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# Quantification of microbial activity in subsurface environments using a hydrogenase enzyme assay

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The subsurface biosphere is the largest microbial ecosystem on Earth. Despite its large size and extensive industrial exploitation, very little is known about the role of microbial activity in the subsurface. Subsurface microbial activity plays a fundamental role in geochemical cycles of carbon and other biologically important elements. How the indigenous microbial communities are supplied with energy is one of the most fundamental questions in subsurface research. It is still an enigma how these communities can survive with such recalcitrant carbon over geological time scales.

Despite its usually very low concentration, hydrogen is an important element in subsurface environments. Heterotrophic and chemoautotrophic microorganisms use hydrogen in their metabolic pathways; they either obtain protons from the radiolysis of water and/or cleavage of hydrogen generated by the alteration of basaltic crust, or they dispose of protons by formation of water.

Hydrogenase ($H_2$ase) is a ubiquitous intracellular enzyme that catalyzes the interconversion of molecular hydrogen and/or water into protons and electrons. The protons are used for the synthesis of ATP, thereby coupling energy-generating metabolic processes to electron acceptors such as carbon dioxide or sulfate. $H_2$ase activity can therefore be used as a measure for total microbial activity as it targets a key metabolic compound rather than a specific turnover process. Using a highly sensitive tritium assay we measured $H_2$ase enzyme activity in the organic-rich sediments of Lake Van, a saline, alkaline lake in eastern Turkey and in marine subsurface sediments of the Barents Sea. Additionally, sulfate reduction rates (SRRs) were measured to compare the results of the $H_2$ase enzyme assay with the quantitatively most important electron acceptor process.

$H_2$ase activity was found at all sites, measured values and distribution of activity varied widely with depth and between sites. At the Lake Van sites $H_2$ase activity ranged from ca. 20 mmol $H_2$ $cm^{-3}$ $d^{-1}$ close to the sediment-water interface to 0.5 mmol $H_2$ $cm^{-3}$ $d^{-1}$ at a depth of 0.8 m. In samples from the Barents Sea $H_2$ase activity ranged between 0.1 to 2.5 mmol $H_2$ $cm^{-3}$ $d^{-1}$ down to a depth of 1.60 m. At all sites the SRR profile followed the $H_2$ase activity profile until SRR declined to values close to the minimum detection limit ($\sim$10 pmol $cm^{-3}$ $d^{-1}$). $H_2$ase activity increased again after SRR declined, indicating that other microbial processes are becoming quantitatively more important. The $H_2$ase and SRR data show that our assay has a potential to become a valuable tool to measure total subsurface microbial activity.