

## Microbial potential and carbon cycle in deep aquifer of the accretionary prism of Southwest Japan

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The accretionary prism situated along the Pacific side of Southwest Japan forms thick sediments. The sediment contains deep aquifers that anaerobic groundwater is accumulated. In addition to the anaerobic groundwater, it has been reported that dissolved natural gases composed mainly of methane are present in the deep aquifers. The groundwater and natural gases are collected from deep wells (150-1500 m depth) which are drilled at the accretionary prism. In the past study conducted in a deep well situated Shimada, Shizuoka Prefecture, Japan, it has been shown that methane has been produced by subterranean microbial community in deep aquifer associated with accretionary prism. However, microbial and geochemical studies have not yet been performed at other areas of accretionary prism. In this study, we collected groundwater and natural gases from 14 deep wells of Shizuoka Prefecture, and we performed measurements of physical and chemical parameters, anaerobic cultivations of microbial communities and 16S rRNA gene analysis to understand microbial potential and carbon cycle in subterranean environments of the accretionary prism.

The temperature of groundwater samples ranged from 24.2 to 49.3 °C, and pH was weakly alkaline. Oxidation-reduction potential suggested -325 to -114 mV at all deep wells. Electric conductivity ranged widely from 92 to 2,110 mS m<sup>-1</sup> at each groundwater sample. NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and S<sup>2-</sup> in groundwater was below the detection limit. Dissolved organic carbon (DOC) ranged from <0.3 to 50 mg l<sup>-1</sup>. From componential analysis of the natural gases, methane was predominant gas component at many sites (>90%). On the other hand, we detected several natural gas samples contained a large amount of N<sub>2</sub> (20-50%). Stable carbon isotopic analysis of methane in the natural gases and dissolved inorganic carbon (DIC) in groundwater suggested that methane of biogenic origin are contained in the natural gases at a lot of sites.

Anaerobic incubations using groundwater amended with organic substrates revealed the high potential of H<sub>2</sub> and CO<sub>2</sub> generation by H<sub>2</sub>-producing fermentative bacteria. Furthermore, methane generation by syntrophic consortium of H<sub>2</sub>-producing fermentative bacteria and H<sub>2</sub>-using methanogen was also observed in 3-5 days after the start of incubation.

Bacterial 16S rRNA gene analysis indicated the dominance of H<sub>2</sub>-producing fermentative bacteria. The presence of denitrifying bacteria was also observed at the sites where N<sub>2</sub> is contained in the natural gas samples. In archaeal 16S rRNA gene analysis, H<sub>2</sub>-using methanogens dominant in the groundwater.

From these date, it was shown that carbon cycle that methane has been produced from organic matters which are contained in the sediments by syntrophic consortium of H<sub>2</sub>-producing fermentative bacteria and H<sub>2</sub>-using methanogens exist in wide area of the subterranean environments of the accretionary prism. In addition to methane production, the presence of denitrification using NO<sub>3</sub><sup>-</sup> or NO<sub>2</sub><sup>-</sup> and organic matter or methane was also suggested at a few site.

Keywords: accretionary prism, deep aquifer, methanogenesis, fermentation, syntrophic biodegradation, subsurface environment