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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.

Anonymous Referee #2

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Review of BG paper:"Changes in spectrum and rates of extracellular enzyme activities in seawater following aggregate formation" by Ziervogel et al.

This study reports on extracellular hydrolysis rates in sea water and in aggregates using new approaches alternative to the small substrate proxies generally used to asses hydrolytic activities by sea water microbes. Using six structurally distinct substrates, extracellular hydrolysis rates sea water prior to and after aggregate formation were studied in great detail. It was shown that aggregate formation enhances production/activity of laminarinase and xylanase enzymes within aggregates as well as in the surrounding water of these. In fact, a very large proportion of activity was found in the surrounding

waters of the aggregates presumably due to release of cell-free enzymes. In contrast, activities of chondroitin, fucoidan, and arabinoglactan hydrolysing enzymes were suppressed after aggregate formation as compared to those measured in whole sea water (containing the same particles but in an un-aggregated state). Such experiments greatly improve our insight of the complexity of polysaccharide hydrolysis rates in sea water. The manuscript is well-written, and after some minor revision it is most probably acceptable for publication in BG.

I have a few concerns and questions which I believe should be clarified before final publication: In general, I am missing more details about the incubations. Why did it take 7 to 15 days to perform experiments? Were aggregates up to 7 days old when enzyme activities were measured (although they formed within 1 hour)? Where aggregates incubated under still conditions when hydrolysis rates were measured? Were all aggs from one roller tank concentrated and sedimented in one 15mL vial? If so, is the microenvironment of aggs retained as compared to natural conditions in the water column during incubations? (probably not). How long time is needed to measure hydrolysis rates using the applied methods? Was the "age" (since sampling) of whole sea water and that of aggregates the same in the experiments?

I am wondering about the determination of aggregate volume. Which app. sizes had the aggregates? Were all aggs sampled for DW determination (on one filter?) and their combined volume then calculated from the DW-size relationship published by Alldredge and Gotschalk (1988)? Marine snow is fractal and its porosity therefore increases with increasing aggregate size. Small aggregates have higher volume-specific DW than large aggregates do: the volume-specific DW in a 1 mm large aggregate is ca. 17 μg/mm3 whereas that in a 1 cm large aggregate is 0.22 μg/mm3. This difference is substantial (a factor of 75) (Alldredge and Gotschalk, 1988). Many small aggregates therefore occupy less volume than one large aggregate with an apparently equal volume (as the sum of small ones) does, because the smaller aggregates are more compact. I think this issue should be clarified.

At several places it is stated that “aggregates are heavily colonized by heterotrophic bacteria”, however without reference or without being specific about their enrichment factors relative to the surrounding waters. It is a pity that this paper does not report on any numbers or abundance of heterotrophic bacteria on the aggregates studied, in the whole sea water, or in aggregate-free water. Only at page 11303 (L2-3) it is stated, with reference to an earlier published (parallel?) study in the same area, that bacterial abundance in aggregate-free water was near detection limit. What is considered the detection limit in this respect and how is it defined? – does that mean that all bacteria were attached to aggregates or to the walls of the roller tanks (as many bacteria in coastal waters are motile and attach to surfaces)? If we consider one liter of sea water with an average bacterial abundance of 1E9 cells L-1, and these all attach to aggregates with a total volume of 0.0005 L, this would result in a bacterial abundance on aggregates of 2E12 bac L-1 agg (ignoring any background bacterial abundance on the particles). Although this number is definitely in the higher end of bacterial abundances on aggregates it is not un-realistic in estuaries with many small aggregates. Are there any other data on bacterial abundance on aggregates - maybe from this parallel (?) study - available to cite? Enrichment factors of bacteria on aggregates relative to the surrounding waters are reported to be ca. 100-1000 (reviewed by Simon et al., 2002). These factors are similar to the enrichment factors of enzymatic activities on aggregates relative to those in whole seawater, and could suggest that cell-specific rates averagely are not that different from those in whole sea water?

Paragraph 3.2 P. 11300 L. 21-24 content should be carefully checked. The first statement that hydrolysis rates on a volume-base in aggs are up to 1000-fold higher as compared to agg-free sea water (is that true or is it a mistake?) as well as to whole seawater can be confusing because it only refers to Fig 1. It should refer to Table 1 as well. To me, it appears that the hydrolysis rates of 44 nmol monomer L-1 h-1 was measured for xylan in roller bottles rather than in agg-free water in fall 2008. IN WHOLE SEA WATER , hydrolysis rates of all substrates except for xylan were lower in fall 2008 as compared to the rates measured in spring 2006. In Figure 1, it also appears that,

by far, most enzymatic activity takes place in the agg-free water. This is a very striking result. However, this is not mentioned in the paragraph. As far as I understand, the difference between the black and white bars describes the activity on aggregates (corrected for their contribution by volume). A calculating example may clarify this to the reader: Although hydrolysis rates are app. 70- to 700-fold enhanced on aggregates relative to whole sea water (containing the same POC although un-aggregated) these should comprise 70 * 0.05%=3.5% to 700*0.05%=35% of total rates, only.

Minor changes: I think that the hydrolytic half-lives of commercial enzymes should be referred to as numbers in the abstract as well as in relation to residence time of water in the bay.

P. 11295 L. 7-9: Exudates do not increase coagulation rate. The stickiness of exudates increases the coagulation efficiency. P. 11302 L9-10: “Since the aggregates themselves occupy a relatively small percentage of a given volume of sea water, however, it is important to consider their effects on the surrounding sea water as well. This is not logical. Please rephrase and include that their interactions through small-scale fluxes of microorganisms, solutes, and nutrients to and from the surrounding water are substantial. (Kiørboe and Jackson, 2001; Kiørboe et al., 2001). It is therefore important to consider their effects on the surrounding water as well. P.11302 L. 15: HOWEVER, enzyme production. . .

Interactive comment on Biogeosciences Discuss., 6, 11293, 2009.