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Biogeosciences Discussions

Interactive comment on "Changes in the spectrum and rates of extracellular enzyme activities in seawater following aggregate formation" *by* K. Ziervogel et al.

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Most of the questions/concerns raised by Reviewer #2 were also raised by Reviewer #1, and were addressed as described above in the new material and methods section of the revised manuscript (p. 4-6). Further items: Reviewer 2 asked about the time course of our polysaccharide hydrolysis incubations as well as the age of aggregates: aggregates were fresh (formed within hours in roller tanks) when we started our incubations. We harvested all the (visible) aggregates and divided them into separate incubation vials, now described on p. 5, l. 127-129. The incubations continued over a time course extending up to 20 days because we do not know a prior how long it will take

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a given microbial community to hydrolyze a specific polysaccharide. Our experiments therefore reflect the community's ability (here, microorganisms either associated with aggregates or not) to respond to the addition of a specific polysaccharide substrate; this experimental approach does not reflect an instantaneous hydrolysis rate. Reviewer 2: "Were the age since sampling of whole seawater and aggregates the same?" Water for aggregate and whole seawater incubations was sampled at the same time, although in spring 2006, aggregate incubations were started one week after sampling (we had to return to our home lab) whereas whole seawater incubations were started one day after sampling. In fall 2008, whole seawater and aggregate incubations were started at the same time. Since our data demonstrates the response of the community to aggregate formation as well as substrate addition over a week to 15 days, this time-lag in handling the samples should not affect the outcome. Similar patterns in terms of the spectrum and rates of hydrolytic enzymes at the two different times (Fig. 1) further suggest no significant effect of this time lag on the ability of the microbial communities to respond to substrate addition. Reviewer 2: "Were aggregates incubated under still conditions?" Aggregates were incubated in glass vials that were continuously agitated on a wave table. Reviewer 2:" Were all aggregates concentrated in one vial?" No, they were separated into different vials. Reviewer 2: "Is microenvironment retained?" No, but we are not concerned about this; the conditions in the roller tank (almost no turbulence) are most likely not the same than those in highly dynamic coastal waters. However, aggregates formed in roller tanks are suitable for microbial measurements and the vast majority of experiments measuring hydrolytic activities of natural microbial communities are conducted under stagnant rather than turbulent conditions. Reviewer 2: "Which app. sizes had the aggregates? Were all aggs sampled ..." We have no data on aggregate numbers, sizes, or volumes,) thus we used the linear relationship given by Alldredge and Gottschalk (1988) to calculate the total volume of aggregated material that we pooled together in separate vials in order to relate the measured enzyme activities to an appropriate volume. Possible differences in the volume of single aggregates that were pooled together in one vial are not critical for the expressed hydrolytic activity

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measured in our experiments. We used the phrase “aggregates are heavily colonized by bacteria” in the abstract, introduction and summary as a rather general statement (with reference to Alldredge et al., 1986 in the Intro). We agree that the lack of bacterial abundance data for the fall 2008 experiments is regrettable. However, we now report the data from spring 2006 that we published in the earlier study (Ziervogel and Arnosti, 2008; Environmental Microbiology). This is not a parallel but the same study, as mentioned in the Methods of the original manuscript (p. 6, l. 164). Reviewer 2: “What is considered the detection limit?” We changed the sentence to “aggregate-free abundances were five orders of magnitude lower than . . .”. Our data on cell abundance from spring 2006 suggest that the vast majority of bacterial cells present in the initial seawater were associated with aggregates after the roller table incubation. These data suggest enrichment factors of 10ˆ3and 10ˆ4 of bacteria on aggregates relative to the aggregate-free and whole seawater, respectively. Thus, enrichment factors of cell numbers are two orders of magnitude higher compared to enrichment factors of hydrolytic activities observed in different treatments (Tab. 1). Therefore, differences in cell counts between aggregates and the surrounding water and whole seawater may only in part explain differences in hydrolytic activities.

Paragraph 3.1: we added that rates in aggregates on a volume basis were 70-700 fold higher referring to Fig. 1 as well as Tab. 1; we deleted the first sentence from paragraph 3.2. We also added a sentence about the contribution of aggregate rates to whole seawater hydrolysis, as suggested by the reviewer. L. 194: Hydrolysis rates of laminarin (not xylan) were 44 nM monomer h-1 in agg-free water in spring 2006. L. 203-206: We added a comment on the overall dominant contribution of aggregate-free activity to total roller bottle activity at the end of the paragraph.

Abstract: we added a comment on lifetimes of free enzymes with respect to the water residence time in the Bay. Intro: we corrected the sentence on coagulation efficiency of exudates. Discussion: we changed the first two sentences of the second paragraph as suggested (p. 9, l. 251-255).

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