

## Measurement of denitrification rate in deep subsurface sediments using acetylene blockage method

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In order to evaluate the safety of the geological deposit of high-level radioactive waste, we have to know the activity as well as the numbers and species of indigenous microorganisms. Especially denitrification rate is very important because high-level radioactive waste contains a great deal of nitrate and some researches said in denitrifying condition nitrous acid is formed in the process of reducing nitrate, and this reoxide reduced U(IV) mineral. In this study, we measured the denitrification rate of deep subsurface sediment using acetylene blockage method. Sediment cores were sampled in the depth of 302 m (tuff) and 340 m (mudstone) with drilling water which contained no microorganisms and no oxygen.

Sediment cores were crashed and put into the 240-ml glass bottle. And 10ml filter-sterilized groundwater for drilling water was poured in the bottle at anaerobic chamber. Two kinds of groundwater were prepared; sodium nitrate was added to one sample in order to contain 10mM nitrate and no nitrate to the other. The bottles with slurry were shut tightly with butyl rubber plug pricking with a needle with silicone plug. Then we took out the bottle from anaerobic chamber, and extracted air from the bottles with vacuum pump. After that, Nitrogen gas with 10-% acetylene was injected to them. We put the bottles into the chamber which was set up 25 C degree constantly. After 4 days, we took them out from the chamber, extracted head-space gas with a gas-tight syringe, and measured the concentration of N<sub>2</sub>O gas with gas chromatography equipped ECD detector.

In not only the sample with 10mM nitrate but also with no nitrate addition in the 302-m sample, formation of N<sub>2</sub>O was detected. On the other hand, in the 340-m sample, no N<sub>2</sub>O was detected. This result indicated that tuff layer at the depth of 302 m was suitable for microorganisms to live because of high water permeability, which provided microorganisms with nutrients and electron acceptor.

Now we are doing the experiment in order to evaluate the influence of the addition of organic matter on denitrifying property.