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The in-situ microbial reduction and immobilization of uranium was assessed as a means of preventing the migration of this element in the terrestrial subsurface. Uranium reduction and microbial respiratory activities were evaluated in the presence of exogenous electron donors and acceptors with field push-pull tests using wells installed in an anoxic aquifer contaminated with landfill leachate. Uranium(VI) amended at 1.5 μ M was reduced to less than 1 nM in groundwater in under 8 d during all field experiments. Amendments of 0.5 mM sulfate or 5 mM nitrate slowed U(VI) immobilization and allowed for the recovery of 10% and 54% of the injected element, respectively compared to 4% in the unamended treatment. Laboratory incubations confirmed the field tests and showed that the majority of the U(VI) immobilized was due to microbial reduction. In these tests, nitrate treatment (7.5 mM) not only inhibited U(VI) reduction, but also appeared to remobilize uranium as nitrite was transiently produced. This latter finding was confirmed with field push-pull tests in which 0.6 mM or 7.5 mM nitrate and 1.5 mM U(VI) were injected into sediments which already contained immobilized uranium. After an initial loss of the amendments, the concentration of soluble U(VI) increased and eventually exceeded the injected concentration, indicating that previously immobilized uranium was remobilized as nitrate was reduced. Laboratory experiments using heat-inactivated sediment slurries suggested that the denitrification intermediates, nitrite, nitrous oxide, and nitric oxide, were all capable of oxidizing and mobilizing U(IV). These findings indicate that in-situ subsurface U(VI) immobilization can be expected to take place under anaerobic conditions, but the permanence of the approach can be impaired by denitrification intermediates that can mobilize previously reduced uranium.

B21B-04 0915h INVITED

Assessing Enhanced Anaerobic and Intrinsic Aerobic Biodegradation of Trichloroethene

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Biodegradation of chloroethenes can proceed either anaerobically or aerobically; however, the techniques for monitoring the two pathways are quite different. At the Idaho National Engineering and Environmental Laboratory's Test Area North (TAN), a combination of anaerobic and aerobic biodegradation of trichloroethene (TCE) is being employed for restoration of a large plume of contaminated groundwater. During stimulation of anaerobic biodegradation of TCE through lactate addition, several assessment tools have proven effective for various objectives. Monitoring TCE and its lesser chlorinated degradation products provides a straightforward assessment tool for the occurrence of degradation. It does not, however, provide information regarding the potential for reductive dechlorination, nor progress from less suitable to more suitable conditions. A technique for obtaining this information is monitoring redox-sensitive geochemical parameters such as dissolved iron, sulfate, methane, and oxidation-reduction potential. This approach was demonstrated by the strong correlation of steps in the reductive dechlorination pathway to redox conditions at the TAN site. Yet another tool is required to determine adequacy of conditions for efficient dechlorination. Dechlorination efficiency appears to be dependent upon the predominant electron donor utilization (or fermentation) process occurring at any given time, an observation consistent with thermodynamic considerations. Thus, monitoring of added electron donor and intermediate product concentrations can help determine an efficient operations strategy. One final tool demonstrated at the TAN site was monitoring stable carbon isotope ratios. As TCE was dechlorinated, a clear fractionation occurred from cis-dichloroethene to

vinyl chloride, and from vinyl chloride to ethene. This fractionation provides a clear signature of reductive dechlorination.

Assessment of aerobic biodegradation of chloroethenes at TAN is more challenging because of the lack of readily identifiable degradation products. The aerobic pathway for TCE consists of cometabolic oxidation by any of several oxygenase enzymes. Intermediates generated in this process are very short-lived. Thus, monitoring of degradation products in the field has not been found to be useful. While disappearance of TCE might suggest aerobic biodegradation, it does not distinguish biodegradation from other processes such as dilution. This issue was resolved at TAN through normalizing TCE concentrations to the concentrations of internal plume tracers. This allowed degradation to be distinguished from physical processes such as dispersion, dilution, sorption, and volatilization. When combined with standard techniques such as enumeration and screening of organisms in site groundwater with the capability to aerobically degrade TCE, this technique provided convincing evidence that intrinsic aerobic biodegradation is occurring at the site, and estimated a rate. Direct measurement of this process remains a challenge, but preliminary work with activity-dependent, fluorescent enzyme probes appears promising. While the appropriate assessment tool depends on the objectives to be met, a combination of tools will almost always be necessary for thorough documentation and implementation of biodegradation of chloroethenes in the field.

