

# Biogenic fabrics in seep carbonates from an active gas vent site in Green Canyon Block 238, Gulf of Mexico

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## Abstract

Carbonate crusts collected from an active methane seep site in the offshore Louisiana of Gulf of Mexico have  $\delta^{13}\text{C}_{\text{PDB}} = -49.16\text{‰}$  to  $-52.29\text{‰}$  and a group of unsaturated 2,6,10,15,19-pentamethylcosane (PML) compounds produced by methane oxidizing archaea (MOA) with  $\delta^{13}\text{C}_{\text{PDB}}$  of PML compounds from  $-107.2\text{‰}$  to  $-115.5\text{‰}$ , indicating that the crusts formed as the result of bacterially catalyzed methane oxidation and sulfate reduction. The fabric of the two flat sides of the crust is distinctly different. The “top” side is composed entirely of microcrystalline calcite while the bottom is composed entirely of “wormy” carbonate cement that could be a random, low fidelity replacement of bacteria. In the interior of the  $\sim 1\text{ cm}$  thick crust microcrystalline calcite is coated by “wormy” carbonate cement, and dispersed pyrite framboids appear to be collaborating colonies of MOA and sulfate reducing bacteria (SRB). We propose that the “wormy” carbonate texture, particularly with microcrystalline calcite and pyrite framboids present, may be a useful indicator of biologically controlled fabrics produced during methane oxidation and sulfate reduction by MOA and SRB.

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**Keywords:** Biogenic fabric; Seep carbonate; Methane oxidizing archaea; Sulphate reducing bacteria; Gulf of Mexico

## 1. Introduction

The area of offshore Louisiana in the Gulf of Mexico is characterized by very active sedimentation and hydrocarbon generation. Hundreds of methane seeps have been found in the area (MacDonald et al., 2002, 2003). Carbonate crusts form in the waning stages of gas venting when the venting rate is slow (Roberts and Carney, 1997; Chen et al., 2004). The very low carbon isotopic ratios of the seep carbonates indicate that they are the byproducts of microbial oxidation of methane (Roberts and Aharon, 1994; Cavagna et al., 1999; Stakes et al., 1999; Peckmann et al., 2001; Campbell et al., 2002; Peckmann and Thiel, 2004; Sassen et al., 2004; Chen et al., 2005, 2006).

Microbial methane oxidation is achieved through the combined metabolism of methane oxidizing archaea

(MOA) and sulphate reducing bacteria (SRB) (Valentine and Reeburgh, 2000). This collaboration of bacterial activity is suggested by fluorescence studies on living MOA and SRB (Boetius et al., 2000; Orphan et al., 2001, 2002; Michaelis et al., 2002; Blumenberg et al., 2004; Knittel et al., 2005). Both MOA and SRB are abundant at seafloor methane seeps. For example,  $3 \times 10^9$  MOA cells  $\text{cm}^{-3}$  and  $6 \times 10^9$  SRB cells  $\text{cm}^{-3}$  are estimated to be present in the upper 5 cm of surface sediments at Hydrate Ridge on the Cascadia margin (Boetius et al., 2000). In the Gulf of Mexico,  $1.5 \times 10^9$  MOA and SRB cells  $\text{g}^{-1}$  have been estimated to populate hydrate-associated sediments, and  $1.0 \times 10^6$  MOA and SRB cells  $\text{ml}^{-1}$  to populate fluids produced by hydrate decomposition (Lanoil et al., 2001). In the Black Sea, Michaelis et al. (2002) reported  $10^{12}$  MOA and SRB cells  $\text{cm}^{-3}$  in bacterial mats up to 10 cm thick, and also show that bacteria cover the surfaces of carbonate chimneys at gas seeps. Biomarkers indicate

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MOA and SRB were present at recent and ancient gas seeps at many locations worldwide (Elvert et al., 2000; Lanoil et al., 2001; Aloisi et al., 2002; Michaelis et al., 2002; Thiel et al., 2003; Zhang et al., 2003; Blumenberg et al., 2004; Peckmann and Thiel, 2004; Stadnitskaia et al., 2005).

Given that MOA and SRB are very abundant and are catalyzing reactions that lead to the precipitation of carbonate and pyrite, and were present in ancient seeps, it is plausible that these microbes could be preserved as carbonate and pyrite as has been suggested. For example, Peckmann et al. (2001, 2002), suggested carbonate filaments that they observed were fossilized MOA bacteria. Popa et al. (2004) suggested pyrite framboids found in anoxic microbial mats associated with venting methane gas that were of microbial origin.

In this paper we take the view that if MOA and SRB bacteria are being fossilized anywhere this should be occurring in the methane seeps of the Gulf of Mexico. In this context we simply describe the carbonate and pyrite forms in seep carbonate samples from an active gas vent site in Green Canyon Block 238 of Gulf of Mexico where MOA and SRB are active today. The samples contain both microcrystalline carbonate that is precipitated by purely inorganic processes and “wormy” carbonate cement that covers the crystals in the sample interior and covers completely one side of the crust. The “wormy” carbonate has a morphology that suggests replacement of bacteria by carbonate. The purpose of this paper is to describe these features which may be a useful geologic indicator of biologically controlled fabrics produced during methane oxidation and sulfate reduction by MOA and SRB.

## 2. Sample and analysis

The seep carbonates described here were collected in May 2002 from an area of Green Canyon Block 238 where methane is seeping across the seafloor which is covered by red-brown and white bacteria mats. The sampling was done during dive 4398 using a clamshell sampler at the end of the robot arm of Johnson-Sea-Link II submersible. The clamshell scoop collected a ~30 cm swath of sediment about 10 cm deep which was placed in a bucket and carried to the surface. The temperature of the site at the time of sampling was ~6.15 °C and the water depth ~700 m.

