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Biogeosciences Discussions

Interactive comment on "Assessing approaches to determine the effect of ocean acidification on bacterial processes" *by* Tim J. Burrell et al.

Anonymous Referee #2

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The authors studied the response of bacterial growth, production and hydrolytic activity to acidification comparing three common acidification methods used in ocean acidification research. They conclude that magnitude and direction of the response may depend on the type of manipulation (acid addition, bubbling, gas-permeable tubing). Unfortunately, they did not evaluate two frequently used methods in ocean acidification research: the addition of high CO2/supersaturated seawater and the addition of bicarbonate. Nevertheless, the effect of the acidification methods on bacterial processes has not been compared so far. The presented data are mostly novel and interesting and the subject area is clearly appropriate for publication in Biogeosciences. The experiments were correctly planned, described and thoroughly carried out. For these reasons I think that the manuscript deserves publication in Biogeosciences. There are, however, a couple of points of relatively major nature – especially in discussion and

conclusion section - that the authors should take into account in a revised version of the manuscript:

The title "Assessing approaches to determine the effect of ocean acidification on bacterial processes" implies a broader perspective than provided by the authors. As a reader, I would expect an assessment of OA methods on various bacterial processes such as growth, secondary production and degradation of organic matter. In fact, those processes were studied and data are provided (although only briefly), but unfortunately not discussed in this context. In my opinion, the authors' focus is to narrow on extracellular enzyme activities and substrate fluorescence. I suggest broadening the discussion of the results including OA effects on bacterial growth and production and changing the conclusion section as well as the abstract accordingly. The authors could also speculate in their discussion whether acidification may have influenced enzyme expression or lifetime therefore indirectly affecting enzyme rates.

The authors studied the effect of pH on substrate fluorescence (MUF and MCA) as well as the effect of substrate addition on seawater pH. The addition of MCA affected seawater pH, while the addition of MUF didn't. This is a very interesting result and to my knowledge has not been shown before. The authors should stress their point that buffering is necessary when determining enzyme rates in general, or at least when using MCA as a marker. In contrast, the effect of pH on MUF fluorescence is well known (e.g. Mead et al. 1955), explicitly written in the Sigma product information and usually considered in enzyme rate measurements. Furthermore, I am not convinced by the authors' proposed effect of pH on MCA fluorescence (see specific comments below).

Mead, J. A. R., et al., The biosynthesis of the glucuronides of umbelliferone and 4-methylumbelliferone and their use in fluorimetric determination of beta-glucuronidase. Biochem. J., 61, 569-574 (1955).

Specific comments:

p. 1 l. 17f: Change to “This study investigated the potential artefacts in determining the response of bacterial growth and activity to ocean acidification, and the relative effects of three different acidification techniques.”

p. 1 l.26ff: From the presented results I would conclude that “bubbling may stimulate carbohydrate degradation and bacterial growth”.

p. 2 l.32ff Add some more information on extracellular enzyme characteristics: Enzymes are considered as the rate limiting step in hydrolysis of HMW-substrate by bacteria. Both enzyme groups consist of several isoenzymes that catalyze the same reaction but may vary significantly in e.g. pH or temperature optimum and sensitivity (e.g. broad range or narrow optimum range). Define” extracellular enzyme”. Do you include cell-attached and particle-attached enzymes or only free enzymes?

p. 2 l.56: What are “indirect influences on longer timescales”? Please specify.

p. 3 l. 66ff: The pH sensitivity of MUF is well known (e.g. Mead et al. 1955 and SIGMA product information). Please clarify this in the text.

p. 4 l. 107: see above

p. 5 l. 130ff: It would be very interesting to see the kinetic curves of the independent tests that the authors mention. Please provide a short table or graph. Enzyme kinetics and maximum velocities may vary from one seawater sample to the other (depending on isoenzymes present in the sample). Did you test enzyme kinetics both, in summer and spring?

p. 5 l. 132f: At which pH did you calibrate MUF?

p. 7 l. 182: Please include data (e.g. as supplementary graph)

p. 8 l. 216: Did you determine pH at the end of the incubations?

p. 8 l. 220: Was there a reason to incubate under artificial light instead of dark incubations?

p. 9 l. 270ff: Can you please give the standard deviation of your calibration? An increase by 4% only seems to me very low and within the experimental detection limit. Previous studies did not detect a significant effect of pH on MCA fluorescence and I would not consider 4% to be significant. It would be useful, if you could provide a graph with the calibration curves of both fluorescent markers at pH 8.1 and pH 7.8!

p. 10 l. 299 I agree that different acidification methods had significant effects on BG activities, but I cannot see a significant effect on LAP activity from the presented data (Figure 1). In p. 11 l. 318 the authors state that, although cell-specific LAP activity showed evidence of a response to acidification, this was not significant in either trial. Please explain/clarify and give statistical evidence. It would be also interesting to see the data for AG and AAP activity.

p. 12 l. 337ff: What about total secondary production rates? How do you explain difference towards the end of trial 2? Can you relate it to changes in BG activity?

p. 13 l. 367: The authors state that the introduction of CO2-air gas mixtures using gas-permeable tubing would be the “most robust technique to investigate the response of bacterial processes to future OA conditions”. This is ignoring the fact that there are more techniques commonly used which were not tested in this study and may be even “more robust”. Furthermore, I would conclude from this study that different techniques may result in different results. They may under- or overestimate certain parameters at the same time but not all parameters equally.

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