

## Storage of biological specimens using the CAS freezing method in the scientific ocean drilling

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[Objectives] The discovery of the subseafloor biosphere from scientific ocean digs has brought attention to research on the biomass distribution and metabolic function of microorganisms throughout the Earth. The specimens collected by the deep sea exploration vehicle "Chikyu" and other drilling platforms are used for a wide range of scientific research related to geoscience and life science. Therefore, these specimens must be appropriately stored and their quality controlled as they are precious scientific assets of the human race. In this research, the storage of low-temperature drilled specimens in the CELLS ALIVE SYSTEM (CAS), which has attracted attention as a freezing technology that does not destroy biological cells, was evaluated.

[Method] Excavated samples obtained during the test voyage of "CHIKYU" were frozen using CAS and stored at  $-80^{\circ}\text{C}$ , and samples refrigerated and frozen at  $4^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$ ,  $-80^{\circ}\text{C}$  and  $-170^{\circ}\text{C}$  (liquid nitrogen tank) were stored at their respective temperatures. After 6 months and 2 years, the number of microbial cells in the sample was counted and compared.

[Results and consideration] As a result of storage, a decrease in the number of microbial cells was observed after storage for 6 months and 2 years, except for the samples frozen in CAS. It was also found that the number of microorganisms in the samples stored at  $-80^{\circ}\text{C}$  and liquid nitrogen decreased further after storage for 2 years, but the number of microorganisms in the samples frozen in CAS did not decrease. CAS applies a weak alternating magnetic field to the sample and vibrates the water molecules to cool them. As a result, freezing occurs after the entire sample is uniformly cooled below the freezing point (supercooling), so that fine and uniform ice crystals are formed in the sample, and it is considered that the microbial cells can be preserved without being destroyed.

This technology is useful for cryopreservation of valuable drilled samples, and is considered to be an indispensable storage method for studying the unknown biosphere under the ocean floor with future advanced analytical techniques.

Key words: Cells Alive System, subseafloor sediment, freezing preservation

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