When the samples were spread out on the deck of the Sea Link, the sediments were found to contain about 20 pieces of carbonate crust mixed with black organic-rich clay that smelled of H<sub>2</sub>S. Each piece of carbonate crust was <~5 cm in lateral dimensions and <~1 cm thick. Both sides of the plate-like crusts show numerous hollows, some of which cut through the crust to form circular holes less than ~1 cm in diameter. Some of the carbonate samples are shown in Fig. 1.

The samples were washed with fresh water at the time of collection. In preparation for analysis they were cleaned again using distilled water, and air dried. The sample was broken with a rock hammer in the laboratory as shown in

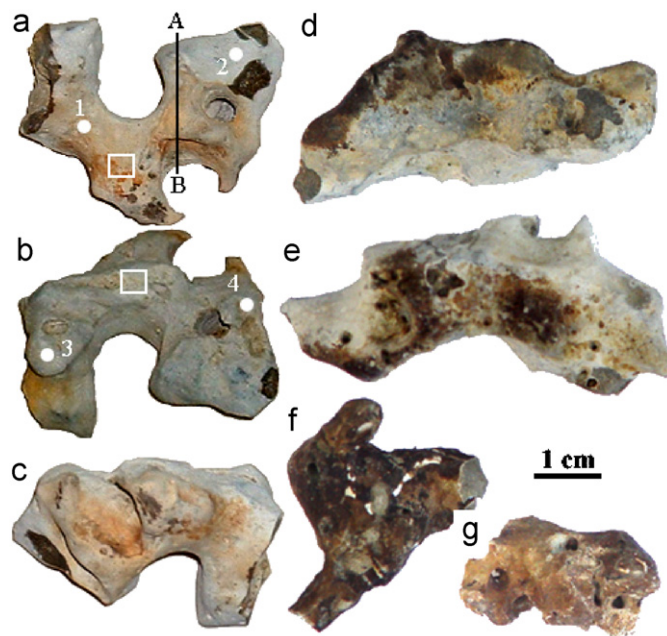


Fig. 1. Seep carbonates sampled on dive 4398 from an active gas vent in Green Canyon Block 238 in the offshore Louisiana Gulf of Mexico. Both surfaces of the plate-like crusts are shown in a and b. Circular holes partially and fully penetrate the crust. White squares show where SEM observations were made on the sample surfaces of side-a (Fig. 2) and side-b (Fig. 3). Line A–B shows where the crust was cut to make a thin section. Optical and back-scattered electron images of this section are shown in Figs. 4 and 7, respectively. SEM images of the broken left corner of side-a are shown in Figs. 5 and 6a. White dots with numbers of 1–4 show the locations where the carbon isotopic data were analyzed in Table 1.

Fig. 1a to expose an interior surface at the left corner: the sample was then cut parallel to line A–B to make a thin section. The sample shown in Fig. 1a and b was observed on both of its flat surfaces (white squares), and in its interior on a break at its left corner and on the thin section cut A–B.

Samples were prepared for Scanning Electron Microscope (SEM) observation by coating them with gold to a thickness of ~200 Å. SEM photographs were taken using the LEO-1530VP SEM operating at 10–20 kV with a 5–9 mm working distance. Optical photographs were taken using a LEICA-DMRX optical microscope. Size estimates were made under the optical microscope using the Leica Qwin Program. The chemical composition of minerals was analyzed by using the JEOL-JXA8100 of the electron probe microanalyzer. Carbonate carbon and oxygen isotopic compositions in permil (‰) relative to Pee Dee Belemnite (PDB) standard were measured by using the GV Isoprime II stable isotopic mass spectrometry with deviations less than 0.01‰ (2σ) for both δ<sup>18</sup>O and δ<sup>18</sup>C values.

## 3. Microscope examination

Figs. 2 and 3 show SEM images of both flat surfaces of the carbonate crust at the white squares in Figs. 1a and b. The two surfaces are very different in character. Side-a,

