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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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Ortega-Retuerta et al. present data of the decomposition and production of transparent exopolymer particles (TEP) in seawater. . ..

Material and Methods The design of the lab experiment could have been improved as TEPs have been exposed to radiation for 18 hours. The period of exposure exceeds a day cycle which limits the natural exposure of TEP on the ocean's surface, e.g. about 12 hours. Through this overexposure, I expect that the rates of decomposition are overestimated. Additionally the PAR was 66 times lower in the lab experiments compared to the outdoor experiment. It needs to be explained, why such much lower PAR has

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been applied for the lab experiments.

We agree with the reviewer that a light regime of 18:6 would not be fully representative of field conditions, although this could be close to 'real' conditions e.g. in summer. We chose this regime simply because we shared the culture room so other incubations were being performed under these conditions and, more significantly, the goal of this paper was not to determine photolysis rates applicable to the field, but first to demonstrate the photoreactive nature of TEP. By contrast, we think that these rates are likely underestimates since we observed no detectable TEP after 36 hours in 2 our of 3 degradation experiments. Thus, a complete TEP degradation in the bottle could have been occurred in a shorter period of time (see discussion (page 11, lines 320-324). In our opinion, the importance of this study resides, therefore, in the fact that we report the existence of TEP photolysis for the first time. However, further work is definitely necessary to explore more accurately the photolysis rates in the field, under different conditions, etc.

Furthermore, the design of the experiments limits conclusions about decomposition/ production rates of TEP within the first 10 cm of the ocean's surface through the direct exposure of the glass bottles. A better approach would have been to do an incubation experiment similar to the approach measuring primary production rates, either incubation in the ocean at different depth's or in an incubation tank with bottles covered with light-shaded foils to simulate light exposure at different depths.

As pointed out by the reviewer, this photolysis process would only occur at this magnitude within the first 10 cm. However, we think that this process should be of great importance given that the sea surface microlayer (SML) may be enriched in TEP (e.g. Wurl and Holmes 2008).

Further problems with the experimental design: P7/L13 I would expect that material with a surface-active character stick to the bottle wall during the incubation. Surprisingly, the authors claim that they did not find significant differences in the concentration

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of the model substances before and after vigorous shaking. I need to question the author's interpretation of the data. First of all, how do the authors perform statistical testing on significance (see P13/L21) in duplicate samples (P13/L15)? Such tests require at least triplicate samples to calculate standard deviation, and therefore the data interpretation is incorrect and no conclusion can be drawn from this tests.

The standard deviation can be calculated by the deviation between the two sample values and the mean value without corrections by n-1. But as the number of replicates is certainly low, and since this is a preliminary methodological test but the differences between light and dark treatments are fairly evident, we have omitted statistics to avoid confusion.

Secondly, according to Fig. 1c, the bottles used for the dark experiments contained more of the model substances than the initial concentrations. How can this be? Pls explain. The authors claim that the concentrations of model substances were not significant different after incubation, but for the alginic acid in the dark exp. it seems the concentration were significant higher than initially (I estimate that by looking at the standard error). Again, the authors need to explain how a standard error is calculated on duplicate samples (see Figure 1 C).

Please see previous comments

Thirdly, the concentration levels chosen for this test are much higher than typical concentration of surface-active substances in natural seawater. Consequently, significant losses through adsorption on the glass wall, e.g. binding to active sites in the glass, at natural concentration levels may be masked through the high concentrations used in the test.

We agree with the reviewer that the concentrations used for this test were high, although within the range of concentrations in some conditions like diatom cultures or blooms (see for instance Corzo et al. 2000 AME 23:62, Ramaiah et al. 2001 MEPS 212:79, Hong et al. 1997, J. Phycol 33:368). However, for the new test performed,

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which is included in this revised version of the MS, we worked with a concentration of Xantan Gum within the ranges of TEP concentration reported in natural conditions.

Last but not least, the concentration of alginic acid after shaking is about half (estimated through Fig 1c) of the concentration measured without shaking. It is not clear to me why losses through shaking can occur? Unless concentrations have been measured in filtered samples after separation of TEP, but it seems that was not the approach.

