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Interactive comment on “Technical Note: Could benzalkonium chloride be a suitable alternative to mercuric chloride for preservation of seawater samples?” by J. Gloël et al.

Anonymous Referee #2

Received and published: 29 September 2015

The main objective of this study, which is written in the form of a technical note, is to test if Benzalkonium chloride (BAC) was as effective as Mercuric chloride (HgCl₂) in the prevention of microbial activity. Microbial activity was evaluated as production/consumption of O₂ during short term incubations. O₂ time course experiments were monitored by O₂/Ar ratios with membrane inlet mass spectrometry (MIMS). Authors worked with natural samples that were collected at different times and had different autotrophic and heterotrophic compositions”

The reasons to replace HgCl₂ with BAC are extremely relevant from an ecological, environmental and health point of view, and I agree with the authors that it is necessary to reduce the use of HgCl₂ due to its toxic nature. However, in order to instigate

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changes in the accepted methodology established by the scientific community, a new technique/method must be presented with irrefutable evidence, and my opinion is that this research is still very limited and does not provide reliability. There are several reasons for this conclusion:

Apparently, BAC had very short term effectiveness, and therefore does not correspond with the concept of preservation. It seems to work as a short-term microbial inhibitor. In the case that the process takes place over a period of a few days, the effect of BAC should be monitored hourly.

Since the authors were evaluating O₂ evolution (respiration and photosynthesis), authors should test the effect of BAC addition on nutrient and dissolved organic matter pools under the specific conditions in which incubation was carried out (e.g. light and darkness).

It is important to state the time of day at which the samples were analyzed, because the O₂ cycle depends on light (for photosynthesis) , Are the samples taking a the same time in each experiment?

It is not sufficient to monitor an experiment lasting from 7-16 days only 4 times, especially as there is a poor understanding of the behavior of BAC

The statistical analysis is not appropriate and the experimental setup is not clear; authors reported duplicate and three treatment s. It is not possible to calculate and ANOVA test. I would suggest that each treatment should be repeated a minimum of 3 times.

The authors did not explain what kind of statistical analysis was performed or how the combined errors in each treatment are estimated; it is very difficult to determine the errors associated with sampling such as avoid contamination by oxygen (since atmospheric levels of this gas is high).

Finally, most of coastal areas have Chl-a > 1 mg m⁻³, thus, why BAC is less efficient in

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productive waters, the added dose was not enough?

Interactive comment on Ocean Sci. Discuss., 12, 1953, 2015.

OSD

12, C796–C798, 2015

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