , where  $y$  represent simulated concentrations,  $x$  measured concentrations and  $a$  the slope of the relationship (not shown). We obtained the following results: for the DEB model:  $a=0.9921$  ( $R^2=0.9925$ ); for the Marr-Pirt model:  $a=0.9916$  ( $R^2=0.9927$ ); for the Monod model:  $a=1.0176$  ( $R^2=0.9876$ ). Globally, each model accurately reproduced experimental data with slope very close to 1 and very good correlation coefficients. Correlation coefficient for the Monod model, although quite high, is lower than those of other models. According to these values, we did not believe as a bonus to calculate the significance of these results. However, it has to be noted that DOC data have been modified for the application of the Monod model as it does not allow production of refractory material (the correlation has been calculated here after data modification). Consequently, this model is in any case unable to reproduce our experimental data sets without any modification and could be automatically “disqualified”. Due to its wide utilisation, we decided to keep it for our BGE

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analysis. We decided to not incorporate this analysis of correlation between models and data in the manuscript because our study does not deal with the capability of a model to reproduce data, but with the interest of using model (1) to determine key processes of bacterial metabolism and (2) to compare experimental results, here focused on the estimation of the BGE. Moreover, even if the criterion would be significantly better for a model, this does not necessarily mean that this model better represents the biology of the studied organism. As an example, a purely statistical model could be more correlated to observations than a mechanistic model; however this statistical model could not provide any insight on processes occurring during an experiment, whereas the mechanistic model could, although the fit is worst.

Discussion:

Page 803, line 17: Were the respiration measurements used for anything else than indicating the maintenance respiration? I would suggest to discuss a little more on maintenance respiration and the problems surrounding it. There have been attempts before that try to measure this.

Reply: Respiration measurements were indeed used to indicate maintenance respiration, but also to highlight the very rapid bacterial response to substrate pulses. According to reviewer comments, maintenance respiration and the problems surrounding it will be discussed a little more in the revised manuscript.

Page 804, line15-18: This sentence is not clear to me. Please rephrase.

Reply: We will replace the sentences “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” (Page 804, L. 13-17 in the original manuscript) by “We showed previously the existence of a maintenance process, characterised by non

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negligible respiration values during starvation periods. Due to the presence of this maintenance, the Monod model was weak in reproducing the experimental dynamics. However, even without the evidence of the maintenance by respiration measurements, which were not considered in the model, this phenomenon was also evident from decreasing POC concentrations during starvation periods, dynamics that can not be produced with the Monod model”

Page 804, line 24: ... maintenance from the reserve, component that would not... Either something is missing here or it's a copy and past error. Please rephrase.

Reply: Our sentence was a little bit confusing. We here compared the Marr-Pirt and DEB models in term of parameter values, and extrapolated what could happen if experimental conditions would be different. We will rephrase this by “For the experiment P, maintenance from the reserve (maintE) in the DEB model was negligibly low. As this parameter constitutes one of the major difference between DEB and Marr-Pirt models (Marr-Pirt model does not comprise any reserve compartment), this could explain why we did not observe dynamical differences between both model simulations for the experiment P. On the contrary, still for the DEB model, maintE was not negligibly low for the experiment SA and was even higher than maintenance from structure (maintV). The Marr-Pirt model realised maintenance only from structure (maintV). Let imagine a pulse experiment with the initial conditions as the experiment SA and over a longer pulse period, and with parameter values from the experiment SA for each model. Because in these conditions maintE » maintV for the DEB model, and because the Marr-Pirt model realised maintenance only from structure, we could observe dynamical differences between both models, the Marr-Pirt model being unable to reproduce the experimental dynamics”.

Page 804, line 25-28: I guess the abbreviations of jEM and jVM are typos as they are nowhere introduced

Reply: Indeed, they are typos. This should be jMEM and jMVM instead of jEM and jVM.

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As we will totally change model abbreviations in the revised version of the manuscript, these will be maintE and maintV.

Page 806, line 5-7: The Carlson and Ducklow citation plus the argument the authors draw from it is repeated on page 807 line 8.

Reply: According to reviewer comment, we will remove the sentence p. 806 L.5-7 in the original manuscript and the reference to Carlson and Ducklow (1995). We will keep this reference in p. 807 L. 8 because the argument was more appropriate there.

Page 807, line 28: It is not clear to me where the authors demonstrate the conversion factors of BP may vary. Please explain this part better.

Reply: In fact, we did not demonstrate that conversion factor of BP may vary, but that the carbon conversion factor (CCF) may vary during an experiment (as explained P. 808 L. 1-4). This CCF is often used to estimate the BP from the difference between final and initial bacterial abundances, multiplied by the CCF. We will reformulate this sentence for a better understanding to readers: “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). BP estimation from bacterial abundances requires the utilisation of a carbon content factor (CCF) to go from bacterial density to bacterial biomass. In this study, we demonstrated that the CCF (defined as the specific POC content in this study), varied from 3 to 38 fmolC.bact<sup>-1</sup> along an experiment. The mean CCF for marine bacteria is often considered to be 20 fgC.bact<sup>-1</sup> (Lee & Fuhrman 1987), corresponding to 1.7 fmolC. bact<sup>-1</sup>. By comparison with our results, the utilisation of this mean CCF would lead to an error of factor 20 when estimating bacterial carbon from bacterial density”.

