



## **Which microorganisms benefit from methane oxidation in seep sediments? - Tracing carbon pathways by isotope labeling experiments.**

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Anaerobic oxidation performed by consortia of archaea and sulfate reducing bacteria is the major sink of methane in marine systems. Although intensive research was done in this field, many questions on this enigmatic metabolism remain unsolved. The main focus of this study was to solve the question as to which organisms can incorporate carbon derived from methane in the AOM zone.

We used seeps sediments from well described areas of the Black Sea, Hydrate Ridge and Gullfaks (North Sea) and incubated them in flow through columns, which were percolated with methane-enriched anoxic seawater as the sole energy source. Several replicate columns received  $^{13}\text{C}$ -labeled methane, others  $^{13}\text{C}$ -labeled carbonate amending both pools by ten percent  $^{13}\text{C}$  carbon. Biomarker analyses of sediments from all sediments incubations showed, that the bacterial biomass, represented by specific fatty acids, was nearly exclusively labeled by  $^{13}\text{C}$  derived from carbonate.  $\text{C}_{16:1\omega 5}$  and its methylation product  $\text{C}_{17:0\omega 5,6}$ , so far solely found in seep related sulfate reducers, showed the strongest labeling signal. The archaeal biomarkers archaeol and hydroxyarchaeol were labeled by both carbon sources in almost equal amounts.

From our experiments we conclude that archaea use methane and carbonate in a ratio of about one to one during biomass assimilation. The sulfate reducers appear to be autotrophs using a yet unknown pathway to gain electrons from the methane oxidizers. Furthermore, we were able to define accumulation and growth rates of biomarker and organisms. Our results show very long generation times of month to years for microorganisms inhabiting AOM zones. This may be caused by the very low energy

yield of anaerobic oxidation of methane. Also, our experiments suggest that AOM is not mediated by syntrophic transfer of an organic intermediate between the microorganisms as no trace of methane-derived carbon was incorporated the sulfate reducers. Hence, redox active electron shuttling remains the most likely connection between the two associated organisms mediating AOM, since hydrogen has been excluded as an intermediate by earlier experiments.