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AEROBIC METHANOTROPHY AT ANCIENT METHANE-SEEPS – A REVIEW

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The inventory of lipid biomarkers of a number of ancient methane-seep deposits has been studied over the last decade (for a review see Peckmann and Thiel, 2004). The authigenic carbonates at seeps that form due to an increase in alkalinity result from microbial activity. The molecular fingerprints of the chemosynthesis-based microbial communities tend to be extremely well-preserved in these seep carbonates. The key process is the anaerobic oxidation of methane, which is performed by consortia of sulphate-reducing bacteria and methanotrophic archaea. Typically, compounds preserved within modern and ancient seep settings comprise ^{13}C-depleted archaeal isoprenoidal (e.g. PMI, archaeol) and bacterial *n*-alkyl lipids (e.g. *anteiso*-C_{15} fatty acid). Besides the occurrence of ^{13}C-depleted isoprenoids and *n*-alkyl-chains in seep settings, ^{13}C-depleted hopanoids have been reported from modern seeps. They have been interpreted to be derived from aerobic methanotrophic bacteria (e.g. Elvert et al., 2000), or from unknown anaerobic bacteria (Thiel et al., 2003). The potential of seep-dwelling anaerobic bacteria to synthesise hopanoids has now been confirmed by Blumenberg et al. (2006), who cultured sulphate-reducing bacteria from a microbial mat from the Black Sea and found abundant hopanoids.

Here, lipid biomarker data are presented from two ancient methane-seep limestones embedded in Miocene strata in Italy and one in Late Cretaceous strata in Colorado, USA. These examples provide strong evidence that methane was not solely oxidized by an anaerobic process. Structural and carbon isotope data reveal that aerobic methanotrophy is more common at ancient methane-seeps than previously noticed. In one Miocene setting, abundant 3β-Me-17β(H),21β(H)-*dihomo*hopanoic acid was found (δ^{13}C −100‰). Most likely, 3β-methylated hopanepolyols, prevailing in aerobic methanotrophs (e.g. *Methylococcus capsulatus*; Summons et al., 1994), were the precursor lipids of this compound. Moreover, more common hopanoids with very similar δ^{13}C values (e.g. 17β(H),21β(H)-*pentakishomo*hopanoic acid methylester; −97‰) have been found in this location as well. A series of isotopically depleted C-4-methylated steranes (lanostanes; −90 to −72‰) in another Miocene seep deposit has been attributed to aerobic methanotrophs (Peckmann et al., 2004). Lanosterol is the most likely precursor of C-4-methylated steranes. These compounds are known to be produced by aerobic methanotrophs, some of which are outstanding among

Bacteria in having the capacity to produce steroids (e.g. Summons et al., 1994). In a Late Cretaceous seep-limestone a suite of conspicuous 8,14-secohexahydrobenzohopanes (−110 to −107‰) has been described (Birgel et al., 2006). These hopanoids have been interpreted as early degradational products of precursor lipids locally produced by seep-endemic aerobic methanotrophs. This interpretation is supported by the presence of ‘regular’ hopanoids that can be discriminated from the unusual 8,14-secohexahydrobenzohopanes by only moderately low ^{13}C-values (−49 to −42‰).

Aerobic methanotrophy is more common at methane-seeps than previously recognised. Our data indicate that anaerobic and aerobic oxidation of methane at ancient seeps occurred in the same setting, probably in close proximity to each other.

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ANAEROBIC METHANE OXIDATION IN EUROPEAN CONTINENTAL SHELF SETTINGS

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Whereas processes and micro-organisms responsible for the consumption of methane in gas-rich sediments on continental slopes and in the deep sea are well known, few studies have investigated such processes in sedimentary settings characterised by low-methane fluxes. To better understand the role of anaerobic oxidation of methane (AOM) as a sink for methane in such settings, and to investigate the microbial ecology of these environments, we conducted geochemical studies on sediments from the Aarhus Bay in the South Eastern Kattegat and the Arkona Basin in the Baltic Sea. For each site, we determined pore water concentrations of methane, sulphate and sulphide and rates of AOM and sulphate reduction (SR). We also investigated the distributions of various lipid biomarkers and applied them as proxies for AOM. To evaluate the impact of AOM on the mineralogy, we measured solid-phase concentrations of redox-sensitive metals such as iron and manganese and investigated the mineralogy using X-ray diffraction and scanning electron microscopy.