B21B-05 0930h

Use of Biotracer Tests to Evaluate the Feasibility of In-Situ Bioremediation

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In-situ bioremediation of organic compounds in the subsurface is increasing in popularity. A biodegradation tracer test, also known as biotracer test, is one approach to determine the feasibility of practicing this method. This test involves conducting a tracer experiment with one or more compounds whose biodegradation properties are known. The mass recovery and transport of the biotracers are compared to a non-biodegradable, conservative tracer to measure the biological activity of the target zone. Field experiments were conducted at three sites. All three sites were contaminated with non-aqueous phase liquids. Biotracers were also used to investigate the response of the system to the addition of perturbations, e.g. the addition of oxygen. The results from these field experiments suggest biotracers are a promising method for characterizing in-situ bioremediation.

B21B-06 0945h

In Situ Respiration and Direct Enzymatic Assays for Assessing Bioremediation

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Laboratory and field studies of in situ bioremediation at sites contaminated with organics or metals have demonstrated that in situ measurements of respiration using start/stop tests or He tracers combined with direct assays of dehydrogenase activity in the sediment are much more effective in determining the efficacy of the bioremediation strategy being used. Sites with high TOC (> 6000 ppm) can have a low oxygen demand if the carbon present is not bioavailable or is recalcitrant to the indigenous microorganisms, as indicated by both respiration rate and dehydrogenase activity. Addition of surfactants or limiting nutrients at these sites increases both the respiration rate and the dehydrogenase activity resulting in a concomitant decrease in the contaminants of concern (TPH, PAH, TCE, DCE, VC, MSW). Similar studies in the laboratory have shown that reduction of chromium is correlated with increased dehydrogenase activity and not to densities of microorganisms or the presence of particular groups as indicated by molecular probes or fatty acid analyses. In situ respiration tests and direct enzymatic measurements of sediment may provide better control of in situ bioremediation processes by indicating need for addition of nutrients, surfactants, or additional carbon/energy supplements.

B21C MC: 120 Tuesday 1020h

Biological Mineralization: Proxies and Processes (joint with OS, PP, MR)

Presiding: P M Dove, Virginia

Polytechnic Institute and State University; J J DeYoreo, Lawrence Livermore National Laboratory

B21C-01 1020h INVITED

Mineralization of the Sea Urchin Skeleton

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The sea urchin possess a calcareous skeleton composed of over 99% magnesian calcite, an enveloping extracellular matrix, and an occluded protein matrix. The most intensively studied skeletal element is the spicule of the embryo. At the 32 cell stage of development a cohort of 4 cells becomes irrevocably dedicated to spicule formation. At the early gastrula stage the descendants of these founder cells form the primary mesenchyme (PMC). The PMCs fuse to form a multi-nucleated syncytium connected by cytoplasmic cables, and the calcitic skeleton is formed within these cables. Our primary concern is with the cellular and molecular mechanisms that support the formation of the mineralized spicules. The import of calcium into the PMCs results in appearance of intracellular vesicles containing precipitated calcium, which is neither very stable nor birefringent, and could be amorphous. The precipitated calcium is vectorially secreted into an extracellular space. This space is almost completely enclosed by cytoplasmic strands, and the mineral is encased in an extracellular matrix. Proteins destined for the extracellular matrix, and for inclusion in the spicule, are present in the Golgi membranes and in small intracellular vesicles. These vesicles apparently deliver the matrix proteins to the growing spicule. Our current view is that the matrix molecules are much more than a passive armature, but are actively involved in precipitation, secretion, and organization of the mineral phase.

