

Pore-water chemistry in clays and shales: Methods and applications

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A suite of techniques to extract pore waters from clays and shales and to analyse them for major ions and isotope ratios have been developed over the last decade. Sophisticated techniques are needed to obtain the full pore-water composition, while simple crush/leach tests and other laboratory protocols provide information on conservative chemical constituents, such as Cl⁻, Br⁻, $\delta^{18}\text{O}$, $\delta^2\text{H}$ and dissolved noble gases. Concentration profiles of these constituents across shale formations are typically curved, indicating a transient state of diffusive exchange with the embedding aquifers. Transport modelling is used to quantify the time scales related to this process, using initial and boundary conditions based on palaeo-hydrogeological evidence [1]. This procedure provides insights on transport processes and on the upscaling of laboratory-derived transport parameters to the formation scale.

A comprehensive set of pore-water data has been obtained from drillcores of the Schlattingen borehole, penetrating a Mesozoic, clay-mineral rich, low-permeability sequence in NE Switzerland. Squeezing tests at 200-500 MPa yielded sufficient water for chemical and isotopic analysis (Fig. 1), in spite of low water contents (3-5 wt.-%). Salinity decreases with squeezing pressure, which is an artefact related to the membrane properties of the rocks. The decline of salinity in the lower part of the profile indicates the presence of a low-salinity boundary below 1000 m depth, leading to out-diffusion of Cl⁻ from the low-permeability sequence. The time scales related to this process are evaluated by transport modelling.

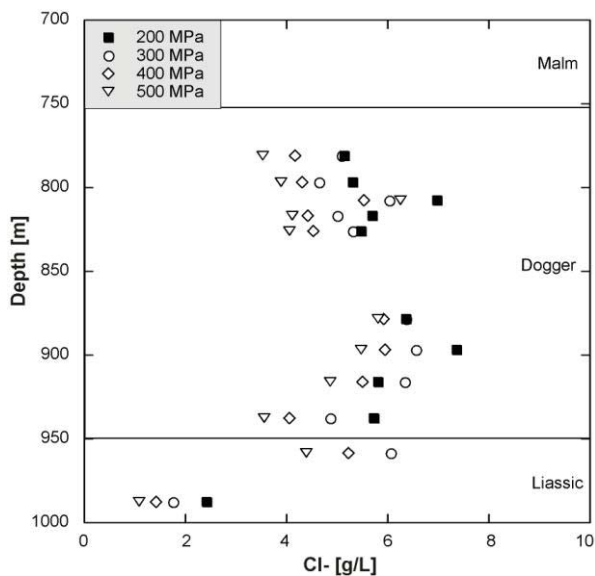


Figure 1: Cl⁻ concentrations in pore waters from the Schlattingen borehole obtained by squeezing experiments

[1] Mazurek *et al.* (2011) *Appl. Geochem.* **26**, 1035-1064.

Investigating marine corrosion communities using tagged pyrosequencing and single cell genomics

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It was recently discovered that members of the Fe(II)-oxidizing *Zetaproteobacteria* (*Zetas*) rapidly colonize mild steel surfaces that represent a ready source of Fe(II) [1], and that a robust microbiologically influenced corrosion (MIC) community develops on steel that includes *Zetas* and *Epsilonproteobacteria* [2]. Here we summarize our efforts to expand our understanding of marine steel MIC bacterial and archaeal communities. We present results from two time series incubations, the first conducted in Great Salt Bay salt marsh in summer 2010, and the second conducted below the marine intertidal zone in Boothbay Harbor, ME in summer 2011.

We used tagged pyrosequencing (V4 region, SSU rRNA gene) to examine bacterial and archaeal communities on mild steel surfaces from the salt marsh time series (2010). *Zetas* were identified in both sediment and steel sample pyrosequencing libraries, and unique *Zeta* OTUs on the steel reflected the numbers observed in the sedimentary *Zeta* communities we analyzed. *Epsilonproteobacteria* (in particular *Sulfurimonas* and *Arcobacter* relatives) were enriched on the steel in comparison with sediments. Unique *Deltaproteobacterial* OTUs present increased with incubation time to near sediment levels, likely reflecting colonization of the steel by Fe(III)-reducing and sulfate-reducing bacteria (SRB). Archaeal results were dominated by a potentially novel order of *Euryarchaeota*, most closely related to the *Thermoplasmatales*. Quantitative PCR of these samples and associated sediments with 16S rRNA gene primers for *Zetas* and functional gene primers for SRB showed that, on average, *Zeta* gene copies in sediments were an order of magnitude less abundant than SRB. *Zeta* gene copies on the steel increased rapidly over the first 10 days, exceeding copies quantified in the sediment by an order of magnitude. The SRB numbers on the steel were 10 fold lower than in sediments during the first days of incubation, but increased to near the sediment levels by 40 days.

A 9 day sample from the 2011 time series was subjected to whole genome amplification. Screening of single amplified genomes (SAGs) for bacteria identified 148 bacterial SAGs; 18 (12%) *Zetas*, 61 (42%) *Epsilonproteobacteria* (near relatives of *Sulfurimonas* sp.), and 46 (32%) *Gammaproteobacteria* (notably 15 relatives of *Hydrogenovibrio* sp., an autotrophic H₂-oxidizing bacterium).

When compared to previous work [2], this study provides evidence for rapid development of a core corrosion community on mild steel. Use of tagged pyrosequencing and single cell genomics enhances our understanding of MIC community richness and provides the possibility of exploring the genomes of individual community members.

[1] McBeth *et al.* (2011) *Appl Env Microbiol* **77**, 1405-12.

[2] Dang *et al.* (2011) *Env Micro* **13**, 3059-74.