

## Synthesis

The goal of this research was to compare geological and biogeochemical processes occurring in two MV provinces, which are located in the Gulf of Cadiz (NE Atlantic) and in the Sorokin Trough (NE Black Sea). These areas are characterized by fundamentally different geological histories and present-day ecological environments. Both are known for the presence of gas hydrates, seep ecosystems, and methane-related carbonates. In each of these MV provinces, an experimental program, which comprised a spectrum of modern geochemical and molecular ecological techniques was applied. An effort was made to determine the possible sources of migrated fluids, especially methane, and to disclose a link between the occurrence of microbes in such extreme environments and their symbiotic partnerships and relation to migrated components from deep subsurface (methane, petroleum products or their mixtures). Microbial anaerobic oxidation of methane (AOM) process and AOM-related carbonate-shaping ecosystems in the geological past (in the Gulf of Cadiz) and within currently active fluid venting environments (in the Sorokin Trough) were investigated. A wide set of hydrocarbon gases and lipid biomarkers was investigated and stable carbon isotope and 16S rRNA gene sequence analyses for MV deposits, pelagic sediments, carbonates and microbial mats collected from different fluid-venting settings within these two areas were performed.

In this thesis it was aimed:

- (1) to integrate and interpret the same set of biogeochemical data from both MV provinces to gain an understanding of deep-fluid dynamics in these areas and to reveal the sources for fluids (hydrocarbon gases) and MV deposits (mud breccias);
- (2) to investigate the environmental impact of seepage phenomena, i.e. study of *in situ* microbial processes involved in the methane cycle, predominantly AOM, and related carbonate formation;
- (3) to compare the obtained information for the Gulf of Cadiz and for the Sorokin Trough in order to understand relationship between geological and biogeochemical regulatory mechanisms of the fluid venting and mud volcanism as well as factors controlling the occurrence, distribution and activity of methane-dependent microbial populations and related carbonate fabrics in both areas.

### 9.1. Sources of hydrocarbon gas and mud breccias

The Sorokin Trough and the Gulf of Cadiz are characterized by different geological histories, tectonic regimes, and the lithology of sedimentary cover. These affect the

composition of the initially formed fluid, fluid venting activity, fluid migration history and, as a result, the fluid composition as it appears at the near-surface sediments. Hydrocarbon gas is one of the dominant components in the MV fluid system. In both MV provinces, the molecular and carbon stable isotope compositions of C<sub>1</sub>-C<sub>5</sub> hydrocarbons are significantly affected by secondary alterations due to migration, mixing and microbial degradation. Despite the first evidence for different maturity properties of gas among the MVs in the Sorokin Trough, the wet gas components in all mud breccias and gas hydrates are related to each other. In general, these wet hydrocarbon gases can be recognized as originally derived from oil cracking and/or biodegradation in the subsurface. In contrast, hydrocarbon gases from two MV provinces within the Gulf of Cadiz can be considered as a consequence of secondary microbial alteration in the subsurface, which affected the  $\delta^{13}\text{C}$  values of C<sub>2+</sub> hydrocarbons. This complicated the assessment of the level of maturity and the original gas source. Nevertheless, the presence of high molecular weight gaseous hydrocarbons indicated that gases are in part thermally mature.

Hence, the hydrocarbon gas data indicate that an admixture of non-microbial, thermogenic hydrocarbon gases characterize migrated fluid from both areas. This indicates that the original source for gaseous fluids in both MV provinces locates in the deep subsurface since the gas generation window was established at ca. 105-155°C to 175-220°C (Pepper and Corvi, 1995). Hydrocarbon gases from both MV provinces represent a complex of redeposited, secondary migrated, mixed with biogenic gas, and microbially altered gas mixtures. In this respect, the redeposition of hydrocarbon gas in the Gulf of Cadiz would be expected within the Olistostrome or in relation with the diapir bodies or with submarine escarpments. The redeposition of the hydrocarbon gas at shallow subsurface in the Sorokin Trough already proved by numerous geophysical surveys in the area which detected many signs of the gas presence at ~ 250-300 m below the seafloor surface (Ivanov et al., 1998; Bouriak and Akhmetzhanov, 1998; Krastel et al., 2003).