There exists in both the Aarhus Bay and the Arkona Basin a sulphate-methane transition zone (SMTZ) that is characterised by maximum AOM rates and intense SR. Biomarkers in the sediments are diverse and include compounds deriving from higher plants (*n*-alkanes and *n*-alkanols), phytoplankton (e.g. long-chain alkenones and alkyl diols) and microbes (isoprenoidal and non-isoprenoidal diethers, isoprenoidal hydrocarbons, hopanes and fatty acids). Here, we place emphasis on the distributions of specific archaeal and bacterial biomarkers, namely archaeol and the structurally similar C_{33} dialkylglycerol diethers (DGDs; putative SRB biomarkers), *sn*-2- and *sn*-3- hydroxyarchaeol, 2,6,10,15,19-pentamethylicosane (PMI), and branched C_{15} and C_{17} fatty acids. The co-occurrence of archaeol, *sn*-2- and *sn*-3- hydroxyarchaeol and PMI is characteristic of methane-rich sedimentary basins and has been reported for numerous cold seep sites (Niemann et al., 2006; Pancost et al., 2001b) and a variety of DGDs and structurally-related fatty acids are useful biomarkers for SRB involved in AOM (Blumenberg et al., 2004; Pancost et al., 2001a).

In the Aarhus Bay sediments, the abundance of *sn*-2-hydroxyarchaeol and PMI varies significantly with depth. At the SMTZ, the concentration of *sn*-2-hydroxyarchaeol increases dramatically from below detection limit to > 900 ng g^{-1} and PMI abundances increase by more than one order of magnitude to 50 ng g^{-1}. Although archaeol is present throughout the sedimentary column and no significant changes in concentration with depth occurs, phospholipid-bound archaeol was detected only in sediments at and below the SMTZ. Thus, the relatively recalcitrant archaeol is probably not a useful biomarker for active AOM, but its phosphorylated (or glycolated) equivalent and the labile hydroxyarchaeol isomers likely are. Bacterial C_{33} DGD abundances also vary significantly with depth, and an increase in concentrations occurs in sediments from just above the SMTZ. In the Arkona Basin sediments, a similar suite of biomarkers was present (with the exception that neither *sn*-2- nor *sn*-3- hydroxyarchaeol were detected in any of the samples), and similar changes occur in the abundances of specific archaeal and bacterial biomarkers in the vicinity of the SMTZ.

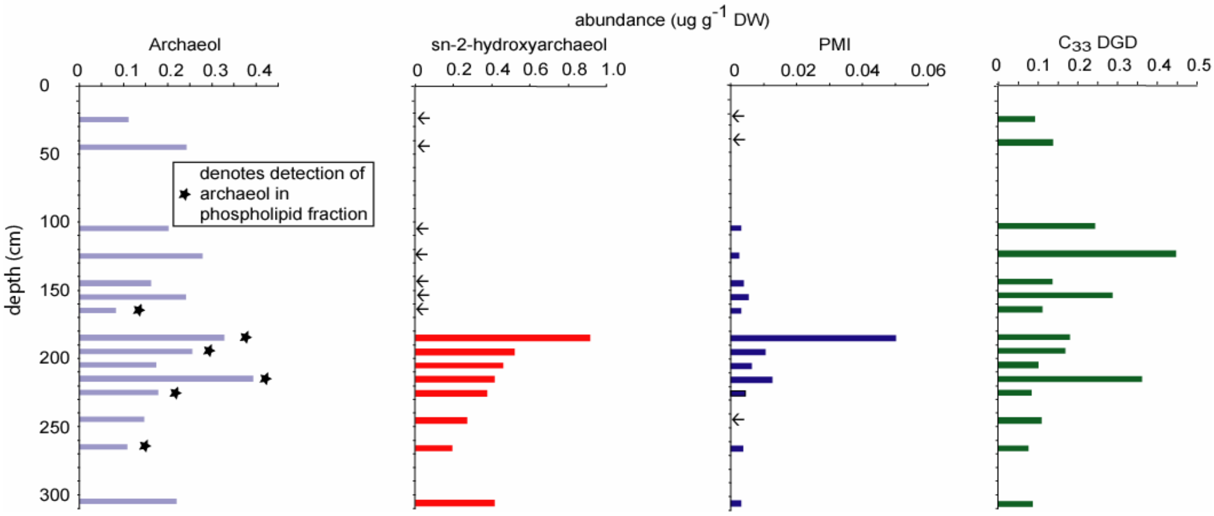

Figure 1. Abundance profiles for specific archaeal and bacterial biomarkers in Aarhus Bay sediments.

These biomarker distributions suggest that microbial communities similar to those found in cold seeps exist at the SMTZ of these low-methane flux settings. Furthermore, the lower abundances of lipid biomarkers at the low-methane flux settings – up to more than one order of magnitude lower – are indicative of smaller/less active communities mediating AOM.

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CRUDE OIL DEGRADATION BY MICROBIAL MATS

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Coastal cyanobacterial mats develop at the water-sediment interface in shallow environments such as estuaries, lagoons or sheltered sandy beaches where they stabilize the sediment. Evidence supporting the biodegradation capacity of these consortia for the elimination of crude oil residues is still limited. In addition to biodegradation, coastal spilled oil undergoes several physico-chemical alteration processes such as evaporation, water-washing or photo-oxidation.

