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Stable Carbon and Hydrogen Isotope Fractionation Associated with the Anaerobic Oxidation of Methane in ANME-Enrichment Cultures

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The anaerobic oxidation of methane (AOM) is one of the major sinks for methane on earth. The current model suggests that AOM is performed by methanotrophic Archaea (ANME) associated with sulfate reducing bacteria, metabolizing methane via reverse methanogenesis. While carbon and hydrogen isotope fractionation associated with the aerobic oxidation of methane are well known, little is known about the isotope fractionation during AOM. Microbial oxidation of methane is accompanied by a strong fractionation against the heavier C and H isotopes, resulting in a ^{2}H (deuterium)- and ^{13}C- enriched residual methane. Field studies investigating isotope gradients of methane in sediment porewater yielded fractionation factors for AOM ranging from 1.002 to 1.014 for carbon (αC methane-methane) and 1.120 to 1.157 for hydrogen (αD methane-methane). However, isotope fractionation can be influenced by several environmental and microbial factors such as temperature, substrate availability, the microbial species and their enzymes involved. Here we provide the first fractionation factors for methane in highly active, sediment-free AOM cultures enriched in different types of ANME-consortia. The sediment-free AOM cultures are dominated by consortia of ANME-2 archaea or ANME-1/-2 and sulfate reducing bacteria of the *Desulfosarcina/Desulfococcus*-group. No methanogenesis was detected in replicate incubations during this short term experiment. The fractionation factors for carbon and hydrogen associated with AOM both revealed a stronger fractionation than previously reported from field studies. This study was part of the BMBF/DFG GEOTECHNOLOGIEN project MUMM II and provides new insight into the microbial impact on the isotopic composition of methane in marine environments.