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Intact preservation of environmental samples by freezing under an alternating magnetic field

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The study of environmental samples requires a preservation system that stabilizes the sample structure, including cells and biomolecules. To address this fundamental issue, we tested the cell alive system (CAS)-freezing technique for subseafloor sediment core samples. In the CAS-freezing technique, an alternating magnetic field is applied during the freezing process to produce vibration of water molecules and achieve a stable, super-cooled liquid phase. Upon further cooling, the temperature decreases further, achieving a uniform freezing of sample with minimal ice crystal formation. In this study, samples were preserved using the CAS and conventional freezing techniques at 4, -20, -80 and -196 (liquid nitrogen)oC. After 6 months of storage, microbial cell counts by conventional freezing significantly decreased (down to 10.7% of initial), whereas that by CAS-freezing resulted in minimal. When *Escherichia coli* cells were tested under the same freezing conditions and storage for 2.5 months, CAS-frozen *E. coli* cells showed higher viability than the other conditions. In addition, an alternating magnetic field does not impact on the direction of remanent magnetization in sediment core samples, although slight partial demagnetization in intensity due to freezing was observed. Consequently, our data indicate that the CAS technique is highly useful for the preservation of environmental samples.

Keywords: Subseafloor sediment, Freezing, Cell Alive System