Biogeosciences Discuss., 6, C4693–C4701, 2010
www.biogeosciences-discuss.net/6/C4693/2010/

Biogeosciences Discussions

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

J. Piontek et al.

Judith.Piontek@awi.de

Received and published: 11 March 2010

Response to referee #1

We are grateful to referee #1 for helpful comments. He/she supports the idea of our study, but requested some experimental details and suggested some points to improve data analysis and interpretation. In the following, every point will be addressed in detail, explaining why we agree or disagree.

1. How many parallels have been used for CultExp I? How were incubations mixed? Loss of POM due to aggregation? CultExp I was conducted in duplicate incubations per CO2 treatment. The 10 L-Nalgene bottles were carefully, but thoroughly mixed twice a day and prior to samplings. This information will be added to the manuscript.

Since incubations were mixed and macroscopic aggregates were not observed, loss of POM due to aggregation seems very unlikely.

2. Field Assay II: sufficient replication for statistical analysis? We summarized the results of all experiments for the statistical analysis. This is mentioned in the caption of Fig. 1, where the results are displayed. Hence, significance was tested with four pairs by means of paired t-tests. We will add a paragraph to the Materials and Methods section to better explain the statistical tests and the loss calculation (see point 7).

3. Loss of polysaccharides during dialysis due to bacterial degradation? In general, dialysis of samples for polysaccharide analysis was conducted at 0°C. Furthermore, tests with samples derived from phytoplankton cultures (including bacterial cell numbers in the magnitude of 106 ml-1) were conducted to exclude loss of polysaccharides during dialysis and hydrolysis. During these tests an aliquot of the samples was spiked with a polysaccharide solution of known concentration. Subtracting the sugar concentrations of samples without polysaccharide addition from those of spiked samples resulted in the amount of polysaccharide added prior to dialysis and hydrolysis. Results of the test will be published in detail in: Engel & Händel, Simultaneous determination of neutral-, amino-, and acidic sugars in marine polysaccharides using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection, submitted.

4. Incubation time for MUF-assays (3-5 hrs) seems to be quite long. Substrate concentrations (1 μM) are rather high. De novo synthesis of glucosidases during MUF-assays? For the MUF-assays neither incubation times were exceptionally long nor was the substrate concentration "rather high". 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 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-ïĄć-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). A concentration of 1 μM for the MUF-substrate analogue was chosen, since it corresponds to the magnitude of natural polysaccharide concentrations (e.g. Myklestad and Børsheim, 2007). This information will be added to the manuscript. "Rather high" concentrations for MUF-assays would be saturating concentrations. Saturation of extracellular glucosidase in marine environments 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). To the best of our knowledge, there is not any publication indicating substantial de novo synthesis of extracellular enzymes during standard MUF-assays as conducted in our experiments.

5. Why did not the authors measure bacterial production to test whether differences in glucosidase activity affected bacterial production or growth? Bacterial production was determined in our study (see table below; this information can be added to the manuscript if decided by the editor), but we do not feel confident about the data due to methodological inconsistency among our experiments. Unfortunately, different radiotracers were used and samples were processed by filtration or centrifugation. Although the measurement of bacterial production was accurate for each individual experiment, it is not appropriate to compile the data of the four experiments and to compare them after conversion into carbon units.

Experiment Radiotracer/Method Incorporation of thy and leu (integrated over incubation time) Incubation time (d) CultExp I 3H-thymidine/ filtration PD 73.9 $\pm$ 2.2 nmol l-1 FO 108.7 $\pm$ 0.2 nmol l-1 30 CultExp II 3H-leucine/ centrifugation PD 103.1 nmol l-1 FO 98.1 nmol l-1 13 FieldAssay I 14C-leucine/ centrifugation PD 338.0 $\pm$ 1.4 nmol l-1 FO 500.5 $\pm$ 18.2 nmol l-1 12 FieldAssay II 3H-thymidine/ filtration PD 14.8 $\pm$ 4.3 pmol l-1 h-1* FO 15.0 $\pm$ 5.1 pmol l-1 h-1* 8

* For FieldAssay II, bacterial production cannot be integrated over time, because the

experiment was conducted under steady-state conditions.

Nevertheless, we think that the interpretation of our results about pH-effects on glucosidase activity and polysaccharide degradation is valid, because our conclusions are not based on potential effects on bacterial biomass production. Our implication that the respiratory CO2 production will increase due to higher glucose availability at lowered pH arises from the fact that glucose-derived carbon is incorporated only with low efficiency into biomass by marine bacterioplankton (Bianchi et al., 1998; Skoog et al., 2002).