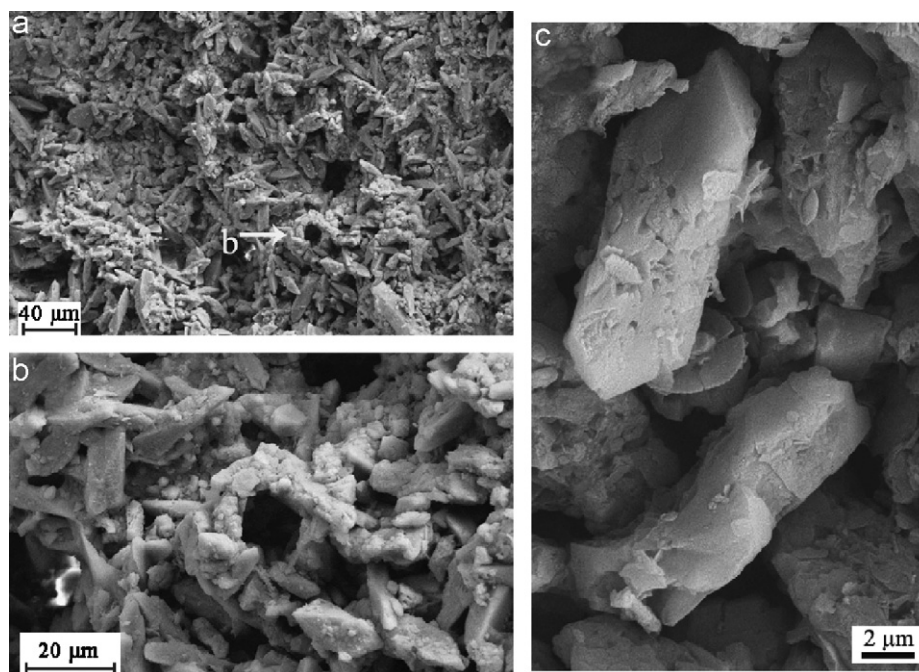


Fig. 2. SEM images observed on the side-a of the seep carbonate crust. (a)–(c) SEM photomicrographs of an intergrowth of  $\sim 4\mu\text{m}$  calcite crystals (microcrystalline calcite) on side-a of the crust marked as a white square shown in Fig. 1a that are occasionally penetrated by small,  $< \sim 10\mu\text{m}$ , holes surrounded by elongated, vertically oriented, euhedral calcite crystals. (b) An enlargement at the position of the white arrow in (a). (c) Further enlargement of clean (no cement coating) elongate euhedral carbonate crystals from a position out of the field view in (a).

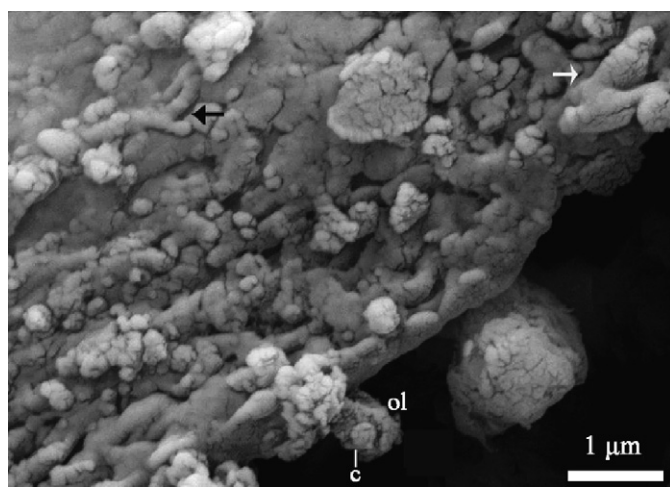


Fig. 3. SEM photomicrograph of the other side (side-b) of the crust observed in an area marked as a white square shown in Fig. 1b. No elongate euhedral calcite crystals are present. Rather the surface is composed of aggregated carbonate grains. Many of the grains are round. One appears to have a central core (labeled “c”) surrounded by an outer shell (labeled “ol”). Other aggregates have a worm- or column-like form (black and white arrows).

shown in Fig. 2, is an intergrowth of  $\sim 4\mu\text{m}$  calcite crystals (microcrystalline calcite) that is occasionally penetrated by small,  $< \sim 10\mu\text{m}$ , holes. The holes are surrounded by elongate, vertically oriented, microcrystalline euhedral calcite crystals. Side-b is also of carbonate composition (see below) but has no euhedral calcite crystals (Fig. 3). Rather it consists of aggregated carbonate particles that are

mostly  $0.6\text{--}1\mu\text{m}$  in diameter. Many of the aggregates are round, and some appear to have a central core (labeled “c” in Fig. 3) that is surrounded by an outer layer or shell (labeled “ol” in Fig. 3). Other aggregates have a worm- or column-like form (black and white arrows in Fig. 3). Higher magnification investigation of side-a (Fig. 2) shows none of the carbonate particles that aggregate to form the entire surface on side-b are present on side-a.

Fig. 4 shows a transmitted light optical image of the interior surface of the sample along A–B. Pyrite framboids that are  $\sim 3\text{--}8\mu\text{m}$  in diameter (average  $\sim 5\mu\text{m}$ ) are dispersed within the carbonate. Fig. 5 shows SEM images of the interior surface of the sample on the corner break of the sample in Fig. 1a. Under the higher SEM magnification of this image the carbonate cement can be seen to be coating the surface of microcrystalline calcite. It can also be seen that the cement is composed of particles  $\sim 0.1\text{--}0.5\mu\text{m}$  in diameter that have a spherical, oval, or rod shape. These particles occur singly and as clumps that merge into sheets. Some of the sheets show the constituent particles fairly distinctly, but in others the particles merge so completely that it is difficult to discern them. X-ray diffraction shows that, overall, the sample is 90.1% calcite, 6.7% clay, and 2.7% quartz (Chen et al., 2005). Electron microprobe analysis shows that the microcrystalline calcite is 47.99% CaO, 1.37% MgO, and 0.37% FeO and the carbonate particle cement is 43.16% CaO, 1.26% MgO, and 0.46% FeO (Table 1).

The pyrite framboids are shown in detail in Figs. 6 and 7. Fig. 6a shows framboids from the broken left corner of the

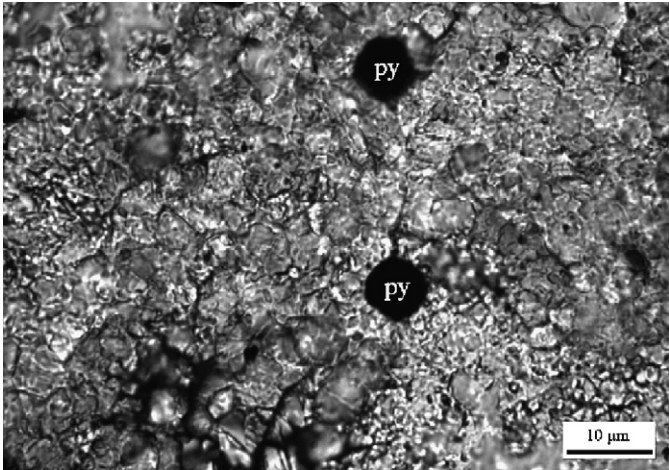


Fig. 4. Transmitted light microscope image of a thin section cut along A–B in Fig. 1. It shows ~5 μm pyrite spheroid (labeled “py”) dispersed in a matrix of microcrystalline calcite that is cemented by a fabric of smaller filament-like calcite.

