Biogeosciences Discuss., 12, C2808–C2812, 2015
www.biogeosciences-discuss.net/12/C2808/2015/

Biogeosciences Discussions
Open Access

Interactive comment on "Optimising methodology for determining the effect of ocean acidification on bacterial extracellular enzymes" *by* T. J. Burrell et al.

Anonymous Referee #1

Received and published: 14 June 2015

General comment:

This manuscript describes experiments that have been performed in order to optimize the procedure of extracellular enzyme activity measurements during ocean acidification studies. Enzyme activities were determined using fluorogenic model substrates (with MUF and MCA) following Hoppe (1983); ocean acidification was simulated by addition of HCl, or by direct bubbling with CO2 gas. The study suggests two main actions for optimization, i.e. addition of buffer solution to the fluorogenic model substrate in order to stabilize the sample pH and the application of gas-permeable-silicon tubing for introducing CO2 into seawater.

While both suggestions could be appropriate for some experiments, I am not convinced that the results of this study represent an 'optimal procedure', or can be generalized as 'best practice' approach. Moreover, it remains unclear, which method needs to be improved. The enzyme tests conducted in this study differ largely from previously published studies, and methods often applied for ocean acidification studies, e.g. addition of small volumes of high CO2 seawater, or addition of CO2-supersaturated seawater and addition of bicarbonate were not evaluated. On the other hand, much of the information and conclusions given in this study have been published before or are well known, so that the amount of new information may be insufficient to justify a stand-alone publication.

There is important information missing in the manuscript. First, was the MUF and MCA calibration done with pH adjusted standard solutions? This is how previous studies that investigated ocean acidification effects on enzyme kinetics accounted for the - well known- effect of pH on these chemicals. Second, were the tests to evaluate the need of buffering MCA and MUF solutions conducted with seawater? Third, how relevant is the effect of the chemicals on seawater pH and the pH effect on the fluorophores at the concentration range usually applied for investigations of enzyme kinetics (i.e. concentrations an order of magnitude lower than tested here)? Earlier studies investigated the effect of pH on MUF and MCA solutions for concentrations applied during OA studies and found either no effect (Endres et al. 2014), or could account for the effect by calibration with pH-adjusted standards (Piontek et al. 2010).

Specific comments:

5845, line 3-5: It is well known that pH has an effect on MUF and MCA solutions. This information is even given in the chemical's fact sheet. Previous acidification studies have accounted for this pH effect by using standard solutions that have the same pH as the acidified seawater sample (e.g. Piontek et al. 2010), or tested the effect of pH on the substrate at applied, and more realistic range of substrate concentration (Endres et a. 2014). Were the substrates in this study not calibrated against standard solutions

BGD
12, C2808–C2812, 2015

at the respective pH? This information is missing.

5845, line 6: A widely used approach is the addition of small amounts of CO2 saturated seawater to acidify a sample. This approach is missing here.

5846, line 4-5: Was biofilm growth investigated after the incubations? Attached bacteria may have released enzymes as well.

5846, line 19-21: This means that the plate was open and samples + substrate analogues were incubated in the plate reader for 3h? What about outgassing from the wells? Especially in the low pH treatments; was the pH development in the single wells controlled over time?

5846, line 23-24: Again, was the pH effect accounted for when calculating enzyme kinetics? If the standard solutions were not set to the applied pH, the calculations would be wrong! Were the enzyme rates given in Fig. 1 and 2 corrected for pH effects?

5848: 2.3.1 'The effect of pH on fluorophore fluorescence diluted in two different solutions was investigated, the organic solvent 2-methoxyethanol (Sigma-Aldrich) as well as 0.1M Tris/HCl.' It is unclear if this test was conducted in seawater, or not, as this description only refers to Tris/HCl buffered solutions and methoxyethanol solutions at different pH. Please, clarify. Seawater itself is buffering. Thus, in order to conclude on the necessity to buffer seawater for enzyme kinetics, the tests need to be conducted with seawater.

5848, line 18: A substrate concentration of 1600 μmol is much higher than usually applied in marine enzyme studies. Of course the effect of the substrate on the pH will depend of the substrate concentration. What is the effect at realistic ranges? A substrate concentration of 10-200 μmol L-1 is commonly found to be saturating in marine samples (e.g. Hoppe et al., 1983; Baltar et al., 2009; Maas et al., 2013; Piontek et al., 2013; Endres et al., 2014).

5849, paragraph 2.3.3. I am not sure, if I understood this test correctly. Was it tested

BGD
12, C2808–C2812, 2015

if addition of a solution with a specific pH would affect a solution with the identical pH? What if the solution wasn't buffered; would mixing of two identical pH solutions create something different?

5849, line 10: what was the final concentration of the Tris/MCA substrate?

5850: Why was the method of addition of CO2 supersaturated water not included in longer incubation test to identify the best method for acidification studies?

5850, lines 12-15 and 5856-85858: The test of seawater acidification methodology is not conclusive. The applied methods do not only differ in the way of acidification, but also differ in the way of water treatment. Thus, in order to differentiate between the acidification effect and the treatment effect itself each treatment needs an appropriate control; i.e. bubbling with ambient air in addition to bubbling with high CO2 in order to differentiate between effect of bubbling and effect of CO2, a silicone tube with ambient seawater pH in addition to the one with high CO2 to account for the increased surface area by the tubes and to identify the true acidification effect. I don't think that bubbles in seawater can be regarded as 'artefact'. Imagine a surface ocean without bubbles! Hence, it would be a mistake to generalize the conclusion that bubbling with higher pCO2 air is an inferior methodology compared to the silicone tubing approach (there are no silicone tubes in the ocean). What this simply shows is the need for a proper control; the effect of high CO2 can also be identified in a bubbled assay when compared to a bubbled assay with ambient air. Otherwise one compares apples and oranges.

5851, lines 14-16: were the cells fixed before freezing, i.e. with GDA?

5853, paragraph 3.1.: The results given in this section are difficult to follow and cannot be evaluated. The full dataset is not provided (no figure or table). This is critical since the study concludes that buffering with Tris/HCl is necessary. In the text, it is unclear what results are compared, e.g. non-buffered MUF at 40000 nM with Tris buffered MUF at 200nM? Also, in the method section it says that MUF fluorescence was determined at 4000, 20000 and 40000 nM, but not at 200nM...?. . .. However, as far as I understood

BGD
12, C2808–C2812, 2015

the pH effect becomes smaller with decreasing MUF and MCA fuorescence. So, is there a real need for optimizing this method under naturally low deliberation of MUF and MCA ? Figure 1 shows activities of 0.3 nM L-1 h-1..... Another factor that also has to be considered is a potential effect of the buffer on microbial activity. Was this tested?

5854: The authors speculate about enzyme efficiencies and substrate affinities. As km and Vmax should be available from the enzyme kinetics performed, this could be directly tested (rather than speculated).

Interactive comment on Biogeosciences Discuss., 12, 5841, 2015.