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Interactive comment on “Acidification increases microbial polysaccharide degradation in the ocean” by J. Piontek et al.

J. Piontek et al.

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Received and published: 11 March 2010

Response to referee #2

We thank referee #2 for providing a number of valuable suggestions. Referee #2 supports the idea of our study and our approach to combine measurements of glucosidase activity with the analysis of polysaccharide concentrations. He/she mentions the novelty of our results in current research on ocean acidification. In his/her general comments referee #2 notes that data on “prokaryotic heterotrophic production, respiration, prokaryotic structure measurements might be very useful” to improve the interpretation of our data set. In general, we agree with the referee that data on other parameters like bacterial respiration, production, and community composition would be helpful. However, we were limited by our incubation volumes and by manpower during our ex-

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periments. Bacterial production was determined in our experiments, but we do not feel confident about the data due to methodological inconsistency among our experiments (see response to referee #1 for a detailed explanation). Nevertheless, we think that the interpretation of our results about pH-effects on glucosidase activity and polysaccharide degradation is valid.

In the following, specific comments will be addressed in detail, explaining why we agree or disagree.

p. 11380, l. 9-11. Degradation of polysaccharides was followed under present-day pH and under seawater pH expected for the future ocean. I suggest adding some information about the pH expected for the future ocean.

We will add this information.

p. 11381-11382. About experimental designs of CultExp I and II described in Materials and Methods: Are the cultures of *Emiliana huxleyi* made under axenic conditions? If yes, no problem. If not, how to identify the part of bacteria coming from the culture to the natural bacterial assemblage.

The cultures of *Emiliana huxleyi* were not axenic. Although we increased the bacterial cell numbers by the addition of natural North Sea bacterioplankton prior to dark incubation, we cannot exclude that some opportunistic strains that were already present in the *E. huxleyi*-cultures contributed to total bacterial activity. This is a limitation of our culture experiments that does not apply to our field studies. The big advantage of our culture experiments was that high amounts of labile organic matter including high shares of polysaccharides could be produced from controlled phytoplankton growth.

You have used several concentrations of nitrate (50 and 30 μM) and phosphate (3 and 1 μM) and several illumination conditions (200 and 300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Explain why.

Nutrients: Initial nutrient concentrations in the cultures of *E. huxleyi* did not affect bac-

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terial activity during the degradation phase. In both experiments, cultures of *E. huxleyi* were harvested when cells suffered from nutrient depletion. Light intensities: Different light intensities were applied for technical reasons. Growth of *E. huxleyi* at 15°C (as applied in our experiments) is light-saturated at 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Nielsen, 1997). Hence, light saturation was achieved in both culture experiments. *E. huxleyi* does not show signs of photoinhibition, even at irradiances of 1700 - 2500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Paasche, 2002).

p. 11381, l. 28-29. Please add the same units than in line 16 (i.e. μM)

The unit will be changed.

p. 11383, l. 22. Authors have used 1 μM as a final concentration for ectoenzymatic measurements. Could you justify this choice? Is it the saturation concentration for your experiment? Have you tested it? If yes, precise that. If not, it is difficult to compare your different results.

A concentration of 1 μM for the MUF-substrate analogue was chosen, since it corresponds to the magnitude of natural polysaccharide concentrations (Kaiser and Benner, 2009). This information will be added to the manuscript. The substrate concentration of 1 μM was non-saturating. Saturation of extracellular glucosidase in marine systems can be expected for substrate concentrations in the range of 50-250 μM (Nausch et al., 1998; Hoppe and Ullrich, 1999; Alonso-Saez et al., 2008; Celussi et al. 2009).

Why authors have incubated 3-5 hours? Are the authors tested with time series experiments? In general for such ectoenzymatic analyses time series at different concentrations are performed in order to determine both incubation time and saturated concentration!

Hoppe, who developed the method (Hoppe 1983), usually incubated samples for 3 hours or more in laboratory experiments and field studies (Hoppe et al., 1988; Hoppe et al., 1993; Hoppe and Ullrich, 1999). In our study, highest glucosidase activity was

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achieved at future-ocean pH in CultExp I. Here, hydrolysate was produced at a mean rate of 17.7 nmol l⁻¹ h⁻¹ at future-ocean pH. Accordingly, after 3 hours of incubation 53 nmol l⁻¹ MUF- α -glucopyranoside were enzymatically cleaved, corresponding to 5% of the added amount. Hence, incubation times of 3-5 hours did clearly not result in substrate depletion during our experiments. In several field studies on in-situ activities in temperate waters MUF-assays needed to be incubated 8 hours or longer due to low bacterial activities (e.g. Baltar et al., 2009).

p. 11384, l. 2. Same remark than before.