6. Higher enzyme activities were not induced by differences in bacterial abundances. Could this be an indication for aggregation? Aggregation did not affect glucosidase activities in our study, since there was not any formation of macroaggregates in our experiments. The referee is right that an experimental study has recently shown effects of aggregation on some enzyme activities in the surrounding seawater (Ziervogel et al., BGD 6, 11293-11316, 2009), but results were obtained from an experiment with intense formation of macroscopic aggregates on roller tables

7. p. 11387, l. 4-5: Higher rates of extracellular glucosidases significantly accelerated the degradation of polysaccharides at lowered seawater pH (Figs. 1-3). This statement is not really supported by the given data. We think it is! We showed a direct relationship between seawater pH and glucosidase activity (Fig. 3) and significantly higher losses of polysaccharides at higher glucosidase activity in acidified incubations (Fig. 1).

Why did the authors use different methods to change pCO2? We applied different methods to change the seawater carbonate chemistry to exclude any impact of the manipulation mode on our experimental results. Accordingly, we mentioned on p. 11380, l. 14-20:

"In current marine research, the biological response to elevated seawater pCO2 and biogeochemical consequences are mainly investigated by perturbation experiments, in which different approaches are used to manipulate the seawater carbonate chemistry

(Gattuso and Lavigne, 2009). In our experiments, reference incubations representing present-day pH conditions were compared with acidified incubations that exhibited pH values projected for the future ocean. Manipulation was carried out by both CO2 aeration and acid addition to exclude impact of the manipulation mode."

The experimental manipulation of the seawater carbonate system is a critical issue in research on ocean acidification. From our point of view, the fact that pH effects on the enzymatic polysaccharide turnover were consistent in experiments with different CO2 manipulation modes supports our conclusions.

Why are there no error bars for CultExp II and FieldAssay I? For CultExp II no replication was accomplished, while for FieldAssay I samples from incubations in triplicate per CO2 treatment were pooled for polysaccharide analysis (as mentioned in the caption of Fig. 1).

How did you determine the % loss of the different parameters? As mentioned in the caption of figure 1 that displays losses of polysaccharides and POC (p. 11397):

"...losses were calculated subtracting the residual from the initial concentration..."

We will add this information to the Materials and Methods section (see also response to point 2).

Acidification in FieldAssay I may have led to precipitation of organic matter, could that explain the low POC value given in table 1? Acidification in FieldAssay I was accomplished by the addition of some drops of 0.1% hydrochloric acid to 10 litres of seawater with intermediate mixing. Thereby, pH was lowered by 0.3 units (see also Tab. 1 and Materials and Methods section). This treatment does not result in the precipitation of organic molecules. Precipitation of organic compounds from seawater requires much stronger acidification, e.g. 5% final concentration of trichloracetic acid. Field Assay I was conducted in the Bay of Biscay in May, 2007. The POC concentration given in Tab. 1 is not low for this study site. During the cruise 2007, POC surface concentra-

tions along a shelf break transect from the west of France to the south of Ireland ranged between 6 and 20 μM. POC concentrations along the transect were in the same range in the years 2006 and 2008 (Harley et al. 2009; de Bodt 2010, PhD thesis).

8. Why did not you plot increase in glucosidase activities vs. loss in polysaccharides? We always compared two pH treatments (present-day and future-ocean pH). Therefore, data could not be tested for a direct relationship between glucosidase activity and polysaccharide loss for individual experiments. A summary of all experiments seems not to be reasonable in this case, because the relative loss of organic matter depends not only on rates of extracellular enzymes, but also on the concentration and the composition of the organic material. Differences in both organic matter concentration and composition existed in particular between culture experiments and field assays.

9. p. 11388, l. 12-14: An increased C-supply may be insignificant when other nutrients such as N and P are limiting. At the end of a phytoplankton bloom C is usually not the limiting bacterial substrate! The impact of N- and P-availability on bacterial glucose utilization is discussed directly after this statement (p. 11388, l. 15-p. 11389, l.2)!