The organic matter composition of the mud breccia matrixes in the MVs from the Sorokin Trough and the Gulf of Cadiz exhibit clear differences. These differences reflect the location of source strata of the erupted material. Despite the proximate location of the studied MVs in the Sorokin Trough, biomarker lipid distributions revealed different mud breccia sources in these structures. This indicates different subsurface-sedimentary depths of defluidization, which are directly linked to the location of fracture zones or diapiric folds. MVs from the Gulf of Cadiz are located relatively distant from each other within tectonically different parts of the Gulf. The lipid biomarker compositions from mud breccia matrixes in all studied MVs showed strong compositional resemblance and similar thermal maturity properties of the organic matter. This indicates related source strata for the erupted material,

similar depths of defluidization, and the extension of common litho-stratigraphic units through the Gulf.

Comparison of lipid biomarkers and hydrocarbon gas data from both areas sheds new light on the question what triggers the eruption of a MV: (i) hydrocarbons or (ii) fluidized sediments or (iii) a mixture of both? The maturity properties of organic matter in mud breccias from MVs in the Gulf of Cadiz and in the Sorokin Trough generally indicate that the source strata for the erupted sediments are located at shallower depth of burial than the source strata for the hydrocarbon gases. Accordingly, our results support the notion that the formation of the MV fluid starts with the release of overpressured hydrocarbons, followed by the mechanical entrainment of encountered sediments.

### *9.2. Anaerobic oxidation of methane*

At cold vents, the composition of fluids and seepage rates generally varies considerably. This directly affects the diversity and abundance of seep-thriving biota. The anoxic water column in the Black Sea eliminates the possibility for the presence of oxygen-dependent macro- and microorganisms, thus limiting microbial inhabitants to only obligate anaerobic species. In contrast, oxygenated waters in the Gulf of Cadiz promote the development of substantially more diverse suite of (micro)organisms. The main outcome of the applied lipid biomarker and 16S rDNA sequence study is that AOM is the dominant biogeochemical process in MVs from both areas and archaea of the ANME groups have been detected in all studied locations. However, the intensity of AOM processes, which was tentatively suggested from the distribution and contents of AOM-derived lipid biomarkers, and the location of AOM zones within the sediments are notably different in the MVs from the Sorokin Trough and from the Gulf of Cadiz. Presently active fluid venting characterizes the Sorokin Trough. In gas saturated mud breccias and sediments with gas hydrates, the highest AOM activity was detected. Besides, lipid biomarker distribution profiles and 16S rRNA gene sequence data revealed that in the MV deposits from the Sorokin Trough, AOM is restricted within the ca. 20 cm zone with archaea belonging to the ANME-1 cluster. The ANME-2 archaeal group was not detected, which does not prove their total absence; it indicates a significant dominance of archaea from the ANME-1 guild in these presently active methane-venting habitats. In contrast, the lack of strong upward fluid transport and the absence of gas escape into the overlying water column characterize the Gulf of Cadiz. In the studied locations, MV deposits showed unexpectedly low concentrations of lipids specific to AOM, suggesting lower intensities of anaerobic methanotrophy. AOM-derived lipid biomarkers were detected through the ca. 240 cm of the mud breccia section, tracing the presence of both archaeal clades. In the site where methane concentration was the highest,

AOM zone was close located to the sediment surface. In contrast, sampling locations with relatively low methane concentrations showed shift of AOM deeper into the sediments. Hence, the results of this study and inspection of published data suggest that the distribution and composition of anaerobic methanotrophs in the studied MV deposits are ruled by the fluid inflow, i.e. methane flux.

### *9.3. Carbonate shaping ecosystems*

In local seepage environments in the Sorokin Trough and in the Gulf of Cadiz, microbial system development results in the growth of specific seep-carbonate fabrics. Carbonates were collected from principally different methane-seepage settings and include: (i) carbonate crusts associated with two different types of living microbial mats from active seepage and gas hydrate localities from the Sorokin Trough and (ii) a carbonate crust from a non-active MV in the Gulf of Cadiz. Compared with the studied Black Sea's carbonates/microbial mats, the crust from the Gulf of Cadiz was considered to be a relict of the past seepage activity.

The common feature among all studied carbonates is the significant role of AOM-related microorganisms in their formation. However, the diversity of anaerobic methanotrophs varied among the crusts. Lipid biomarkers and 16S rDNA sequence data revealed the presence of archaea affiliated with the ANME-1 clade in all carbonates from the Sorokin Trough, whereas carbonate crust from the Gulf of Cadiz shows the presence of both guilds. Since the crust from the Gulf of Cadiz was examined in detail, changes in the archaeal communities were traced along the carbonate, evaluating changes in the local environments during the precipitation of the crust.