For this first test of TEP adhesion to bottle walls we measured the concentration of the model substances by weighting filters before and after filtering a known amount of solution. Since the number of replicates is low (as also indicated by the reviewer), the dispersion between measurements was rather high using this approach. In fact, the alginic acid was not substantially lower after shaking than before shaking in one of the duplicates. However, the visible difference between samples irradiated and non-irradiated (see Fig. 2) is, in our opinion, marked enough to suspect photolysis.

Unfortunately, test on losses through adsorption on glass wall is important to interpret the further results, and I consider the resulting uncertainties as rather high.

To solve these uncertainties we have performed a new test on TEP adherence to bottle walls (please see the M&M section of the revised version of the manuscript). From this experiment we can conclude that TEP loss due to adhesion to the walls is a minor process, and may be even lower in quartz bottles (see enclosed figure).

Chemical and Biological Analyses P8/L12 Samples for dissolved mono- and polysaccharide have been filtered through a GF/F filter. GF/F filter do not have a well-defined pore size as filter membranes (such as polycarbonate membranes). I am concern with the use of GF/F filter in this study, as TEP may not retain on this filter, and be part of the filtrate and therefore contribute to the mono- and polysaccharide concentration. Consequently, the concentrations of TEP pre-cursor material may be overestimated.

For these analyses, we filtered water through pre-combusted GF/F filters, whose ac-

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tual pore size is 0.5 μm, close to the 0.4 μm filters that are used for TEP analyses. Therefore, the fraction of TEP that should have passed the filters would be minima. However, due to the flexibility of these particles, some of them can go through smaller pores that their actual size, which would lead to a slight overestimation of dissolved polysaccharides. The purpose of our study, however, was not to estimate real concentrations of TEP precursors in the experiments, and, indeed, total carbohydrates are not fully representative of acidic TEP precursors, but monitor changes in carbohydrate concentrations associated to changes in TEP. Anyway, we can conclude from our results that dissolved polysaccharides were not the final products of TEP photolyisis.

Results

Through the lack of the estimation of adsorption of surface-active substances on glass walls, the interpretation of the results is challenging. Before publication, I strongly suggest to repeat the test on glass wall adsorption.

Following the reviewers' suggestions we have repeated the test on glass adsorption, but in this case we have stained the bottles walls directly with alcian blue instead of shaking (see M&M of the revised version).

P9/L19 I disagree as according to Fig.2, the TEP concentration in Exp.1 with +UVB increased from a incubation time of 1.5 days to 3 days.

The increase observed in experiment 1 from 1.5 to 3 days was not statistically significant (see error bars), and was probably due to some growth of bacteria. Anyway, the overall decrease in TEP during the whole incubation time was clearly significant (see Table 2)

P9/L21 The sentence does not make sense as it says that by excluding UVB TEP decreased at lower rates in Exp. 2 but no significant changes were observed in Exp. 2 and 3. Pls clarify.

We made a mistake in this sentence. There were no significant differences over time

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respect to dark treatments in experiments 1 and 2. In experiment 3, the significant differences (see Table 2) are probably due to the increase in TEP in dark treatments. However, the decrease in TEP in experiment 2 in –UVB treatment itself (without comparison to dark treatments) is significant. However, we not include this last test to avoid too much complexity that would lead to confusion. We have rewritten this sentence.

Discussion

P12/L9 “We obtained an average TEP photolysis rate of 31% d , which would yield complete TEP photolysis in ca. 3 days under UVB radiation. However, TEP photolysis rates were even faster, at least 69–71% per day in 2 of our experiments, leading to a complete loss of TEP in around 35 h or less. “ This statement is misleading as in natural seawater decomposition and production of TEP may occur under different conditions. For example, TEP is produced at depths without significant exposure to radiation, and ascend to surface through positive buoyancy or adsorption on bubbles. That creates an upward flux of replenishing TEP reservoir in surface water, which likely exceeds decomposition as TEP has been found to accumulate in microlayers (as stated in the introduction of this paper). So I feel above statement is misleading and requires further discussion of TEP cycling.

We agree with the reviewer that in the field more processes, not only direct photolysis, govern TEP dynamics. Indeed, we include in the discussion section the potential replenishment of TEP in the sea surface microlayer (SML) by new ascending particles (page 13 line 374-377). However, the replenishment of TEP in the SML would possibly enhance the photolysis rates, as these are likely dependent on the initial TEP concentration or ‘availability’ in the SML. We think that our work is important in highlighting for the first time TEP susceptibility to photolysis, but indeed more effort is needed to study TEP photolysis dynamics in the field.