"The fate of glucose monomers taken up by bacterioplankton depends on the nutrient availability and the physiological state of the bacterial cell. Up to 60% of glucose consumed by marine bacterioplankton gets remineralized by respiration in nutrient-poor regions (Rich et al., 1996; Bianchi et al., 1998). The proportion of respired glucose is significantly lower in nutrient-rich areas, where appropriate nitrogen and phosphorous sources fulfil bacterial growth demands (Bianchi et al., 1998). In order to balance the increased availability of labile carbon, bacteria are able to utilize inorganic nitrogen (Kirchman et al., 1990; Kirchman, 2000). In particular actively growing marine bacteria act as sink for inorganic nitrogen, when an easily utilizable carbon source like glucose is available (Goldman and Dennett, 1991). The increased bacterial consumption of inorganic nutrients in response to increasing labile carbon availability changes the partitioning of inorganic nutrients between bacterioplankton and phytoplankton and leads to lower phytoplankton biomass production (Thingstad et al., 2008). Hence, it must be

assumed that also increased glucose availability resulting from enhanced glucosidase activity at lowered seawater pH can stimulate bacterial competition for mineral nutrients and can mediate secondary effects on autotrophic production in the ocean."

You should have measured bacterial production directly. It would have been also interesting to measure protease activity since this would not only increase C- but also N-supply. see above (response to point 5)

10. May incubation have resulted in changes in bacterial community structure? It has been shown previously that ocean acidification may change the composition and the transcriptome of bacterial communities. Link to literature? Referee #1 is right that incubation experiments investigating biological activities and biogeochemical aspects of natural marine communities have limitations. Nevertheless, they are a valuable tool to test effects of changing environmental conditions (e.g. temperature, pCO2, substrate/nutrient supply) that cannot be easily simulated by in situ-studies. We used bacterioplankton communities of different sites (Bay of Biscay and North Sea) and all experiments consistently revealed increased polysaccharide degradation at lowered pH. Allgaier et al. (2008), who investigated the effect of changing pCO2 on the bacterial community during a mesocosm experiment, found that changes in pCO2 (and thus in pH) induced changes in the community structure of free-living bacteria, but these changes did not significantly affect bacterial activity. We will include this paper into our discussion.

11. The discussion remains rather speculative since the authors did not measure bacterial production, respiration and sedimentation. This point probably refers to section 4.3, where we discuss some implications of our findings for the cycling of organic matter in the future ocean. A major objective of research on ocean acidification is to identify potential feedback mechanisms to increasing pCO2 and temperature based on simulation experiments. Extrapolating experimental results to natural systems always bears considerable uncertainties. Nevertheless, we feel confident that our extrapolations are justified. Our major implications are âĂć reduced export due to the enhanced degrada-

tion of particulate carbon at lowered pH âĂć increased respiratory CO2 production due to higher glucose availability at lowered pH. These conclusions emerge from our experimental results of âĂć higher enzymatic activities at lowered pH âĂć higher losses of polysaccharides and POC at lowered pH in conjunction with relevant publications: âĂć Smith et al., 1992: extracellular enzymes drive particle solubilisation; âĂć Baines and Pace, 1991: polysaccharides can account for more than 50% of phytoplankton primary production; âĂć Rich et al., 1996, Skoog et al., 2002, Bianchi et al., 1998: high uptake rate constants underline the importance of glucose as carbon and energy source for marine bacterioplankton, less than 20% of bacterial production is supported by carbon derived from consumed glucose, up to 60% of glucose taken up by bacteria is respired.

12. Experiments have been short-term experiments. Since bacteria can rapidly adapt to changes in environmental parameters longer experiments need to be run or at least the point of physiological adaptation needs to be discussed. We chose incubation times from 8 to 30 days for our experiments, because semilabile organic matter, including polysaccharides, is degraded during days to weeks by bacterioplankton in the ocean. Furthermore, short-term incubation as conducted in our study reduces the risk of artefacts, e.g. due to changes in the community composition (see also point 10). We agree with referee #1 that it is useful to discuss the aspect of potential physiological adaptation. We will add a paragraph to our discussion.

Additional comments: Fig. 1 is rather confusing. We do not follow the referee's argument

Fig. 2: data should not be summarized. The summary of data was necessary to meet requirements of the statistical test.

Fig. 4: Highly hypothetical since no respiration, BPP, and export have been measured. Effects of increased glucosidase activity due to acidification may be compensated by higher polysaccharide production and N and P limitation, which would result in accumulation of POC and subsequent C-export. This scheme illustrates the feedback mech-

anism inferred from our experimental results (see also point 11). Therefore, it does not include potential counteracting processes that were explored by recent research on ocean acidification, e. g. increased phytoplankton exudation and aggregation. At the current state of knowledge, it is impossible to predict the prevailing feedback of the marine biota to rising CO2. Since recent papers on potential changes in marine biogeochemical cycles do not consider bacterial activity (e.g. Arrigo 2007), figure 4 is useful to underline the importance of the heterotrophic bacterioplankton and the need to include it into research on global change impacts. We will add a paragraph to discuss in more detail interactions of acidification effects on bacterial organic matter degradation with effects on autotrophic organic matter production.

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