Biogeosciences Discuss., 6, C4079–C4081, 2010
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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 #3

Received and published: 22 January 2010

[General comments]

This manuscript presents interesting information on the influence of aggregates on hydrolysis of organic molecules in aquatic environment. The topic is very important for our better understanding of whole system of carbon biogeochemical cycle. The data and discussion are worthy to be published however the manuscript needs to have some revision.

[Specific comments]

[1] In this manuscript, the method for aggregate formation and measurement of hydrolysis activity are not described very well. I found detail explanation of these methods in

their previous paper (eg, Ziervogel and Arnosti, 2008) however, it is better to explain some essential things in this manuscript; otherwise, it is difficult for readers (including me) to catch up the experimental situation. [1-1] How long and how fast (speed) did you rotate the water tank to form aggregates? [1-2] How did you separate "aggregates" and "water surrounding aggregates"? [1-3] How did you determine the dry weight of aggregates? (After drying, it may not be suitable as a sample to measure enzymatic activity.) [1-4] To calculate the hydrolysis activity (per hour) in Table 1 and Fig 1, how long did you incubate the sample with fluorescent-labeled polysaccharides (= how long did you monitor the changing of molecular weight to calculate the rate shown here? On which time point did you get the actual data?)? In the first paragraph of p11299, "The total time course of hydrolysis experiments varied between 7. . . ", what do this mean? "the maximum hydrolysis rates reported here were usually measured at time points within the first week of incubations," means the rate is "average rate" during the first 7 (or less?) days? [1-5] Was the hydrolysis activity in whole seawater measured at the same time with the rotated sample? (The sample seawater was kept in the same kind bottle with rotated seawater, under same temperature, and just without rotating?) Or does the hydrolytic activity in "whole seawater" mean the initial value before rotating?

[2] Do you have data for bacterial abundances in the whole seawater, aggregates, and aggregates-free seawater? It is better to show the bacterial abundance (and community structure, if possible) in each fraction to strengthen the discussion. Besides, per cell activity in each fraction may also give us interesting information as well as bacterial abundances. P11305-line2: "bacterial abundances in aggregate-free seawaters . . . were near detection limit" means almost all bacteria in the original seawater were attached on aggregates after rotating?

[3] From the Fig 1, hydrolysis of laminarin was enhanced mostly in the aggregate-free seawater, while that of xylan was enhanced in/on aggregates, although both hydrolysis activities increased due to aggregates formation. Please include a statement and discussion for this interesting fact. I think this fact is related to the statement on p11305-

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line13 “The relative contribution of cell-free enzymes to total hydrolytic activity varies among enzymes”.

[4] Fig 2: Why don’t you show the same kind of graph for “aggregates”? I suppose this graph can demonstrate the characteristics of spectrum of enzyme activity in/on aggregates compared to the seawater.

[5] The authors try to assess the active lifetime of cell-free enzymes. P11305-line18 $\sim$ p11306-line9: I agree the point that natural extracellular enzymes are “likely better tuned to work in extracellular environments” and the attaching to the particle would “likely enhance their hydrolytic lifetimes”. However, in natural environment, cell-free enzymes are target of proteases, as authors mentioned in p11305-line19. This experiment was carried in ASW, which did not contain any proteases (and other enzymes). Of course it is difficult to assess the lifetime of enzymes in natural seawater containing natural proteases, so that, as the first step, the experiment in ASW is reasonable. However, it is better to mention this difference between natural seawater and ASW and possibility of being shorten the lifetime of enzyme by natural proteases in seawater than in ASW.

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