B21C-02 1035h INVITED

Silica Synthesis by Sponges: Unanticipated Molecular Mechanism

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Oceanic diatoms, sponges and other organisms synthesize gigatons per year of silica from silicic acid, ultimately obtained from the weathering of rock. This biogenic silica exhibits a remarkable diversity of structures, many of which reveal a precision of nanoarchitectural control that exceeds the capabilities of human engineering. In contrast to the conditions of anthropogenic and industrial manufacture, the biological synthesis of silica occurs under mild physiological conditions of low temperatures and pressures and near-neutral pH. In addition to the differentiation between biological and abiotic processes governing silica formation, the biomolecular mechanisms controlling synthesis of these materials may offer insights for the development of new, environmentally benign routes for synthesis of nanostructurally controlled silicas and high-performance polysiloxane composites.

We found that the needle-like silica spicules made by the marine sponge, *Tethya aurantia*, each contain an occluded axial filament of protein composed predominantly of repeating assemblies of three similar subunits we named "silicateins." To our surprise, analysis of the purified protein subunits and the cloned silicatein DNAs revealed that the silicateins are highly homologous to a family of hydrolytic enzymes. As predicted from this finding, we discovered that the silicatein filaments are more than simple, passive templates; they actively catalyze and spatially direct polycondensation to form silica, (as well as the phenyl- and methyl-silsesquioxane) from the corresponding silicon alkoxides at neutral pH and low temperature. Catalytic activity also is exhibited by the silicatein subunits obtained by disaggregation of the protein filaments and those produced from recombinant DNA templates cloned in bacteria. This catalytic activity accelerates the rate-limiting hydrolysis of the silicon alkoxide precursors. Genetic engineering, used to produce variants of the silicatein molecule with substitutions of specific amino acid sidechains, in conjunction with computer-assisted

molecular modeling and biomimetic synthesis, allowed us to probe the determinants of catalytic activity and confirm the identification of the amino acid sidechains required for hydrolysis of the silicon alkoxides. If, as suggested by the data of others, silicic acid is conjugated with organic moieties after its transport into the cell, the catalytic mechanism described here may be important in biosilicification by sponges.

As is often the case, we have been better able to answer mechanistic questions about "how" silica can be formed biologically, than "why" the diversity of structures is elaborated. Studies of spicule formation during cellular regeneration in *Tethya aurantia* reveal that synthesis of the larger silica needles (megascleres) and smaller starburst-shaped microscleres may be independently regulated, presumably at the genetic level. The spatial segregation of these morphologically-distinct spicule types within the sponge further suggests an adaptive significance of the different skeletal elements.

B21C-03 1050h INVITED

Stable Isotopic Signatures of Microbially Formed Carbonates and Metal Oxides: Temperature Proxies for Biomineralization in Low Temperature Sedimentary Environments

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Microorganisms exist and mediate geochemical processes in almost all low-temperature sedimentary environments. A variety of authigenic minerals can be formed directly or indirectly by microbial activities. Carbon and oxygen isotopic compositions of these minerals may indicate depositional temperatures at the time of mineral precipitation. A review of isotopic fractionations associated with biogenic magnetite and siderite will be provided. These biogenic minerals show temperature-dependent carbon and oxygen isotopic fractionations under laboratory conditions. However, the temperature-dependent fractionations can be complicated by variation in solution chemistry. An understanding of microbial activity, solution chemistry, mineralogy, and isotope biogeochemistry at the systems level should provide better insight into the mechanisms for biogenic mineral formation in natural environments.

URL: <http://web.missouri.edu/~geoscsc/research.html>

B21C-04 1105h INVITED

Mg and Sr Incorporation in Foraminifer Shells: Patterns, Controls and Applications.

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The incorporation of Mg and Sr in planktonic and benthic foraminifer shells is important for paleoceanographic research because of the potential to record physical and chemical changes in the oceanic environment. Pelagic shells are 99%+ CaCO₃, and abundances of Mg and Sr are typically ~0.1%, requiring sensitive quantification methods such as ICP-MS or AES.

