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High-pressure continuous-incubation studies of sediments containing highly active communities of anaerobic methanotrophs

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The anaerobic oxidation of methane (AOM) coupled to sulfate reduction in marine sediments is an important sink in the global methane budget. However, the biochemistry of methane oxidation and assimilation by anaerobic methanotrophs is not yet fully understood and knowledge about the regulating factors is very limited, because no pure cultures of methane-oxidizing anaerobic organisms have yet been obtained. According to the net reaction of AOM the concentration of aqueous methane is expected to be a key variable for the reaction thermodynamics and kinetics. Methane is poorly soluble under atmospheric conditions. Hence, we sought to develop and implement high-pressure methods to increase the concentration of dissolved methane and to closer simulate some *in-situ* deep-sea environmental conditions in the laboratory.

We have established a unique multiple-phase continuous-incubation system which is designed to simulate conditions at gas seeps, hydrate systems or other habitats that are characterized by high activities of aqueous gases. The system is designed to be operated in a wide range of pressure (up to 45.0 MPa) and temperature (up to 230˚C). We can continuously sample all relevant fluids and biomass from the experiments and thus are able to precisely determine turnover rates and analyze the chemical composition of the fluid. It is now possible to precisely study the impact of even small concentrations of various compounds on AOM metabolism and to monitor compounds that might be present at very low concentrations at either steady state under optimum conditions or at transient state during shift experiments.

In long-term continuous-incubation studies with dilute biomass prepared from micro-

bial mats from *Black Sea* at 15.0 MPa with approximately 60 mM of aqueous methane we observed AOM rates of 60 μMol·l^{-1}·h^{-1}. From the increase of activity over time we estimate a doubling time of less than 40 days. We further observed that the high turnover rates could be sustained at sulfide concentrations up to 17 mMol·l^{-1}. Without substrate supply over 60 days neither sulfide nor methane production could be observed. This shows that sulfide production is exclusively fueled by methane. Furthermore, it suggests that starving biomass is not degraded at a significant rate which was further confirmed by analysis of volatile fatty acids. After the starvation period of 60 days the organisms could readily be reactivated by supplying methane. No lag phase in AOM was observed.

Using samples from highly active methanotrophic communities from different habitats such as the *Mediterranean Sea*, *Hydrate Ridge* off *Oregon* and the *Gulf of Mexico* *w*e will further perform microbiological and biogeochemical experiments to further investigate the mechanism and regulation of AOM and to estimate AOM-specific parameters (K_m, r_{max}, μ_{max}).We also pursue long-term cultivation strategies to produce highly enriched sediment-free biomass to be used in biochemical and molecular studies. This work was carried out in the framework of the BMBF.-DFG Geotechnologies program “Methane in the GeoBioSystem, project “MUMM”.