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Biogeosciences Discussions

Interactive comment on "Effects of ultraviolet B radiation on (not so) transparent exopolymer particles" *by* E. Ortega-Retuerta et al.

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Reply to interactive comments of anonymous reviewer 2 on "Effects of ultraviolet B radiation on (not so) transparent exopolymer particles" E. Ortega-Retuerta et al.

Anonymous Referee #2 Received and published: 7 September 2009

General comment This manuscript addresses a very interesting issue. As noted by the authors, nonballasted TEP (even TEP that are not sufficiently ballasted) migrate upward and accumulate at the surface, and hence, become exposed to intense solar radiation. Since TEP play major roles in marine biogeochemical cycling, via their key role in aggregation processes and, thus, vertical fluxes, an alteration of TEPs' properties or a lysis linked to UVB exposure might control their implications in pelagic

processes.

This manuscript is well written and the objectives are clearly exposed. However I am not totally convinced by the interpretation of the results. What I am most concerned about is the one-way interpretation given to the data. I argue that there are alternative processes that may potentially explain the decrease of the TEP concentration observed during the incubations, and that they should be discussed. One process in particular may easily explain the observed decrease and weaken the photolysis hypothesis. As written by the authors, "non-ballasted TEP migrate upward and accumulate at the surface". In a bottle, this mechanism may promote the formation of a biofilm on the wall of the bottles. TEP may stick to the inner wall of the bottles, as TEP in the surface microlayer sick to glass plate samplers, and be lost for subsequent assessment of their concentration in the bottle. Actually, this mechanism may explain why the dissolved mono- and polysaccharide (DTCHO) concentrations also decreased in the +UVB treatments of all experiments. One may even hypothesize that UVB increases TEP stickiness. Such a modification of TEP properties would lead to the formation of a "strong" and resistant biofilm. As a result, the observed decrease would not be due to photolysis, but to the increase loss rate of suspended TEP due to wall attachment.

We agree with the reviewer that the formation of a biolfilm adhered to bottle walls would represent an alternative loss of TEP in the experiments. But, in the case this process was significant compared to photolysis, we would have observed a decrease in TEP also in the dark treatments, which was not the case. However, to reinforce the experimental results of the UV-photolysis hypothesis and to rule out the potential significance of biofilm, we have included additional methodological tests. We checked the potential formation of a biofilm in the different material (quartz vs. borosilicate) and light conditions (full solar spectrum vs. dark). We demonstrated that, even using the same material, there are differences in TEP concentration after solar light exposure that cannot be attributed to TEP adhesion to walls. Please see new M&M and enclosed figure above.

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The methodological tests conducted should have solved this, but I totally share Referee # 1 concerns about the high uncertainties related to the results of these tests. I am especially concern about the efficient of the shacking method to detach a biofilm.

We have included a new experimental test to monitor the potential formation of a biofilm. Please see new M&M.

Question Did the authors make some microscope slides to check whether TEP looked like flakes after 1.5 or 3 days of incubation, or during the methodological tests? The presence of such flake-like shapes after shaking the bottle may well indicate the formation of biofilms at the inner surface of the bottles.

We did not make these slides directly from the bottles. However, in the new experimental test, we immersed a borosilicate slide per bottle in the dark treatments (three in total) to check potential TEP adhesion to the walls following Bar-Zeev et al. (2009) technique. Although we found TEP adhesion in two of the three slides, the superficial coverage was really low and hard to quantify without an image analyzer. The three replicates were different, so no conclusive results can be shown from this analysis.

Miscellaneous In some occasions, the results are botched up and only the 'positive' results are put forward. For instance: - Page 9 (7607), last paragraph: The authors state that "TEP concentrations were very low or undetectable after 1.5 days and at the end of each experiment (3 days)." On figure 2, we can see that it is not the case for Exp 1, where TEP concentration is around 30 _g XG eq L-1 (it even increased between day 1.5 and day 3).

