Quantitative molecular techniques to study the abundance and activity of microorganisms in the marine deep subsurface

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The Ocean Drilling Program Leg 201 Peru Biosphere, dedicated to studying deep sub-surface marine microbiota, focused on the abundance and activity of microorganisms in marine deep sediments of the Peru Continental Margin and the Equatorial Pacific down to 400 m below seafloor. Currently, we are applying fluorescence in situ hybridization (FISH) and real-time Polymerase Chain Reaction (rt-PCR) to quantify bacteria and their activity in ODP Leg 201 samples. The rt-PCR approach for quantification of microorganisms promises to be more sensitive than FISH or MPN under subsurface conditions. Experimental design of rt-PCR permits the quantification of phylogenetic groups or single species of microorganisms by amplification of 16S rDNA(rRNA) molecules. The metabolic state of microorganisms can be determined by the quantification of mRNA of functional genes. We have studied the detection limits, reproducibility, and applications of the method, measuring the expression of 16S rRNA and dissimilatory (bi)sulfite reductase (DSR) mRNA in *Desulfobacterium autotrophicum* during in vitro growth. Using DSR as phylogenetic and activity marker, we will study the *in situ* activity of sulfate reducing bacteria in the sub-seafloor biosphere.

Activity coefficient and polymerization of aqueous silica based on high-*PT* solubility measurements

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We have made high-*PT* solubility measurements on several mineral assemblages which buffer aqueous SiO_2 at a wide range of concentrations, in order to define more closely the activity-concentration relations. Solution equilibria at 800 °C and 12 kbar were determined on zircon ($ZrSiO_4$, ZRC)-baddeleyite (ZrO_2, BDY), enstatite ($MgSiO_3$, EN)-forsterite (Mg_2SiO_4, FO), and kyanite (Al_2SiO_5, KY)-corundum (Al_2O_3, COR) assemblages from both the SiO_2-undersaturated and -oversaturated directions on synthetic crystalline materials. The NaCl-graphite piston-cylinder apparatus was used with welded Pt capsules containing H_2O+initial SiO_2 and a perforated inner Pt capsule containing the mineral assemblages. Solubilities were determined by weight changes of the inner capsule after a run (solubilities of components other than SiO_2 were below detection). The SiO_2-buffering reactions, higher and lower bounds on the equilibrium SiO_2 molality (m_h and m_l), and the mean SiO_2 activity coefficient (γ_S) derived from the Zotov and Keppler (Chem. Geol., 2002) dimerization model, are given in the table below.

Reaction	ΔG^o kJ/mol	m_h molal	m_l molal	γ_s
ZRC=BDY+SiO_2	16.31	0.0632	0.060	1.090
2 EN=FO+SiO_2	9.17	0.285	0.267	0.527
KY=COR+SiO_2	2.05	1.027	0.940	0.333
QTZ=SiO_2	0	1.500		0.277

$\Delta G°$ at 800 °C and 12 kbar of each reaction (with quartz: Holland and Powell, J. Meta. Geol., 1998) and quartz solubility (Manning, Geochim. Cosmochim. Acta, 1994) are necessary inputs.

The equilibrium constant controlling dimer (d) formation from monomers (m) at constant *T* and *P* is:

$$K = (X_d/X_m^2) \bullet a_{H2O}^{\Delta n}$$

where a_{H2O} is the activity of H_2O and Δn is the net change in hydration number. This model with Δn=1 fits all of our solubility brackets very well with K=169, except for the ZRC-BDY assemblage, whose very low SiO_2 solubility gives an impossible negative K and $\gamma_s > 1$. This may indicate a different solution behavior at very low SiO_2 concentration.