These aspects are important for designing adequate remediation strategies of coastal oil spills such as that in the Arabian Gulf after the 1991 war. Intensive cyanobacterial growth on the oil top was observed soon after the spill (Sorkhoh et al., 1992). Crude oil was toxic for the cyanobacterial grazers but not for these photosynthetic organisms. This process was proposed to be a first step towards natural bioremediation (Radwan et al., 1999) but partial oil elimination was also attributed to the combined effects of physico-chemical weathering and microbial degradation (Sauer et al., 1998). Other reports indicated that cyanobacterial building mats in the Saudi coast led to preservation of oil residues (Barth et al., 2003).

The present work is addressed at gaining insight into the potential of microbial mats for crude oil degradation as compared to the physico-chemical processes. For this purpose analysis of samples collected in the Saudi Arabian Gulf coast after the 1991 oil spill in selected areas containing or devoid of microbial mats and development of microcosm experiments for exposure of microbial mats to oil pollution (Fig. 1).

The preliminary results arising from these experiments have shown that water weathering leads to more effective and rapid elimination of hydrocarbons amenable to washing and oxidation than microbial mat metabolism. Mat degradation occurs at a much lower rate than the weathering processes resulting in an apparent effect of preservation of low molecular weight aliphatic and aromatic hydrocarbons. This is not the case, however, for hydrocarbons containing nitrogen atoms (Garcia de Oteyza et al., 2006).

Microbial mats are also able to enhance elimination, at least in part, of long carbon chain hydrocarbons such as the C_{24}-C_{30} *n*-alkanes that are not very much affected by water weathering. Higher viscosity constitutes a limitation for the physico-chemical transformation of the oils through water weathering but not for biodegradation from microbial mats.

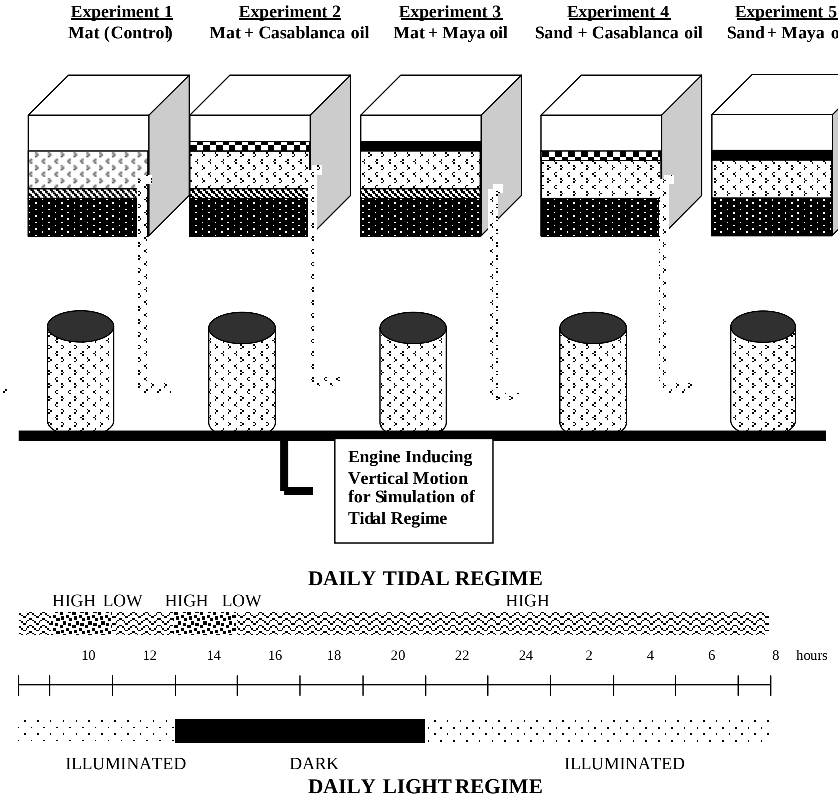

Figure 1. Experimental setups for the simulation of the oil degradation by microbial mats under tidal regime. 1: Development of the microbial mat without oil pollution: ▓ sand, ▨ microbial mat, ░ water. 2: Degradation of Casablanca oil (▦) with microbial mat. 3: Degradation of Maya oil (■) with microbial mat. 4: Transformation of Casablanca oil without microbial mat. 5: Transformation of Maya oil without microbial mat.

In the context of marine coastal oil spills, the results suggest that whereas the occurrence of microbial mats may delay the elimination of the most volatile crude oil fracions, higher degree of transformation of the less volatile oil constituents should be expected when these consortia of microorganisms are present.

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