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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.

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1) The reviewer asks whether using a glycine-ammonium buffer (pH 10.5) would be beneficial prior to measuring fluorescence. As the reviewer notes, the pH effect for the MUF substrate was previously known.

The objective of our research was to investigate if there was a pH dependency in the MCA assay, and whether our assay required buffering. We have shown that for this substrate it is important to control pH. We wished to maintain a constant and defined pH throughout the incubation as well as the fluorescence measurement, as the specific activity of enzymes' can vary with pH. Thus we favored buffering at a pH relevant to enzyme activity, rather than allow pH to "roam" during the incubation, with potential variations in product formation between experiments.



2) The reviewer notes that fluorescence was determined at the same wavelengths for MUF and MCA, but the fluorescence spectra of the two molecules are different. The review asked whether this slight difference could have any effect on the results and conclusions?

For robustness we used filter blocks in our sea-going plate reader. 365 nm excitation and 460 nm emission wavelengths was the only block available that covered the correct wavelengths. Although we may be slightly off the ideal excitation and emission wavelengths, which raises our limit of detection, this should not alter the results or conclusions, unless there was something else in the system giving an emission at that wavelength and whose emission was pH responsive. We ran trials with natural seawater to ensure there was no inherent interference with fluorescence. Variation in the wavelengths used for MUF & MCA also exists within current literature. For instance, Chrost (1992) used the same MUF excitation and emission as we report, but used 380 nm excitation and 440 nm emission for MCA (as stated by the reviewer), while Hoppe et al. (1988) used 365 excitation and 445nm. Christian & Karl (1995) used 360 nm excitation and 447nm emission for MUF, while both Mass et al. (2013) and Piontek et al. (2009, 2010, 2013) used 355nm excitation and 460 nm emission for both MUF & MCA. In our study, it was important to use wavelengths used by others for consistency and comparison of responses.

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