

MIS027-11

Room:201A

Time:May 22 17:00-17:15

## Thermodynamic control on anaerobic oxidation of methane below the sulfate-methane interface

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The methane flux from marine environment accounts for only 2% of the global methane flux, because most of methane in anoxic marine environment is microbiologically consumed by anaerobic oxidation of methane (AOM). In the AOM, sulfate is used as a terminal oxidant in the following net reaction:  $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$ . Molecular biological and biogeochemical studies demonstrated that microbial consortium of anaerobic methanotrophic archaea (ANME) and sulfate reducing bacteria (SRB) is the major candidate for AOM reaction. The metabolic process of AOM is speculated to be an intermediate shuttling between ANME and SRB, which account for a transfer of reducing equivalent or organic carbon compound directly derived from methane. AOM was considered to be restricted to the sulfate-penetrated sediment due to the close coupling between methane oxidation and sulfate reduction. However the previous study showed that single phylogenetic group of anaerobic methanotrophs, ANME-1, was dominant in the sediments underlying the sulfate-methane interface (SMI). In this study, we focused on the ANME-1-mediating process of the interaction between sulfate reduction and methane oxidation, and the environmental control in marine sediment off Joetsu, Japan. Control factor and putative AOM reaction mechanism of ANME-1 occurring below the SMI were discussed from the perspective of thermodynamic and kinetic constraints. ANME-1-dominated sediment was rich in methane and alkalinity but was poor in sulfate and hydrogen sulfide, suggesting that net AOM reaction was thermodynamically and kinetically favorable. However, absence of consortium-like structure composed of ANME and syntrophic bacterial partner suggested the AOM proceeded independently of direct association with sulfate reduction. Considering the concentration of molecular hydrogen in the porewater, methanogenesis can be thermodynamically more favorable than sulfate-independent methane oxidation. ANME-1 might act as methanogen in deep sediment below the SMI.

This research is supported by MH21 Research Consortium JAPAN.

Keywords: archaeal methanotroph (ANME), anaerobic oxidation of methane (AOM), gas hydrate, Japan Sea