Table 1  
Chemical composition of the microcrystalline calcite and the cement that coats it shown in Fig. 3 from electron probe analysis (wt%)

| Analyzed locations | CaO   | MgO  | FeO  | CO <sub>2</sub> | Total |
|--------------------|-------|------|------|-----------------|-------|
| 1 Crystal          | 44.80 | 1.33 | —    | 50.38           | 96.51 |
| 2 Crystal          | 48.36 | 1.44 | 0.34 | 48.38           | 98.51 |
| 3 Crystal          | 48.41 | 1.47 | 0.55 | 46.2            | 96.64 |
| 4 Crystal          | 50.39 | 1.23 | 0.23 | 44.3            | 96.15 |
| Mean               | 47.99 | 1.37 | 0.37 | 47.32           | 96.95 |
| 5 Cement           | 39.06 | 1.11 | —    | 50.2            | 90.36 |
| 6 Cement           | 43.66 | 1.30 | 0.57 | 53.62           | 99.16 |
| 7 Cement           | 46.75 | 1.36 | 0.34 | 47.18           | 95.63 |
| Mean               | 43.16 | 1.26 | 0.46 | 50.33           | 95.05 |

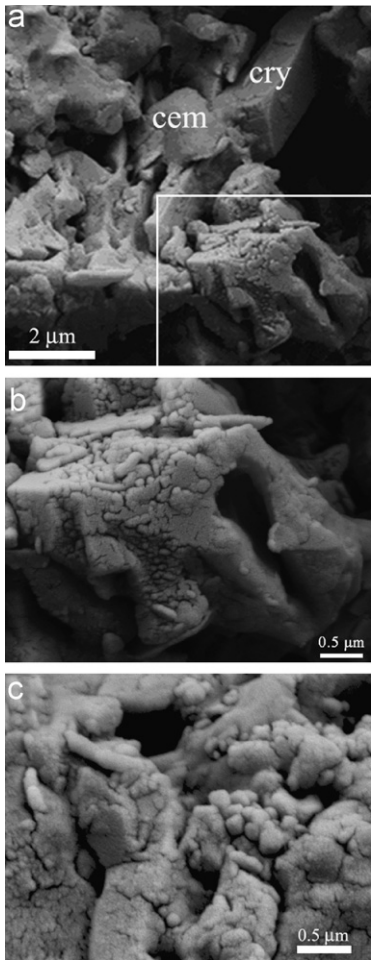


Fig. 5. SEM images from the interior of the carbonate crust showing the carbonate consists of calcite crystal lath and cement coating on calcite crystals. The cement (labeled “cem”) as coating on the crystals (labeled “cry”) is mainly composed of ~0.1–0.5 μm diameter carbonate particles of spheroids, ovoids and rods. (b) is an enlargement of (a) at the position of the white rectangular area.

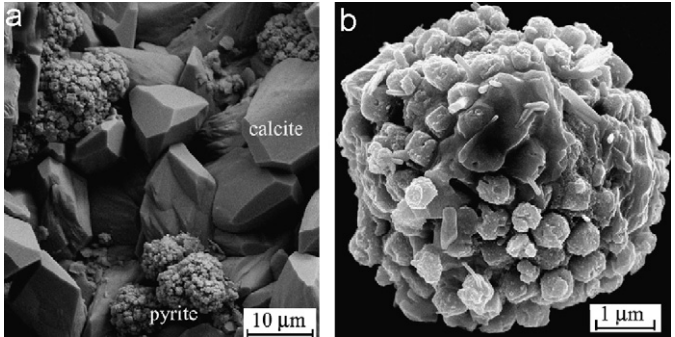


Fig. 6. SEM images of the pyrite framboids. (a) is a SEM image of pyrite framboids on the broken left corner of side-a shown in Fig. 1a. These pyrite framboids consist of overgrowth cubic crystals of pyrites <1 μm. (b) is SEM images of a single framboid that consists of numerous smaller pyrite spheroids with small unknown mineral crystals. The smaller spheroids tend to take on a roughly cubic form.

Fig. 1a sample shown that are ~3–8 μm in diameter and composed of smaller spherical particles that are ~0.5 μm in diameter (Fig. 6b). Fig. 7 shows framboids from the thin section cut along A–B in Fig. 1a. The framboids comprised of particles show a center and an outer layer (Figs. 7a–b). The center is composed of numerous small (white) pyrite and (black) calcite particles, and the outer layer is composed of numerous small (grey to white) pyrite particles and rods. Electron microprobe analysis (Table 2) shows both the center and outer layers are pyrite with a little calcite. The center is 39.79% Fe, 48.58% S, and 1.78% Ca, and the outer layer 37.52% Fe, 46.97% S, and 3.44% Ca. The morphology is very similar to that of living colonies of MOA and SRB bacteria that arrange themselves in this zoned spherical form to collaborate in sulfate reduction and methane oxidation (Figs. 7c and d).

