1. PUBLISHABLE SUMMARY

The anaerobic oxidation of methane (AOM) coupled to the reduction of sulfate (SO_4^{2-}) is a microbial process that largely prevents the emission of CH_4 to the atmosphere in marine environments. Researchers from different disciplines have made great strides towards understanding this process. In-depth knowledge on the microorganisms responsible for the process and the metabolic pathway is still limited to the availability of a pure culture or defined consortium of the microorganisms involved, or at least on the availability of sufficient amounts of highly enriched cultures. In this project, two distinct reactors were designed and constructed for the enrichment of microorganisms conducting AOM. To overcome slow growth rates, in one reactor, an external membrane was used which allows efficient retention of biomass. In another reactor, biomass retention was obtained via biomass attachment to sponges and the reactor was designed as trickling packed bed. In both reactors, the membrane bioreactor (MBR) and the packed bed bioreactor (PBR), sulfate reduction with concurrent sulfide production was recorded only after about 200 days.

After 400 days of reactor operation, the biomass from the MBR was tested for its capacity to conduct AOM. When this biomass was incubated anaerobically with ^{13}C labeled CH_4, ^{13}C labeled CO_2 was produced (the ^{13}C-CO_2 production rate was 0.1 ‰ d^{-1}) indicating AOM by this biomass. Illumina high-throughput sequence analysis of 16S rRNA genes showed that the sediment retrieved from Ginsburg mud volcano in the Gulf of Cadiz and used as inoculum for the MBR reactor contained mainly *Thermococcales* (85%) and Marine group II (10%) as archaeal components, and ANME were not detected by the method used. However, after about 360 days of reactor operation ANME-1 sequences were detected, albeit at low abundance (about 1.6% of the archaeal community). The low relative abundance of ANME after long reactor operation times is consistent with ANME below detection in the inoculum and the reported low growth rates of this microorganisms. Fluorescence in situ hybridization (FISH) analysis of the MBR reactor after ca. 450 days showed the presence of ANME-2 in this reactor enrichment.

The biomass from the PBR could not be tested for AOM using ^{13}C labeled CH_4. However, analysis of 16S rRNA gene sequences showed that 2.8% of the achaeal community of the PBR was ANME-1. Although the ANME community abundance was low, the results of the PBR show that similarly to the MBR, ANME enrichment is feasible in these type of reactors despite that in the inoculum (i.e., sediment from Ginsburg mud volcano) ANME were below detection by the approach utilized.

Considering the logistic difficulties to accessing and retrieving deep marine anaerobic sediments having high AOM activity and hence having high abundances of anaerobic methanotrophs, this project explored the AOM potential of a shallow sediment retrieved from Marine Lake Grevelingen, The Netherlands which was better accessible. Marine Lake Grevelingen is a former estuary with a water depth of ~ 40 m and salinity of 31 g/kg, which is separated from the North Sea by a dam. High rates of deposition and degradation of organic matter have resulted in methane rich anoxic sediments which combined with sulfate from sea water renders the site a potential niche for AOM. To assess the presence of AOM activity in those sediments, pore water chemical analysis, serum bottle incubation for activity tests, and microbial analysis by 16S rRNA gene sequences and FISH were conducted.

AOM was evident at depths of 5 to 15 cm in the sediment with a steep decline in CH_4 concentrations from ~5 mM at 20 cm depth to negligible at the sediment surface; concurrently, sulfide concentrations increased to 5 mM. In vitro incubations with CH_4 and SO_4^{2-} showed sulfide production coupled to the consumption of sulfate at approximately equimolar ratios at 150 days. In contrast, sulfate and sulfide concentrations remained constant in biotic and abiotic control incubations. ANME and archaeal cells were detected by 16S rRNA gene sequencing and observed by FISH. Using three independent approaches, this study, for the first time provides evidence for the occurrence of AOM in sediments of Marine Lake Grevelingen.