The difference in sulphate concentration is likely one of the causes for the different suite of authigenic minerals present in the AOM-carbonates of the Sorokin Trough and in the Gulf of Cadiz. For instance, compositional analyses of numerous carbonates from the Sorokin Trough revealed the presence of calcite, with the predominance of high Mg-calcite, and dolomite (Mazzini et al., 2004). Aragonite, which is common carbonate mineral in seepage locations, was not detected at all. In contrast, aragonite is the dominate carbonate mineral in the carbonate crust from the Gulf of Cadiz. At fluid venting sites, the precipitation of calcite is favoured over aragonite when low sulphate concentrations occur in the system (Burton, 1993; Naehr et al., 2000; Aloisi et al., 2002). Low sulfate environments (ca. 18 mM compare with the ocean of 30 mM) are typical for the Black Sea bottom waters (Murray et al., 1991). Accordingly this observation indicates that anaerobic methanotrophy only serves as a partial source for the bicarbonate and the compositional variety for the resulted carbonates are partially dictated by the sulphate reduction and probably by other microbial activities impacting on cycles of other elements.

In both areas the methane, lipid biomarker and 16Sr rRNA gene sequence data revealed that the carbonate forming ecosystems are located below the seafloor and the precipitation of methane-related carbonates *via* AOM takes place beneath the sediment water interface, i.e. where methane and sulfate are abundant. AOM processes are most active within the neoformed carbonates and are substantially reduced in the hosting sediments. The carbonate growth direction likely follows the pathways of migrated methane although the presence of anoxic water column in the Black Sea allows the upward accretion of carbonate. In the Gulf of Cadiz, the carbonate growth is ultimately happening in a downward direction.

Overall, the study of different fluid venting environments in the Sorokin Trough and in the Gulf of Cadiz revealed different intensities and duration of AOM, most likely forced by intensity of local seepage activity and by composition of migrated fluid.

#### *9.4. Factors that regulate biogeochemical processes in the Sorokin Trough and in the Gulf of Cadiz*

The major cause for different fluid venting activity reflected in AOM intensities and carbonate formation is the different marine geotectonic settings of the studied areas. The regional topography and distinctiveness of the seafloor at the Sorokin Trough and at the Gulf of Cadiz can be indicative for type and modes of seepages. For instance, the occurrence of pock-marks, fissure outflows of mud breccias, or mud volcanoes at the seafloor specifies different physical factors leading the formation of such different seep-system scenery. In other words, relatively low-pressure events with less dense fluid will cause pock-marks topography. A high-pressure event with outburst of high-density material is required for the formation of a mud volcano. Thus, development of mud volcanoes is related to episodic high-pressure relief of deep fluids.

Both areas are known for mud volcanism and associated phenomena. However, the AOM signal in the mud breccias from the Gulf of Cadiz is considerably lower than in the MV deposits from the Sorokin Trough. This suggests low AOM rates which indicate the absence of intensive fluid venting and methane influxes in the Gulf, confirmed by the hydrocarbon gas data. The Sorokin Trough is known to be a currently active fluid venting area which results in dense AOM communities. Gas saturated sediments are ubiquitous in the Trough and AOM even takes place in the water column. Unlike, in the Gulf of Cadiz, fluid venting activity is distributed locally and often the most active sites are the craters of MVs. The phases of MV activity in response to past and present geo-tectonic regimes or/and (palaeo) environmental events are not known for both MV provinces. Fields of outcropping methane-related carbonate material in the Gulf of Cadiz mark the episode of active defluidisation in the geological past. Besides, this period of seeping should last over a relatively long time span,

since the length of these chimneys are in some cases reach 0.5 m (Kenyon et al., 2002; Díaz-del-Río et al., 2003). It is still enigmatic when such episode took place in the Gulf's history, and which factors acted as a trigger. Nevertheless, this can testify different modes of fluid venting in the Gulf of Cadiz and in the Sorokin Trough. Likely, the absence of fluid supply in the mud breccias from the MV in the Gulf of Cadiz can be explained by an abrupt and brief period of MV eruptions associated with emissions of fluid saturated mud breccias followed by fast consumption of introduced methane and other organic and inorganic components. Accordingly, after some time, mud breccias in the Gulf are in "fluid-flux-starvation" environments. In the Sorokin Trough, gas saturated sediments are ubiquitous and AOM even take place in the water column. The fluid inflow is constant and some parts of the Trough are characterized by the presence of so called "gas front" (Ivanov et al., 1998). This determines the presently steady energy source environments and thus flourishing of chemosynthetic biota and associated mineral formation.