A research of the metabolisms of nitrogen in deep subsurface environment - mainly about the nitrate reduction -

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1.Introduction

In our previous study, the reduction of nitrate was found from tuff and mudstone sampled from more than 300-m depth in the Tertiary sedimentary rock area. In another analyses, e. g. anion analysis of pore water squeezed from core and genetic analysis using 16S rRNA, the existence of the metabolisms mainly using nitrogenous chemicals, especially denitrification. In this study, we measured denitrification rate using various substrates, and elucidate what indigenous microorganisms metabolize.

2.Materials and Methods

We used acetylene blockage technique for measuring denitrification activity. Rock sample and water were added to the autoclaved 67-mL vial bottle. We plugged sample bottles with a butyl lubber plug and took these bottles out from the anaerobic chamber. And we removed head-space gas from each bottle with a vacuum pump equipped hypodermic needle, and injected gas mixture, 0.1 v/v acetylene in nitrogen gas, so that the inside of the bottles would have slight positive pressure. After the injection of the gas mixture, the bottles were put into the chamber which was set up 25 degrees Celsius constantly. Rock cores were sampled in 6 depths (293m, 300m, 302m, 324m, 340m, 350m). Water used for incubation was ultra-filtered ground water drown from the well near the boring place. 10 mM of nitrate and several organic compounds (acetate, lactate, glycerol, succinate) and gases (hydrogen gas, methane) were added to the water for substrate. If the sample contained several substrate had higher denitrification rate than that contained only nitrate, we made another sample contained only one kind of substrate in order to clarify the real substrate.

3.Result and Discussion

Samples in 300-m, 302-m, 350-m had high denitrification rate. 300-m and 302-m sample were very soft tuff sample enough to destroy the structure easily, so it was easy for ground water to pass through this layer and for microorganisms to live there. 350-m mudstone layer had larger pore space than another layer. In both tuff layer and 350-m mudstone layer, hydrogen gas was consumed most for denitrification, and we found that fermentation was the major process of denitrification there. And in 350-m sample, the promotion of denitrification using methane was found, and this phenomenon suggested the possibility of anaerobic methane oxidation