Mg/Ca values range from 0.5 mmol/mol in cold planktics and benthics to ~5 mmol/mol in tropical planktics, with some species (*Orbulina universa*) having even higher values. The main control on Mg incorporation is temperature, but pH and salinity also exert small influences, presumably through calcification rate. The Mg/Ca content of the primary ontogenetic calcite can be altered by the addition of so-called gametogenic calcite, generally deposited in deep, colder waters. After deposition on the seafloor, dissolution becomes the main influence, with progressively lower Mg/Ca values in more dissolved samples. This loss appears to occur by preferential loss of the more Mg-rich portions of the shell, although the details remain unexplained.

Sr/Ca values range from 0.9 in some benthic species (*Uvigerina* spp.) to 1.6 mmol/mol in some planktics. Culturing results suggest that temperature, salinity and pH all exert a weak control (i.e., 1% per °C) on shell Sr, presumably through a kinetic effect. The main control appears to be related to environmental differences. For example, comparison of Sr/Ca in *Neoglobobulimina pachyderma* from plankton tows and cultures with core-top specimens indicates that the latter have significantly higher values, presumably due to deep crusting, perhaps added with a much higher calcification rate. This observation clearly demonstrates that Sr/Ca is not simply related to a single physical parameter as temperature.

Downcore records of shell Mg/Ca and Sr/Ca reveal substantial variability that can be correlated with known paleoceanographic change. For Mg/Ca, observed variations can largely be explained by climate-related variations in temperature. For Sr/Ca, it appears that observed variations related to secular

changes in seawater Sr/Ca, but this cannot be fully substantiated without a more complete understanding of primary and post-depositional controls on shell composition.

URL: <http://www.geol.ucsb.edu/~lea/>

B21C-05 1120h INVITED

Application of Foraminiferal Calcium Carbonate Chemistry to Proxy Past Ocean Conditions: key roles of Biomineral Formation and Dissolution

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The use of the shell chemistry of foraminiferal calcite and aragonite for seawater paleothermometry and paleochemistry rests on confidence in the calibrations established to link metal uptake or isotope fractionation by the marine biogenic calcium carbonate to modern seawater composition. The early pioneering work in this field led to a period of application, principally of the establishment and expansion of stable oxygen and carbon isotope studies. But new work on trace metals in foraminifera has led to an upsurge of interest in, and reappraisal of, what controls metal uptake and the extent to which foraminifera do a good job in recording and revealing the oceans secrets. Two issues are considered: (i) understanding how secretion by the organism takes place and the chemical consequences; (ii) how dissolution changes the initial chemistry. The early work on ultrastructure shows that different, often progressive, layers are formed ranging from anhedral microgranules to euhedral crystallites; and that there is sequential and preferential dissolution of the different textural forms. This forms some basis for understanding, for example, the heterogeneity in foraminiferal Mg/Ca and how this is affected by dissolution. In general, warmer species (higher Mg/Ca) show greatest 104 line broadening and depth (or carbonate ion) related dissolution is accompanied by a decrease both in Mg/Ca and line broadening. Variation in calcification rate has been linked to changes in carbon isotope and Sr/Ca, perhaps through a carbonate ion effect. This is also seen from size fraction data. There is also variability in shell mass both from initial calcification history and from dissolution, and both affect shell chemistry. Shell mass is also associated with changes in normalised size and accompanied by chemical changes. One clear effect of Mg heterogeneity is in its effect on calibrations for thermometry and the extent to which phase differences between temperature (dissolution-corrected Mg/Ca) and ice volume (temperature-corrected oxygen isotopes) can be defined. Most paleoceanographic proxies are complex and are affected by primary and secondary factors which is why multi-proxy approaches are increasingly used. Understanding why and how foraminifera incorporate trace elements through biological mineralization will help aid expansion of this important field.

