



# FILTRATION IN THE BENZALKONIUM CHLORIDE ADDITION METHOD FOR WATER SAMPLE PRESERVATION IN RADIOCARBON ANALYSIS OF DISSOLVED INORGANIC CARBON

Hiroshi A. Takahashi<sup>1</sup>, Koh Kakiuchida<sup>2</sup>, Wan Hong<sup>3</sup>, Masayo Minami<sup>4</sup>

<sup>1</sup> Geological Survey of Japan, AIST, Tsukuba 305-8567, Japan (h.a.takahashi@aist.go.jp)

<sup>2</sup> Graduate School of Environmental Studies, Nagoya University, Nagoya 464-8601, Japan

<sup>3</sup> Geochemical Analysis Center, KIGAM, Daejeon 34132, Republic of Korea

<sup>4</sup> Institute for Space-Earth Environmental Research, Nagoya University, Nagoya 464-8601, Japan

## Abstract

Dissolved inorganic carbon (DIC) concentration and carbon isotopic ratio ( $^{14}\text{C}$  and  $\delta^{13}\text{C}$ ) in natural waters often change during sample preservation due to the microbial activity in water sample. As an alternative to mercury-based conventional sterilization, our group tested benzalkonium chloride (BAC). However, it has been confirmed that BAC is not sufficiently effective for certain samples, including seawater (Takahashi et al., 2019; Takahashi and Minami, 2022). While the underlying mechanism remains unclear, it has been suggested that the high salt content of the water samples might contribute to the reduced efficacy of BAC (Takahashi et al., submitted). García et al. (2001) reported that biodegradation of BAC occurred due to marine bacterial populations, therefore, seawater likely contains microorganisms that BAC cannot inactivate. It should also be noted that BAC in water may be removed primarily by adsorption onto sludge rather than by biodegradation (Zhang et al., 2015). In this study, we examined the effect of suspended matter in water samples as one way of resolving these problems in using the BAC addition for sample preservation.

The investigation focused on the decline in bactericidal efficacy of BAC in the presence of mud in preserved samples. This decline was examined through a comparative analysis of seawater samples with and without mud, following BAC addition. Seawater samples, exhibiting no visible suspended particulate matter, were collected at the Pacific coast area. The mud were obtained from the tidal flat at the Bay area of the Pacific coast and subsequently sterilized in an autoclave at 120°C for 1 hour prior to use. The results demonstrated that the DIC underwent a significant change during the preservation process in samples containing mud, while the change in DIC was minimal in samples without mud. This finding suggests that the presence of mud might mitigate the bactericidal effect of BAC. However, given the assumption that mud contains a substantial number of microorganisms, it is plausible that the addition of mud to the samples, if the sterilization process was not complete, could have led to a considerable increase in the microbial population. This increase in microorganisms relative to the BAC could have been contributed to the observed change in DIC. Further verification is necessary to reach a definitive conclusion.

The presence of mud appears to affect the effectiveness of BAC in suppressing DIC changes when the BAC was added for water sample preservation, whether due to the mud itself or the large amount of microorganisms contained in the mud. However, filtration is expected to improve the situation regardless of the underlying cause. A combined procedure involving BAC addition and filtration has been proposed as an effective method for suppressing biological DIC changes in  $^{14}\text{C}$  analysis, including in seawater samples (Takahashi and Minami, 2024). Presumably, filtration plays a pivotal role in mitigating biological DIC changes in  $^{14}\text{C}$  analysis when using the BAC addition technique for water sample preservation. Further research is needed to ascertain the optimal pore size for filtration and to assess the potential ramifications of filtration on other issues.



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