4. Carbon isotopes and biomarkers

Isotopic and biomarker data are often useful in determining how carbonates were deposited. The carbonate in our sample has strongly negative values, with δ<sup>13</sup>C<sub>PDB</sub> ranging from –49.16‰ to –52.29‰ (Table 3). These low isotopic ratios indicate that the carbonate

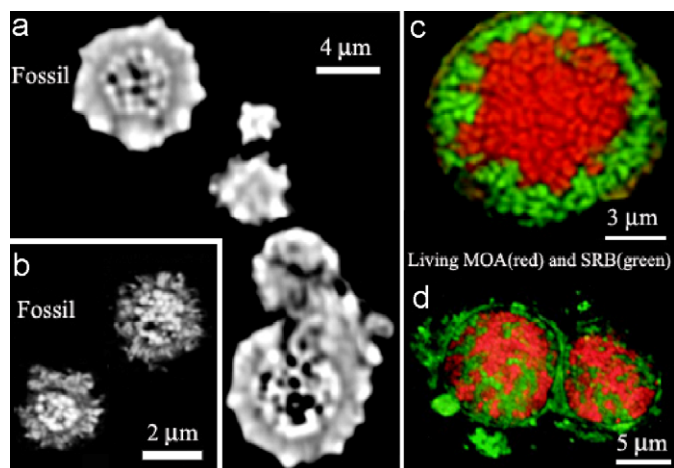


Fig. 7. Back-scattered electron images (a) and optical reflected light microscope images (b) of pyrite framboids taken from the A–B thin section show a center and an outer layer similar to living collaborating colonies of MOA and SRB (c and d). (a) and (b) show that the center of the pyrite framboids is composed of numerous small (white) pyrite and (black) calcite grains, and the surrounding outer layer is composed of numerous small (grey to white) pyrite grains and rods. (c) and (d) show colonies of living MOA (red) and SRB (green) 1–11 μm in diameter, imaged by laser scanning confocal microscopy (Orphan et al., 2002; Knittel et al., 2005). The living MOA cells aggregate in a core that is fully or partially surrounded by SRB cells. The size and form of the living MOA–SRB consortia and pyrite framboids are remarkably similar.

Table 2

Chemical compositions of the inner core and outer rim of pyrite framboid shown in Fig. 4 are determined by electron probe analysis (wt%)

| Analyzed locations | Na   | S     | Ca   | Fe    | Total |
|--------------------|------|-------|------|-------|-------|
| 1 Center           | 0.22 | 50.54 | 0.37 | 41.93 | 93.06 |
| 3 Center           | 0.20 | 46.09 | 4.07 | 36.95 | 87.31 |
| 5 Center           | 0.29 | 49.12 | 0.89 | 40.49 | 90.79 |
| Mean               | 0.24 | 48.58 | 1.78 | 39.79 | 90.39 |
| 2 Outer layer      | 0.26 | 45.17 | 3.31 | 36.50 | 85.24 |
| 4 Outer layer      | 0.25 | 45.28 | 6.25 | 34.75 | 86.53 |
| 6 Outer layer      | 0.28 | 50.45 | 0.76 | 41.31 | 92.80 |
| Mean               | 0.26 | 46.97 | 3.44 | 37.52 | 88.19 |

carbon came from bacterial oxidation of methane (Roberts and Aharon, 1994; Cavagna et al., 1999; Stakes et al., 1999; Peckmann et al., 2001; Campbell et al., 2002; Peckmann and Thiel, 2004; Sassen et al., 2004).

Song and Wang (2006) analyzed the biomarkers of our seep carbonate samples and the mud collected with them. The total mass chromatograms of aliphatic hydrocarbons from the carbonates and the associated mud sediments show that there is a group of unusual compounds distributed between  $nC_{22}$  and  $nC_{24}$  and centered on  $nC_{23}$  (Fig. 8). The mass spectra of these compounds are characterized by a common  $m/z$  69 base peak and a second base peak of  $m/z$  123, and their ionic molecular mass peaks are 350, 348, 346, 344, and 342, respectively (Fig. 9). These peaks are identified as a group of unsaturated 2,6,10,15,19-pentamethylicosenes (PMIΔ) con-

Table 3

Carbon and oxygen isotopic composition of seep carbonate crusts

| Samples                                        | $\delta^{13}C_{PDB}\%$ | $\delta^{18}O_{PDB}\%$ |
|------------------------------------------------|------------------------|------------------------|
| Analyzed locations of crust shown in Fig. 1a–b |                        |                        |
| 1 S-4-Side-a-1                                 | −51.95                 | 4.06                   |
| 2 S-4-Side-a-2                                 | −50.72                 | 3.46                   |
| 3 S-4-Side-b-3                                 | −52.22                 | 4.53                   |
| 4 S-4-Side-b-4                                 | −52.29                 | 4.35                   |
| Other crusts collected from same site          |                        |                        |
| S-4-2                                          | −50.86                 | 4.56                   |
| S-4-3                                          | −49.16                 | 3.15                   |
| S-4-4                                          | −51.17                 | 3.33                   |
| S-4-5                                          | −49.83                 | 3.21                   |
| S-4-6                                          | −50.80                 | 4.17                   |
| S-4-7                                          | −51.00                 | 2.90                   |
| S-4-8                                          | −49.89                 | 2.74                   |
| S-4-9                                          | −50.46                 | 3.14                   |
| S-4-11                                         | −50.59                 | 4.29                   |
| S-4-12                                         | −49.62                 | 2.96                   |
| S-4-13                                         | −49.46                 | 3.21                   |
| S-4-14                                         | −49.60                 | 3.74                   |
| S-4-15                                         | −50.53                 | 3.20                   |
| S-4-16                                         | −49.94                 | 3.71                   |
| S-4-17                                         | −49.53                 | 3.73                   |
| S-4-18                                         | −49.97                 | 3.58                   |
| S-4-19                                         | −50.08                 | 4.83                   |

