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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 δ^{13}C-CH_4 and δD-CH_4 as well as δ^{13}C-$CH_4$and molecular ratio of $C_1/(C_2+C_3)$ indicated that CH_4 was derived from thermogenic pathway. The values of δ^{13}C-CO_2, however, had higher values and carbon isotope fractionation factors between CH_4 and CO_2(α(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_2O and CH_4. We revealed that concentration of H_2 were kept lower by these co-cultures between fermenters and hydrogenotrophic methanogens and α(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_2O and CH_4 by these co-culture increased (α_H values decreased) with increasing H_2 concentration.