

New insights into AOM by stable isotope probing combined with HISH-nanoSIMS and membrane-derived lipid analysis

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Experiment and Analytical Methods

Sediment-free enrichments of anaerobic methanotrophic archaea (AMNE) and sulfate-reducing bacteria were incubated for 15 days under AOM conditions and with the addition of ¹³C-labelled potential carbon sources such as methane, bicarbonate, acetate, formate, carbon monoxide or methanol. Furthermore we tested the incorporation of ¹³C-labelled methane and ¹³C-bicarbonate in the presence of additional non-labelled carbon sources (e.g. formate, acetate). Substrate-dependent sulfate reduction was tracked during the experiment as sulfide production, and utilization of the ¹³C-labelled compounds was examined as (I) conversion into total particulate organic carbon (TOC) (II) change of isotopic composition of membrane derived lipids and (III) ¹³C/¹²C imaging using Halogen *In Situ* Hybridization - Secondary Ion Mass Spectroscopy (HISH-SIMS [1]).

Results and Discussion

Using HISH-SIMS we detected predominant inorganic carbon uptake into both consortium members (ANME-2/DSS) and into single ANME-1 cells. In the presence of methane, TOC became significantly ¹³C-labelled by the ¹³C-bicarbonate addition; hence we conclude that both AOM partners use autotrophic pathways for carbon assimilation. The addition of formate stimulated sulfate reduction and bicarbonate uptake of the microbial community. For acetate and methanol only methane-independent activity and carbon uptake was observed, whereas CO inhibited AOM activity. We recommend the applied methods as powerful tool-box for the examination of potential carbon sources for uncultivated microorganisms.

[1] Musat *et al.* (2008) *Proc. Nat. Acad. Sci.* **105**, 17861-17866.

The imprint of methane seepage on geochemical processes in cold-water coral mounds on Pen Duick Escarpment, Gulf of Cadiz

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The production of hydrogen sulfide during anaerobic oxidation of methane (AOM) strongly imprints the geological record of cold-water coral mounds on Pen Duick Escarpment, Gulf of Cadiz. This study examines mechanisms of carbonate dissolution and precipitation in coral-containing, hydrocarbon-influenced, siliciclastic sediments on cores from Alpha and Beta Mound retrieved during R/V Marion Dufresne Cruise 169 MICROSYSTEMS. Analyses of solid-phase iron minerals, multi-element pore-water composition and carbonate mineralogy combined with $\delta^{34}\text{S}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotope measurements reveal a strong coupling between post-depositional diagenetic changes with respect to the S and Fe cycle and alternations in the sedimentary carbonate composition. The sediments exhibit an almost complete consumption of reactive Fe-mineral phases due to the formation of iron-sulfide minerals with average degree of pyritization (DOP) values > 0.7. $\delta^{34}\text{S}_{\text{pyrite}}$ values of up to -2‰ indicate that this process is driven by the upward diffusion of ³⁴S-enriched hydrogen sulfide produced during AOM in deeper sediment layers. In the top part of the mounds, this lack of reactive Fe-minerals combined with ongoing, in-situ organoclastic sulfate reduction (non-AOM) leads to a net increase in proton production that is not compensated by Fe-oxide reduction and iron-sulfide precipitation processes, but induces coral dissolution. Additionally, Ca²⁺ and Mg²⁺ pore-water profiles and carbonate mineralogy data suggest the precipitation of high-Mg calcite in deeper layers. Authigenic carbonate precipitation is promoted by the contemporaneous production of DIC from AOM as evidenced by depleted $\delta^{13}\text{C}_{\text{carbonate}}$ signals.