P12/L18 “Consequently, our results indicate that photolysis may at times be a potentially significant loss process of TEP, which should be included in future TEP budgets

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and carbon cycling scenarios." That is indeed true and important for a better understanding of carbon cycling. However the application of the presented data is limited to the upper 10 cm water layer (estimated through bottle diameter). Incubation at different depth/using light-shaded foil would have been useful to obtain similar data for the depths at which penetration of radiation is still significant.

As mentioned above, we think that our results are important in view of an accumulation of TEP in the SML. The approach proposed by the reviewer, although indeed interesting, would also be insufficient as it does not consider vertical movements of TEP through the water column (sedimentation vs floating).

P13/l18 "In the experiment testing TEP formation in the presence of microorganisms, TEP increased greatly under UVB radiation suggesting that UV promotes the production of TEP by organisms." Further discussion on TEP accumulation in the microlayer seems to be important here, as microlayer are often enriched in bacteria abundances. See also (Cuncliffe & Murrell, ISML Journal, doi:10.1038/ismej.2009.69)

We have included this information and reference in the discussion (page 13 line 368-370)

In the present form, I can not recommend this paper for publication, but would like to encourage the author to re-checked the test on wall adsorption, clarify and if necessary to re-do the test with more replicates. I am open for further discussions and clarification on how the test was performed. Getting further data on different exposure level (to simulate radiation levels at different water depth) would improve the relevance of the study.

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

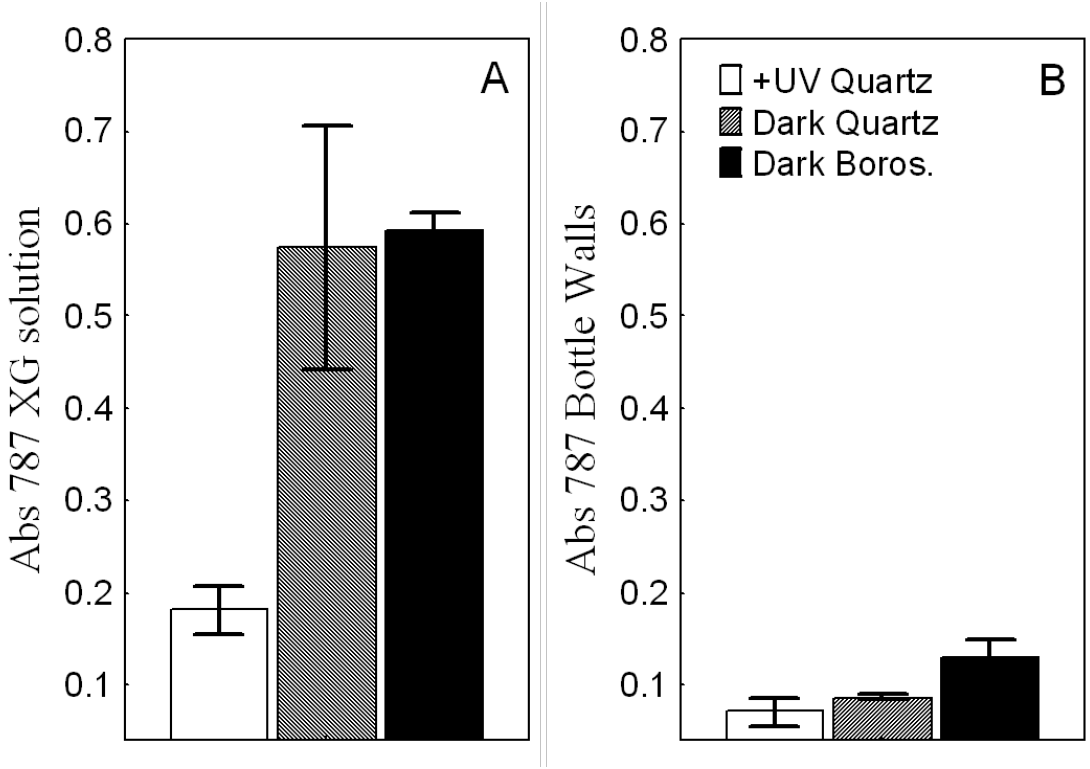

Fig. 1. Differences in Alcian Blue absorption at 787 after 3 days of exposure in the (A) xanthan gum solution and (B) the different bottle walls

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