Page 808, line 3: What is the average CCF generally reported and what is the source of these CCFs?

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Reply: The mean CCF for marine bacteria is often considered to be 20 fgC.bact<sup>-1</sup> (Lee & Fuhrman 1987), corresponding to 1.7 fmolC. bact<sup>-1</sup>. However, great variability in CCF values has been reported, depending on several factors. Bratbak (1985) found globally higher CCF in cultures of *Pseudomonas putida* (from 10.75 to 26 fmolC. bact<sup>-1</sup>) compared to mixed cultures of bacteria collected in an estuary (8.8 to 17.8 fmolC. bact<sup>-1</sup>). However, these values also varied according to the limitation (C, N or P), being generally lower when cultures were C-limited. In our experiments, 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. Vrede et al. (2002) also observed CCF variation in function of the growth phase, being larger during exponential growth phase than during stationary phase. This would mean that CCF should be adapted to the physiological state of bacteria, which depends on substrate availability. It has also been shown that CCF is higher at higher temperature (from 0.4 fmolC. bact<sup>-1</sup> at 10°C to 87 fmolC. bact<sup>-1</sup> at 26°C) (Jimenez-Mercado et al., 2007). This study also demonstrated that the BGE, directly calculated from changes in POC and CO<sub>2</sub>, increased with temperature. However, when the BGE was calculated using measured cell abundances and the commonly used CCF of 20 fg C per cell (instead of the direct measurements of POC), the trend reversed. This demonstrated the difficulty of working with this conversion factor, especially because its value may vary in function of the environmental/experimental conditions. As this conclusion is directly related and relevant to our study on BGE determination, it will be added to the revised manuscript.

Litterature cited:

Bratbak G (1985) Bacterial biovolume and biomass estimations. *Applied and Environmental Microbiology* 49:1488-1493

Jiménez-Mercado A, Cajal-Medrano R, Maske H (2007) Marine heterotrophic bacteria in continuous culture, the bacterial carbon growth efficiency, and mineralization at excess substrate and different temperatures. *Microbial Ecology* 54:56–64

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Lee S, Fuhrman JA (1987) Relationships between Biovolume and Biomass of Naturally Derived Marine Bacterioplankton. *Applied and Environmental Microbiology* 53:1298-1303

Vrede K, Heldal M, Norland S, Bratbak G (2002) Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. *Applied and Environmental Microbiology* 68:2965-2971

Page 808, line 12: There are more recent papers on seasonal variations in BGE from the field than the review cited.

Reply: As mentioned above, we will improve our discussion to include more papers on temporal and spatial dynamics in BGE estimates from the field: “Several authors discussed BGE temporal variations (Lemée et al., 2002; Reinthaler et al., 2005; Lee et al., 2009). They all estimated BGE as  $BGE = BP / (BP + BR)$ , where BP was estimated either from bacterial abundance and CCF or from short incubations with 3H-leucine, and BR from Winkler method with short incubation periods and an assumed RQ value of 1. Although study sites were clearly different (tropical coastal waters, open NW Mediterranean Sea and Southern North Sea) BGE ranged from <1% to 43% for all studies. Maximal values were observed in September-October in surface waters of the NW Mediterranean Sea (Lemée et al., 2002) whereas BGE were maximum in spring in the Southern North Sea (Reinthaler et al. 2005). Factors regulating BGE seem to vary according to study site: it has been shown that substrate quality was the most important factor regulating BGE in tropical coastal waters (Lee et al., 2009), whereas BGE was negatively correlated to bacterioplankton richness in the Southern North Sea (Reinthaler et al., 2005). Although numerous other measurements were realised at each sampling in the NW Mediterranean Sea (nutrients, DOC concentration, chl a, primary production), regulatory mechanisms of BGE could not be identified in this investigation. Our results demonstrated that the only fact that DOC concentration (the labile part of DOC) may vary suddenly affect BGE values, which could be an explanation for results of Lemée et al. (2002)”.

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Cited literature:

Lee, C. W., Bong, C. W., and Hii, Y. S.: Temporal variation of bacterial respiration and growth efficiency in tropical coastal waters, *Appl Environ Microb*, 75, 7594–7601, doi:10.1128/AEM.01227-09, 2009.

Lemée, R., Rochelle-Newall, E., van Wambeke, F., Pizay, M. D., Rinaldi, P., and Gattuso, J.-P.: Seasonal variation of bacterial production, respiration and growth efficiency in the open NW Mediterranean Sea, *Aquat Microb Ecol*, 29, 227–237, 2002.

Reinthal, 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.

The figures are too small, particularly fig 4 and 5

Reply: Size of figures will be increased in the revised version of the manuscript.

Fig 4. Why did the authors change the sequence of presenting P and B. I would suggest to leave the original sequence of presentation with B first and than P.

Reply: The sequence of presentation will be changed for Fig. 4 and 5 in the revised manuscript, by starting to present experiment B.

Fig. 5. Why did the authors change the lines for P and B? I suggest to change B to solid and P to dashed.

Reply: We will change B to solid and P to dashed lines in Fig. 5 in the revised manuscript.

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