This point refers to the method description of tests with purified glucosidase. Here, a concentration of 50 μ M substrate analogue was used. The reaction velocity of purified glucosidase in our tests was an order of magnitude higher than that of natural glucosidase assemblages, because the concentration of purified glucosidase was an order of magnitude higher. To compare the response of purified and natural glucosidase to decreasing pH, we chose a non-saturating concentration also for the assays with purified glucosidase. Pre-tests with concentration kinetics revealed a final concentration of 50 μ M to be non-saturating, but high enough to accomplish 2 hours of incubation at the different pH values.

p. 11385, l. 28. Change “was calculated four the four...” by “was calculated for the four...”

Of course this will be corrected. Thanks for your careful reading!

p. 11386, l. 26-27. Authors referred to Grossart et al. (2006), another study studying the effect of ocean acidification on heterotrophic marine bacterioplankton. However, authors did not compare with this study. Why? Your paper will benefit to compare both results.

The two studies are difficult to compare. Grossart et al. (2006) studied bacterioplankton activity during the growth and decline of a phytoplankton bloom, while three of our

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four experiments were conducted as degradation experiments in the dark. Hence, in the study of Grossart et al. (2006) degradation occurred under continuous autotrophic production of labile organic matter, while in our experiments organic matter losses due to bacterial degradation were not replaced by organic matter input. Therefore, Grossart et al. (2006) could not clearly differentiate between pH-effects and effects induced by substrate availability. Nevertheless, we will follow the referee and include the study of Grossart et al. in more detail into our discussion.

p. 11387, l. 27-29. p. 11388, l. 1-2. Authors claim that the most significant effects of acidification can be expected for the degradation of POM in the twilight zone. I think that is very interesting but it is not well understandable as it is written.

We expect the most significant effects in the twilight zone, because high polysaccharide fractions in sinking organic matter are exposed to high bacterial abundances and activities. Our concluding statement mentioned by the referee is explained by the prefixed sentences (p. 11387, l. 22-29): “In the ocean, enhanced extracellular glucosidase activity at lowered seawater pH will impact the carbon turnover, when organic matter contains high fractions of polysaccharides. Intense production of polysaccharides included in phytoplankton biomass and exudates occurs during bloom events (...). Hence, the most significant effect of acidification can be expected for the degradation of freshly produced POM that becomes subject to bacterial degradation when sinking through the twilight zone after phytoplankton blooms.” We will improve the sentences for clarification.

p. 11390, l. 2-4. “Also the accelerated degradation of dissolved polysaccharides can reduce the carbon removal from the surface ocean, since a considerable fraction of organic matter is exported in dissolved form during mixing events (Carlson et al., 1994).” This is unclear. Please better explain.

Carlson et al. (1994) showed that organic carbon export in the temperate and sub-polar ocean is not mediated by the sinking of large particles alone. The vertical annual

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carbon budget could be closed, when high amounts of DOC exported to the deep ocean by convective mixing were included. The pool of DOC exported by deep mixing events in winter contains considerable amounts of semilabile material left over from the previous year's blooms, thus including high proportions of polysaccharides. We will add a more detailed explanation to the discussion.

Figure 1. CultExp II was not performed in replicate so there are no error bars but why are there no error bars for FieldAssay I performed in triplicate (p. 11382, l. 15).

FieldAssay I was conducted in triplicate, but for the polysaccharide analysis samples from replicate incubations needed to be pooled. This is mentioned in the caption of figure 1. Therefore, values given for the losses of combined glucose and total polysaccharides in figure 1 represent a mean value of three parallel incubations, but a standard deviation could not be calculated.

Figure 2. No error bars are given for some glucosidase activity. Why? Any replicate?

In figure 2, error bars are given for all experiments except for CultExp II that was not conducted in replicate.

Interactive comment on Biogeosciences Discuss., 6, 11377, 2009.

BGD

6, C4702–C4707, 2010

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