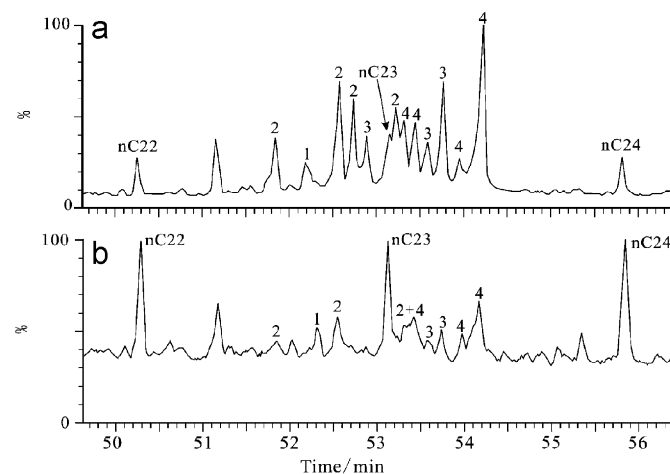


Fig. 8. Chromatograms of aliphatic hydrocarbons from the black mud (a) and the seep carbonate (b) show a group of unsaturated 2,6,10,15,19-pentamethylicosane (PMIΔ) compounds that are biomarkers for anaerobic methane oxidation (Song and Wang, 2006).

taining 1–5 double bonds. These PMIΔ compounds are characteristic of anaerobic MOA (e.g. Pancost et al., 2001; Zhang et al., 2003; Peckmann and Thiel, 2004; Stadnitskaia et al., 2005). The carbon isotopic compositions of PMI:2, PMI:3, and PMI:4 compounds are −107.2‰, −115.0‰, and −115.5‰ PDB, respectively. The very low carbon isotopic ratios of the PMIΔ compounds indicate that these compounds were derived from anaerobic methane oxidation (Blumenberg et al., 2004; Reitner et al., 2005; Pape et al., 2005; Bouloubassi et al., 2006).

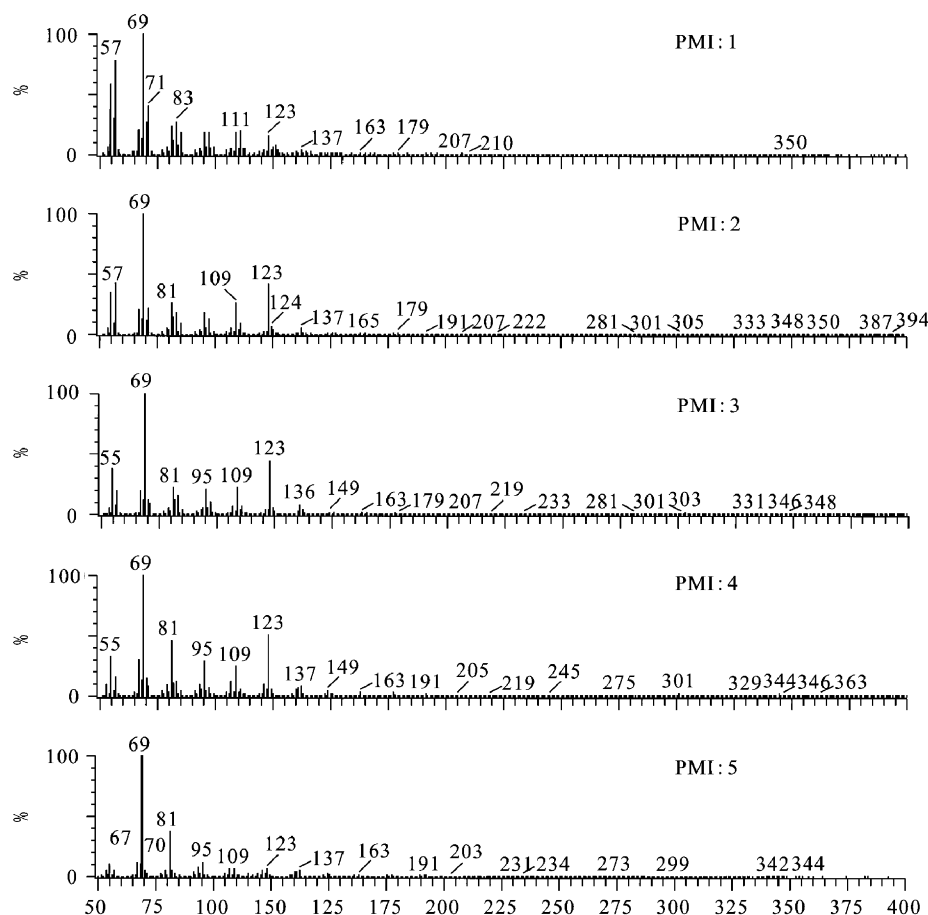


Fig. 9. Mass spectra of 1–5 double bond 2,6,10,15,19-pentamethylicosenes (PMI:1 for one double bond, PMI:2 for two double bonds, PMI:3 for three double bonds, PMI:4 for four double bonds, and PMI:5 for five double bonds) (Song and Wang, 2006).

