

Carbon and hydrogen isotope fractionation by methane production in co-culture between fermenters and hydrogenotrophic methanogens

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The sedimentary layer in the southern part of Japan is accretionary prism which includes enriched organic matters derived from sediment on oceanic plate. There is geothermal deep-aquifer in which a large amount of methane (CH₄) dissolved. Because of importance of CH₄ both as a greenhouse gas and as natural gas fuel, revealing the process of CH₄ production in the deep-aquifer is required. In our previous study based on 16S rRNA genes analyses, we had revealed that hydrogenotrophic methanogens and H₂-producing fermentative bacteria are dominant in the deep-aquifer.

In this study, anaerobic enriched cultures with this groundwater amended with organic substrates which achieved co-cultures between fermentative bacteria and hydrogenotrophic methanogens and cultures with this groundwater under H₂+CO₂ (80:20, v/v) condition in which hydrogenotrophic methanogens produce CH₄ by CO₂ reduction were incubated at 55 and 65°C. Concentration of H₂, CH₄, and CO₂ were monitored by TCD-GC. In addition, d¹³C-CH₄, dD-CH₄, d¹³C-CO₂, and dD-H₂O values were measured and carbon isotope fractionation factors (*alpha*(CO₂-CH₄) value) and hydrogen isotope fractionation factors (*alpha*_H value) were determined.

In these co-culture between fermentative bacteria and hydrogenotrophic methanogens, H₂ concentration increased at the initial phases, then decreased gradually, and kept low during CH₄ producing, indicating H₂ consumption by hydrogenotrophic methanogens to produce CH₄. *alpha*(CO₂-CH₄) values by these co-cultures were higher (more than 1.065) than that of the cultures under high concentration of H₂ + CO₂ (*alpha*-values were approximately 1.02). On the other hand, *alpha*_H values by these incubation experiments showed that hydrogen isotope fractionation decrease (*alpha*_H values increase) at lower H₂ concentration. We will discuss mainly the relationship H₂ concentration in the cultures and carbon and hydrogen isotope fractionation factors. Additionally, we will mention the comparison with other studies.