We have changed this sentence (page 8, lines 231-233)

- Page 9 (7607), last paragraph: The authors state that "When UVB was excluded (–UVB) TEP decreased at lower rates in experiment 2 and no significant changes were observed in experiments 2 and 3.." On figure 2, we can see that there is a significant increase of TEP concentration in Exp 3. \\

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We have rewritten this sentence (page 8, lines 233-234)

- Page 10 (7608), line14-15: It is stated that the correction for the potential bacterial production of TEP did not change the main results, but the data are not presented. It would be very helpful to see these results. In addition, the correction made for bacteria involvement in the evolution of TEP concentration (Page 10, line 10-12) only considers TEP production by bacteria, while one of the main roles of bacteria would rather be to degrade and mineralize TEP.

We agree with the reviewer that bacteria can both generate and degrade TEP. Bacteria growth was particularly evident in dark treatments, with very few or undetectable growth in UV treatments. Thus, as we observed increases in TEP in the dark treatments, we tried to correct these increases only considering bacterial contributions, not degradation (which would have yielded even higher TEP increases, enhancing the difference between +UVB and dark treatments and hence supporting our results even more conclusively).

- Page 10, line 28-29: The authors state that “In the -UVB treatments TEP photolysis rates ranged from negligible (8 _ 8 % d-1) to 18 _ 2 % d-1”. According to figure 2, there is an increase (not a diminution) of TEP concentration for Exp 3, therefore one cannot assume photolysis.

In effect, we cannot assume TEP photolysis under PAR light in experiment 3. That’s why we state that “photolysis rates was negligible” in this treatment.

Suggestions In my opinion, this manuscript needs additional experiments to confirm the effect of UVB on TEP photolysis, photoinhibition and production. Since sticking material such as TEP can easily attached to the wall of the bottles used during the incubations, particularly if the water is not agitated (as it was the case), an additional experiment should be conducted to check whether or not wall adhesion can be considered as a loss factor. If the presence of a biofilm is detected, the estimation of the bacterial concentration should also be reevaluated since a large fraction of bacteria

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would probably be associated with this biofilm. Finally, since the bottles used for the different treatments are made of different material (quartz and borosiliacte), the authors should check whether TEP attachment to the walls (assuming attachment) varies as a function of the kind of material used.

We have performed additional tests to assess the relevance of biofilm formation in our experimental bottles. Please see new M&M.

One way to test whether the observed apparent decrease in TEP is due to photolysis or to wall adhesion is by monitoring the evolution of the bacterial abundance attached to the inner wall according to incubation time. This can be done by emptying the glass bottles, presumably leaving the biofilm attached to the wall, and using one of the procedures used to dissolved polysaccharidic matrix and free associated particles (e.g., pyrophosphate or methanol) in order to enumerate the fraction of wall-attached bacteria. Since biofilms are composed (among other things) of bacteria and TEP-like material, an increase of wall-attached bacteria during the incubation would most likely also imply an increase of wall-attached TEP. This indirect approach will not give the actual wall-attached TEP concentration, but it will certainly help answering the question whether or not biofilms form on the wall.

In the experiments performed with algal cultures we observed a bacterial increase, but our initial purpose was not to include bacteria in the incubations. Thus, we decided that measuring TEP directly by staining the bottle walls would be more accurate than indirectly measuring bacteria attached to walls. Please see new M&M.

Alternatively, one may also want to try immerging a small glass plate in the incubation tubes and directly scratch the biofilm with a blade (as for the SML sampler) in order recover the biofilm and to directly determine the concentration of wall-attached TEP. The data presented are interesting, but I am afraid that at this stage they do not allow concluding about the effect of UVB on TEP. The effect of other loss factors, i.e. adhesion to the walls, should be investigated more carefully. Therefore, I cannot recommend

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publication, but I strongly I encourage the authors to resubmit this work once the issue of wall adsorption solved.

Interactive comment on Biogeosciences Discuss., 6, 7599, 2009.

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