Stable isotope and microbial analyses of methane-producing process in a geothermal aquifer associated with the subsurface of the accretionary prism, Japan


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The sedimentary layer in the southern part of Japan is accretionary prism which includes enriched organic materials derived from sediment on oceanic plate. There is geothermal aquifer in which a large amount of methane (CH$_4$) dissolved. Since CH$_4$ is important as a greenhouse gas and an important natural gas fuel, revealing CH$_4$-producing process in subsurface environment is required. To understand the process of the CH$_4$ production, we collected the groundwater from the aquifer of 1,189-1,489 m depth, and analyzed by using stable isotope and microbial analyses.

16S rRNA gene analysis showed a dominancy of hydrogenotrophic methanogens in domain *Archaea* and a dominancy of anaerobic heterotrophes to be known to produce H$_2$ and CO$_2$ by fermentation process in domain *Bacteria*. The anaerobic enrichment cultures with the groundwater amended with organic substrates showed that CH$_4$ was produced by co-culture between the fermenters and hydrogenotrophic methanogens. On the other hand, conventional isotopic estimations for the origin of CH$_4$ using $\delta^{13}$C-CH$_4$ and $\delta$D-CH$_4$ as well as $\delta^{13}$C-CO$_2$ and molecular ratio of $C_1/(C_2+C_3)$ indicated that CH$_4$ was derived from thermogenic pathway. The values of $\delta^{13}$C-CO$_2$, however, had higher values and carbon isotope fractionation factors between CH$_4$ and CO$_2$ ($\alpha(CO_2-CH_4)$) were approximately 1.05 to 1.06 indicating the possibility of biogenic CH$_4$ production. Therefore, the origin of CH$_4$ production was estimated as mixing both thermogenic and CO$_2$ reduction from isotopic data.

Furthermore, we incubated these enriched co-cultures and measure stable carbon isotope ratios of CH$_4$ and CO$_2$ and stable hydrogen isotope ratios of H$_2$O and CH$_4$. We revealed that concentration of H$_2$ were kept lower by these co-cultures between fermenters and hydrogenotrophic methanogens and $\alpha(CO_2-CH_4)$ values were higher than that of cultures with the ground water amended with high concentration of H$_2$+CO$_2$. Hydrogen isotope fractionation factor between H$_2$O and CH$_4$ by these co-culture increased ($\alpha_H$ values decreased) with increasing H$_2$ concentration.