B21C-06 1135h INVITED

Understanding the Molecular-Scale Processes of Biomineralization: The Role of Mg²⁺ and Sr²⁺ in Calcite Growth

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Determining the precise role that common inorganic species assume during the complex processes of biomineralization is central to understanding biomineralization strategies and for the unambiguous use of biogenic minerals as paleoclimate indicators. Inorganic impurities influence the solubility, reactivity, and polymorphic expression of biogenic carbonates leading to a dynamic relationship between the evolution of biomineralizing organisms and the long-term chemistry of the worlds oceans. Biological control over the minor and trace element contents of skeletal materials is therefore a fundamental aspect of the strategies employed by organisms to govern mineral formation. These vital effects, however, impact the use of biogenic minerals as tools for understanding past climates. Resolving the chemical and physical factors that control trace element incorporation during calcite mineralization is critical

to providing the inorganic baseline needed for assessing both paleoclimate signals and biological processes. It is now possible to determine the fundamental thermodynamic and kinetic parameters of calcite growth by making direct quantitative measurements of the microscopic processes occurring at the mineral-water interface. These molecular-scale observations are often able to provide crucial information concerning the actual mechanisms of growth that is otherwise unobtainable through macroscopic techniques.

Here we use in situ fluid-cell atomic force microscopy (AFM) to achieve a physical understanding of the role of magnesium and strontium in governing calcite formation. Despite the biogeochemical importance of this system, macroscopic studies have been unable to discern the actual mechanism by which Mg²⁺ mediates calcite growth and morphology. AFM was used to resolve the controversial mechanism of calcite inhibition by magnesium through molecular-scale determination of the thermodynamic and kinetic controls of Mg²⁺ on calcite morphology and growth. Comparison of directly observed monomolecular step velocities to standard crystal growth impurity models demonstrated that calcite growth inhibition was due to enhanced mineral solubility through Mg²⁺ incorporation. Terrace width measurements on calcite growth spirals confirmed that magnesium thermodynamically inhibits calcite growth by independently demonstrating decreased effective supersaturations in the presence of magnesium.

In contrast, the addition of strontium as an impurity in the growth system caused measured step velocities to decrease by a mechanism that corresponds to the step-pinning model of impurity inhibition. Therefore strontium provides a kinetic barrier to calcite growth by physically blocking the migration of monomolecular steps. The results of this study indicate that impurity size and ability to form solid solutions that are isomorphous with the host crystal play a major role in determining the specific mechanism of inhibition of biogenic calcite crystals. Further, these fundamental empirical observations may explain why Mg/Ca ratios have proved to be a more reliable paleothermometer than Sr/Ca ratios in biogenic calcite.

B21D MC: 122 Tuesday 1030h

Geomicrobiology and Biogeochemistry of Gas Hydrate Systems II (joint with A, OS)

Presiding: C Zhang, University of Missouri; B D Lanoil, Univ of California

B21D-01 1030h

Scanning Electron Microscopic Investigations on Natural and Synthetic Gas Hydrates: New Insights into the Formation Process

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We present results of field-emission scanning electron microscopic investigations of gas hydrates from shallow marine sediments of Cascadia margin as well as from synthesis experiments. The natural hydrates were taken by TV-grab sampling during the TECFLUX project on RV SONNE cruises, SO143 and SO148 on the southern summit of Hydrate Ridge. The samples are dominantly methane hydrates with a low content of H₂S (1.5-3.0 vol%). The hydrates develop as pure white ice-like layers in otherwise soft sediment deposits. The synthetic gas hydrates were prepared from pure CH₄ gas at variable pressure and temperature including experimental conditions similar to the natural situation. All synthetic hydrates show a porous microstructure with pore diameters of a few hundred nm (see figure) and grain sizes of a few μm[1]. Samples were transferred to a pre-cooled cryo-stage field-emission scanning electron microscope via an interlock. No decomposition was observed during our work, which was carried out below -165° C in a vacuum of <10⁻⁵ mbar by using an electron beam of 1.0-1.5 keV. The microscope is connected with an energy-dispersive X-ray spectrographic analyzer, which can clearly identify methane in the clathrate structure by detecting the carbon peak in the elemental spectrum.