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

Anonymous Referee #2

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General comments:

The authors investigated short- and longer-timescale microbial community and enzyme activity responses to ocean acidification. This is an important subject, and the authors have performed the important service of separating the physico-chemical effect of pH on enzymes from the biological response of microbial heterotrophs to acidification. From a technical standpoint, the experiments are mostly correctly performed, although the choice of buffers may invalidate the peptidase results (see comment for page 5849). There are several important problems with the writing, as detailed below.

I can't quite tell if this manuscript is meant to be a methods paper, as the title suggests,

or an investigation of the short- and medium-term effect of acidification on enzyme activities. As written, it has elements of both. I do not think it rises to the level of an extensive methods optimization paper. Furthermore, I think the authors have made a valuable contribution by separating the physico-chemical response of enzymes to changing pH, from the biological community response. The title and abstract should be rewritten to more accurately represent the manuscript contents.

The introduction contains numerous factual inaccuracies with respect to the effect of pH on enzymes, often supported by incorrectly-chosen references. These are described in detail below, and must be fixed.

Specific comments:

Page 5842 Line 18-20: This is an incomplete sentence.

Page 5843

Line 16: **ALL** enzymes are pH-sensitive!

Line 17: There are competing definitions: see, for instance, Arnosti (2011) in Ann. Rev. Mar. Sci. The authors are using Chrost's definition, which has never made much sense to me. In my (and other researchers') opinion, it makes more sense to refer to any enzyme that is exposed to the extracellular medium as 'extracellular', whether or not it is attached to the cell membrane. I note that it is often operationally impossible to determine whether an enzyme is attached to a cell (not extracellular, according to the authors' preferred definition) or cell-free but sorbed to a mineral surface (extracellular according to that definition).

Page 5844

*Line 11-14: This section is confused. The Suzuki citation is an editorial about protein carbonylation that does not refer to pH effects at all. If this process is relevant to ocean acidification, the authors should make that case using more relevant references. The Duffy reference is about connexins, a family of transmembrane proteins that exist

(as far as I can tell) exclusively in vertibrates and do not catalyze hydrolytic reactions. Furthermore, the protein structural changes in this paper were apparently a straightforward response to protonation/deprotonation of amino acid side chains, which is the generic mechanism by which pH influences enzyme structure and function. The authors should delete the text starting with "affecting the polar..." and ending with "...or altered substrate affinity."

This point - that, to a first order of approximation, the direct effect of pH on enzymes is only by protonation/deprotonation of amino acid side chains - is important to this manuscript, because it means that we should think of acidification effects on enzymes slightly differently than we do in most ocean acidificaiton studies, in which both pH and pCO2 are important. In the latter type of study, it is crucial to modify pH via pCO2. When we're thinking about enzymes, we do not expect a short-term influence of pCO2 on enzyme activities (apart from the accompanying change in pH), so any observed influence of pCO2 apart from pH is probably due to changes in enzyme expression levels, growth/death of microbial taxa, or other secondary effects.

Line 16: Again, the reference isn't as relevant as the authors suggest. De Paolis and Kukkonen is about binding of model pollutant compounds (pentachlorophenol and benzo(a)pyrene, both relatively small, aromatic compounds) to humic substances. An effect was noticed over large pH step sizes (pH changes of 1.5 units), and the effect was attributed to changes in ionization state of the model pollutant, not humic substances. I'm willing to believe that pH-mediated interactions between humic-bound proteins or amino acids might be important, but the authors would need to demonstrate it using directly relevant literature. Furthermore, if an effect of substrate binding with humic substances was important, it would probably be fairly instantaneous rather than happening over "longer timescales of days". (Note that humic acids precipitate more or less instantly as pH is lowered beyond a threshold, indicating a very fast pH response.)

Line 16-18: This statement requires a relevant reference - or, better, should be left out.

Page 5845

Line 9-10: Well, it depends on the system, doesn't it? In an open system, adding acid will drive down DIC as CO2 equilibrates with the atmosphere.

Line 20: Probably more accurate to say "respond to changes in carbonate species concentrations" and to put the species in brackets to indicate concentration.

Line 24-27: This is an important point, and the authors have stated it clearly.

Page 5846

Line 15: What about adjusting the pCO2 of the experimental treatment headspace, as in (for instance, I believe) Ries et al (2009) Geology 37(12): 1131-1134?

Page 5847

Line 13: Show the data used to determine that 40 uM are "optimal" in the supplementary data. There's no reason not to, and other standard protocols typically use higher concentrations. Steve Alison recommends 80 uM Leu-AMC for ocean water, for instance; see protocol at http://allison.bio.uci.edu/protocols/enzymeprotocolmarine100311.pdf . Steen et al (in Aquatic Microbial Ecology, 2013) found Km values for seawater peptidases in the range of 50-180 uM, and Williams and Jochem (Hydrobiologia, 2006) found Km value for leucyl aminopeptidase of 22-66. It is not clear how the authors' define "optimum", but to approximate Vmax, substrate concentrations must be substantially larger than Km.

Line 23: I'm not clear on how the authors actually calculated Vmax. There are two ways to do it: either to assume that substrate concentrations are much larger than Km (and that the enzyme kinetics do in fact approximate Michaelis-Menten kinetics, which may not always be true; c.f. Steen and Ziervogel (2012) Soil Biology and Biochemistry) in which case one makes the approximation that the observed hydrolysis rate approximates Vmax. This is the most common procedure, although it is not really a calculation. The second method is to use the following equation: Vmax = (v0*(Km+S))/S, where v0

is the observed reaction rate and S is substrate concentration. What did the authors do?

Page 5849

*** TRIS is a dangerous buffer for peptidase studies, because it contains an amine group which might interfere with peptidases' active sites. To avoid having to redo the entire experiment, the authors should compare the activity of coastal peptidases at identical pH using TRIS and another buffer (borate is an option in seawater at around pH 8). The results of this manuscript can only be trusted if there is no major difference in activity between those two buffers.

Page 5853

Section 3.1: Data from this section should be represented as plots or tables rather than inline in text.

Line 5-8: The syntax here is confusing: it reads as if the authors are comparing pH to fluorescence.

Line 21: It is worth noting that the authors used HCl salts of the enzyme substrates. Some investigators use free bases (both are commercially available from Sigma-Aldrich, for some substrates at least). The results here probably only apply to the HCl salts, as seen from the lack of change of pH on addition of the sugar substrates.

Page 5856 Line 1: change to "faster, more"

Page 5858

Line 16-20: This is not a complete sentence.

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