## 5. Summary and discussion

Carbonate crusts  $\sim 5$  cm in lateral dimension and  $\sim 1$  cm thick were collected near methane seeps off the Louisiana coast (Fig. 1). The crusts were examined using optical and SEM microscopy. The dissimilarity of the two surfaces of the carbonate crust is striking. Side-a has microcrystalline calcite pierced by holes (Fig. 2), but side-b has no microcrystalline calcite, only  $0.6$ – $1$   $\mu\text{m}$  diameter aggregates of  $\sim 0.03$   $\mu\text{m}$  diameter carbonate particles (Fig. 3). These spherical aggregates appear to have an inner core that is surrounded by an outer shell (Fig. 3). In the interior of the crusts, spherical and rod-like carbonate particles  $0.1$ – $0.5$   $\mu\text{m}$  in diameter coat the micro-crystalline calcite singly and as clumps that merge into sheets (Fig. 5). Pyrite framboids  $3$ – $8$   $\mu\text{m}$  in diameter are dispersed within the crusts. The framboids are composed of  $0.5$   $\mu\text{m}$  particles grouped into an inner core and an outer shell in a fashion very similar to that observed in living, collaborating colonies of SRB (outer shell) and MOA (inner core) bacteria (Fig. 7). Carbonate carbon isotopic data indicate that the seep carbonate was precipitated as the result of methane oxidation. Biomarkers PMI4 show that MOA

colonized the sample. The PMI4 carbon isotopic signatures show that microbes were present during (and in fact caused) carbonate precipitation.

The aggregated forms of the carbonate particles in the interior of the sample are similar in form and habit to fossilized bacterial colonies described in the literature where spherical, oval, and rod-like particles merge into sheets (Castanier et al., 1999; Folk, 1999; Folk and Rasbury, 2002). Fluorescence studies at Hydrate Ridge surface sediments (Boetius et al., 2000) show that individual MOA and SRB cells are usually spherical particles  $0.3$ – $0.5$   $\mu\text{m}$  in diameter. At Black Sea vent sites (Michaelis et al., 2002; Orphan et al., 2002; Knittel et al., 2005; Reitner et al., 2005) individual MOA cells are cylindrical rods with tabular ends that have a rectangular appearance, and they are  $0.6$ – $1.4$   $\mu\text{m}$  in diameter. Living MOA cells interconnect to form chains, referred to as filaments, up to  $70$   $\mu\text{m}$  long (Orphan et al., 2002). The cements in the interior of the crust show a texture similar to MOA bacterial colonies, but the fossilization, if that is what it is, is not a snapshot of the colonies at any instant of time, but rather a pattern that could represent the replacement of random cells with carbonate over a protracted period of time.

Living MOA cells are known to clump into aggregates that are partially or fully surrounded by a shell of aggregated SRB cells (Blumenberg et al., 2004; Boetius et al., 2000; Orphan et al., 2001, 2002; Reitner et al., 2005). The aggregated consortia of MOA and SRB cells range from 1 to 11  $\mu\text{m}$  and average  $3.2 \pm 1.5 \mu\text{m}$  in diameter (Boetius et al., 2000). The form and dimensions of living MOA–SRB colonies are very similar to the framboids in Fig. 7, and others (e.g. Popa et al., 2004) have suggested framboids found in anoxic microbial mats where methane gas was venting were of microbial origin. Framboids have also been found in seep carbonates from the South China Sea (Chen et al., 2005, 2006).

The zoned carbonate balls dangling from side-b of our sample are at the low end of the size range of living SRB–MOA colonies (Boetius et al., 2000) so they could be collaborating colonies similar to those fossilized in pyrite in the crust interior. Both the bacterial cell size and the colony size are much smaller, however.

The distinct difference between the two sides of the crust is perhaps its most striking feature. The microcrystalline fabric (with no wormy cement) of side-a contrasts starkly with the “biogenic” wormy cement (with no microcrystalline calcite) of side-b. We hypothesize that side-a is the top of the sample and side-b the bottom, and that methane passed around and through the holes in the sample. Collaborating colonies of MOA and SRB on the underside of the crust enriched the pore waters in bicarbonate. Calcite crystals grew where these waters gained access to seawater calcium, especially perhaps where they emerged from small holes in the crust. The higher oxidation state of seawater may have inhibited microbial growth on the top of the crust, while the conditions were just right for their growth on the underside.

If the fabric of the wormy carbonate cements on the interior and “underside” of the crust is the result of a fossilization-like control on carbonate precipitation, the presence of this fabric, particularly with pyrite framboids and microcrystalline calcite also present, could be an indicator of the biological controlled fabrics produced in the methane oxidation and sulfate reduction by MOA and SRB. In other words, if the interest is in inferring geochemical process (in this case biogenically controlled methane oxidation and sulfate reduction), the observation of “wormy” and microcrystalline fabrics may provide a useful kind of fossil evidence. It could be that a weak, inaccurate form of fossilization that preserves only the general fabric could still be very useful. For chemical purposes, it might be useful to generalize our concept of “fossil” to include such low fidelity replacements if they produce recognizable fabrics and interpretations that can be cross-checked by other observations (framboids, microcrystalline calcite, very low  $\delta^{13}\text{C}_{\text{PDB}}$ , and  $\text{PMI}\Delta$ ). Whether “fabric-fossils” is a valid concept will depend on whether observation of a great many other samples shows it is useful.

