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Interactive comment

Interactive comment on "Rapid abiotic transformation of marine dissolved organic material to particulate organic material in surface and deep waters" *by* Paola Valdes Villaverde et al.

Anonymous Referee #1

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In this study, seawater samples were repeatedly filtered, and the POC concentrations in re-filtered samples were assessed. It is important to assess the validity of established technique (here, filtration with GF/F filter), but this manuscript is too immature. The most serious concern is the validity of the discussion on the abiotic aggregation during filtration procedure. The authors considered that detected POC in re-filtered sample is derived from aggregated POC during filtration procedure. However, they did not provide any clear evidence for aggregation. If aggregation occurs within short period as the authors considered, conversion from DOM to POM might easily occur under turbulence in natural seawater as the authors discussed, but I feel suspicious. Many studies had previously shown aggregation process by shear stress. I think timescale

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of those experiments would be several tens minutes to hours. In this study, they said aggregation occurred within 4 seconds, which is quite short. If the authors provide clear evidence, this finding could have implication to change the concept of DOM-POM continuum. I think there is no clear evidence to support the aggregation in such short period. Simply, the particles passing through 1st filter might be trapped in the second filter. As the authors mentioned, structure of GF/F filter would be not uniform. On the contrary to membrane filter, cut-off by GF/F filter would be variable. Therefore, it is probable that a part of particles with size around 0.7 μm is sometimes trapped but sometimes not. In addition, I feel English is sometimes awkward, so the manuscript should be checked by professional correction service.

Introduction The strategy of this study is quite obscure. The authors should clearly state the aim of measurement of bacteria and TEP in introduction section. I cannot clearly understand what is new in this study. They should add more reference.

Line 26: I think the cut-off size is generally 0.7 μm. On the HP in Millipore, the pore size is described as 0.7 μm.

Line 30: I think the term “membrane enclosed particles” is not generally used. The authors did not apply any specific method the particle with and without membrane. So, I believe this term is not appropriate.

Line 34-36: Details of CHN instrument is not important in this study. I recommend deletion.

Line 39-42: Please describe how to treat the sample blank in the previous studies with more detail, because it relates with this study. Were the methods used in this study same with the previous study?

Line 45-47: From this sentence, readers will think that the particles are same regardless the name was different. However, the meanings of the words; EPS, SAG, mucilage, perfect slime, TEP, CSP, were overlapped, but not same. For example, TEPs

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are alcian-blue stainable particles, but CSP are coomassie stainable particles. Please clarify.

Line 47-49: I cannot understand why the authors mention that they do not consider marine snow in this sentence. I recommend deletion.

Line 57-58: Again, I think the authors should not separately describe MEPs and other particles, because this study did not distinguish the particles based on with/without membrane.

Line 60-62: In my understanding, for TEP measurement, filter with pore size of 0.4 μm has been used.

Line 71-74: In the two sentences in this section, the first one introduces aggregation of DOC in surface seawater, but second one is the description about deep sea. How did the author consider the difference of the samples? The ratios of POC/DOC would be considerably different across the depth, so it should be carefully compared.

Line 77-85: The author did not cite any reference in this section. For example, is there any literature about the relative contribution of hydrogels to the POC across the depth? They mentioned less change in hydrogel with depth, but please provide literature. In addition, they considered prokaryote cell passing the first filter is partly retained on the second filter, but is it not just absorption? How do they think the difference of retainment and absorption?

Line 88-89: Please provide reference.

Line 92-102: No literature was introduced this section. They need to carefully provide literature (e.g., termination of aggregation in phytoplankton bloom, acceleration of aggregation with turbulence).

Methods Line 114-119: I think the pressure at 5 kPa is relatively lower than standard protocol. They mentioned it took 1 hour to complete filtration for each filtration step. I feel it is slightly longer. Aggregation would time-dependently occur. If aggregation is important as authors consider, filtration with long period could be an artifact to induce aggregation. How much volume did they filter? In addition, what is Fi? In Fig. 1, POM1, 2 and 3 plus POMi were shown. I understand POM1-3 as sequential filtration. In the figure, I cannot understand what kind of sample is introduced to filtration funnel.

Line 122-137: The information about measurement of POC/PON would be redundant. I recommend more concise. On the other hand, I would like to recommend addition of detection limit and limit of quantification. Probably, the concentration of POC on the second filter would be low, so the detection limit would affect the quality of the data.

Line 158-159: Please provide reference for the value of TEP carbon to XG.

Line 161-165: How long were the samples stored before counting? Please clarify.

Results The quality of figures is bad. Lines and plots are sometimes overlapped.

Line 175-176: I would like to confirm how to treat the sample in the accident. Two samples were measured together, and the values were provided as a half of the measurement, right? If so, I strongly recommend to deletion the data.

Line177-184: When I read these sentences, I thought no difference in the treatment for experiment 1-3. Thereafter, I found difference among 1-3 in legend of Fig. 3. This is quite tricky, so please rewrite.

Line 191-199: First, the presentation of data using Fig. 4 is not appropriate. The log-scale is not useful to compare the data of top and bottom filter, because some plots were overlapped. I recommend boxplot. Difference of POC and PON between top and bottom filter should be statistically analyzed. The increase in the POC/PON ratio with filtration step should be also statistically analyzed. For the data analysis, I think the detection limit would be important. I suspend that POC on some of the bottom filter is under detection limit. Please clarify.

Line 199-203: I think it is difficult to discuss the aggregation due to shear stress based on the increase in bottom/top ratio. For example, if the shape of particles is ellipse, the efficiency of filtration would be not 100%. Natural particles would have complicate shape, so the particles with the size around cut-off of GF/F would sometimes pass, but sometimes retained. After sequence of filtration, most of such particles would be removed.

Line 204-207: The Fig. 5 is bad. Most of symbols and lines are overlapped. So, it is quite difficult to check the datasets.

Line 207-219: The authors showed only geometric means, but the error should be also provided. This study focuses on methodological aspect, so the assessment of confidence is essential.

Line 225-226: What is the “expected range for filters of 0.45 μm”?

Line 226-236: Fig. 7 is also bad quality. The data seems to be highly variable among replication. The author should interpret the data with consideration on technical error.

Line 229: What kind of statistical method was applied?

Line 238-239: These two sentences should be moved to introduction.

Line 224-257: Regarding TEP and bacterial cell number, no information of the concentration was provided. The raw data of the concentration is important to assess the validity of the methods.

Line 246-257: This section should be moved to interpretation.

Interpretation Line 259-262: The reason why the authors considered that aggregation due to fluid shear contribute to retainment of POC. If the reason is only increase in top/bottom ratio, I cannot agree as pointed out in the comment for Line 199-203. Please discuss with more clear evidence. The author should compare with the past study on aggregation under fluid shear. In Drapeau et al. (1994), aggregation under laminar shear flow was observed along time course using Couette device. They showed aggregation, but the timescale is several tens minutes to hours. Aggregation within 4 seconds is quite short. So, if the authors want to say, they need to provide more prominent evidence.

Line 272-274: Regarding aggregation by bubbling, a report had been recently published, in which the timescale to form aggregation is also provided. Wada et al. (2020) Journal of Oceanography, 76, 317-326

Line 282-290: Retainment of bacteria would not be evidence that new hydrogels appeared. There are a variety of organic particles other than bacterial cells, and behavior of those other particles would be different from that of bacteria. In addition, size of bacterial is highly variable. Since data of bacterial abundance was provided as ratios not concentration as pointed out by the comment for Line 224-257, it is impossible to justify whether this study can be generalized.

Line 307-310: Treatment with acid would remove not only inorganic particles. DIC in seawater sample would be absorbed to filter, and acid remove them. The concentration of DIC is generally 1-2 order of magnitude higher than POC and DOC, so it could lead overestimation.

Line 312-326: Here, the authors compared the present study with the previous ones. Such comparison should be done in introduction section, because when I read introduction, I felt the novelty of this study is unclear.

Line 330-334: I think hydrogels would also partly pass through the first filter as well as bacterial cells. I cannot understand why the author only mention aggregation regarding hydrogel.

Line 335-337: Please describe more detail of the literature; Maske and Garcia (1994). How much amount of carbon was absorbed? Is it possible to explain the results in this study from the amount of carbon absorbed in the literature? Was the seawater used in the literature comparable with the present study?

Line 339-341: The connection between this and past sentence is unclear. What is the reason why the author provided the assumption?

Line 343-350: The possibility that the authors proposed is not limited to bacteria. I feel strange why they put this information just before the discussion about bacteria.

Line 359: What does "this assumption" imply here? Please clarify.

Line 363-371: I also think bacterial cell trapped on 2nd filtration would be small, and carbon content per one cell could be low. Therefore, estimate of carbon in bacterial cells in Fig. 9 would be not reliable, and the discussion based on Fig. 9 is meaningless.

Line 372-378: Again, less contribution of bacteria is not evidence of aggregation. The authors should provide evidence.

Line 385-386: If shear stress during filtration really affect the concentration of TEP, as the author described, interpretation of TEP is difficult. However, the authors did not show clear evidence in this study.

Line 400-401: The difference in staining between Gum Xanthan and natural gels is not problem, because Gum Xanthan is just a standard compound. Problem is the ratio of TEP-carbon to Gum Xanthan equivalent is variable among samples.

Line 405-416: I cannot follow the authors' thought in that they described rapid and non-biogenic phase transition is supported. What is the direct evidence for this finding? In the section from 405-415, they introduce the previous reports, but suddenly said "Our data support ∼". Here, what is the "our data"? POC, TEP or bacteria? Please explain step by step.

Line 420-424: The proposed idea of the authors is not sufficiently proved.

Line 441-450: Many uncontrolled factors are considered in this section. If the condition on board has serious effect on the data, this study has serious problem in the viewpoint of reproducibility, because no one can treat sample with same manner in this study.

Line 444: I think reviewers' comment should not be directly mentioned in the main text.

Line 451-458: The authors should carefully interpret the results. Aggregation should be supported by clear evidence. Throughout this manuscript, I cannot find any clear evidence for aggregation.

Line 459-479: As the author mentioned, effect of turbulence on biogeochemical cycle has been recently attracted phenomenon. However, in this study, they did not provide any information on turbulence or shear stress. In addition, I think aggregation in quite short period is suspicious.

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