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## References

- Aloisi, G., Bouloubassi, I., Heijs, S.K., Pancost, R.D., Pierre, C., Damste, J.S.S., Gottschal, J.C., Forney, L.J., Rouchy, J., 2002.  $\text{CH}_4$ -consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters* 203, 195–203.
- Blumenberg, M., Seifert, R., Reitner, J., Pape, T., Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. *Proceedings of the National Academy of Sciences of the United States of America* 101, 11111–11116.
- Boetius, A., Ravensschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B.B., Witte, U., Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–626.
- Bouloubassi, I., Aloisi, G., Pancost, R.D., Hopmans, E., Pierre, H., Damsté, J.S.S., 2006. Archaeal and bacterial lipids in authigenic carbonate crusts from eastern Mediterranean mud volcanoes. *Organic Geochemistry* 37, 484–500.
- Campbell, K.A., Farmer, J.D., Des Marais, D., 2002. Ancient hydrocarbon seeps from the Mesozoic convergent margin of California: carbonate geochemistry, fluids and palaeoenvironments. *Geofluids* 2, 63–94.
- Castanier, S., Metayer-Levrel, G.L., Perthuisot, J., 1999. Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view. *Sedimentary Geology* 126, 9–23.
- Cavagna, S., Clari, P., Martire, L., 1999. The role of bacteria in the formation of cold seep carbonates: geological evidence from Monferrato (Tertiary, NW Italy). *Sedimentary Geology* 126, 253–270.
- Chen, D.F., Cathles, L.M., Roberts, H.H., 2004. The chemical signatures of variable gas venting at hydrate sites. *Marine and Petroleum Geology* 21, 317–326.
- Chen, D.F., Huang, Y.Y., Yuan, X.L., Cathles, L.M., 2005. Seep carbonates and preserved methane oxidizing archaea and sulfate reducing bacteria fossils suggest recent gas venting on the seafloor in the Northeastern South China Sea. *Marine and Petroleum Geology* 22, 613–621.
- Chen, D.F., Feng, D., Su, Z., Song, Z.G., Chen, G.Q., Cathles, L.M., 2006. Pyrite crystallization in seep carbonates at gas vent and hydrate site. *Materials Science and Engineering: C* 26, 602–605.
- Elvert, M., Suess, E., Greinert, J., Whiticar, M.J., 2000. Archaea mediating anaerobic methane oxidation in deep-sea sediments at cold seeps of the eastern Aleutian subduction zone. *Organic Geochemistry* 31, 1175–1187.
- Folk, R.L., 1999. Nannobacteria and the precipitation of carbonate in unusual environments. *Sedimentary Geology* 126, 47–55.
- Folk, R.L., Rasbury, E.T., 2002. Nanometer-scale spheroids on sands, Vulcano, Sicily: possible nanobacterial alteration. *Terra Nova* 14, 469–475.
- Knittel, K., Losekann, T., Boetius, A., Kort, R., Amann, R., 2005. Diversity and distribution of methanotrophic archaea at a cold seep. *Applied and Environmental Microbiology* 71, 467–479.

- Lanoil, B.D., Sassen, R., La Duc, M.T., Sweet, S.T., Neilson, K.H., 2001. Bacteria and archaea physically associated with Gulf of Mexico gas hydrates. *Applied and Environmental Microbiology* 67, 5143–5153.
- MacDonald, I.R., Leifer, I., Sassen, R., Stine, P., Mitchell, R., Guinasso Jr, N., 2002. Transfer of hydrocarbons from natural seeps to the water column and atmosphere. *Geofluids* 2, 95–107.
- MacDonald, I.R., Sager, W.W., Peccini, M.B., 2003. Gas hydrate and chemosynthetic biota in mounded bathymetry at mid-slope hydrocarbon seeps: Northern Gulf of Mexico. *Marine Geology* 198, 133–158.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jorgensen, B.B., Widdel, F., Peckmann, J., Pimenov, N.V., Gulin, M.B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science* 297, 1013–1015.
- Orphan, V.J., House, C.H., Hinrichs, K.U., McKeegan, K.D., DeLong, E.F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* 293, 484–487.
- Orphan, V.J., House, C.H., Hinrichs, K.U., McKeegan, K.D., DeLong, E.F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediment. *Proceedings of the National Academy of Sciences of the United States of America* 99, 7663–7668.
- Pancost, R.D., Hopmans, E.C., Sinninghe-Damste, J.S., The MEDINAUT Shipboard Scientific Party, 2001. Archaeal lipids in Mediterranean cold seeps: molecular proxies for anaerobic methane oxidation. *Geochimica et Cosmochimica Acta* 65, 1611–1627.
- Pape, T., Blumenberg, M., Seifert, R., Egorov, V.N., Gulin, S.B., Michaelis, W., 2005. Lipid geochemistry of methane-seep-related Black Sea carbonates. *Palaeogeography, Palaeoclimatology, Palaeoecology* 227, 31–47.
- Peckmann, J., Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology* 205, 443–467.
- Peckmann, J., Reimer, A., Luth, U., Hansen, B.T., Heinicke, C., Hoefs, J., Reitner, J., 2001. Methane-derived carbonates and authigenic pyrite from the northwestern Black Sea. *Marine Geology* 177, 129–150.
- Peckmann, J., Goedert, J.L., Thiel, V., Michaelis, W., Reitner, J., 2002. A comprehensive approach to the study of methane-seep deposits from the Lincoln Creek Formation, western Washington State USA. *Sedimentology* 49, 855–873.
- Popa, R., Kinkle, B.K., Badescu, A., 2004. Pyrite framboids as biomarkers for iron–sulfur systems. *Geomicrobiology Journal* 21, 193–206.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., Thiel, V., 2005. Concretionary methane-seep carbonate and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology* 227, 18–30.
- Roberts, H.H., Aharon, P., 1994. Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: a review of submersible investigations. *Geo-Marine Letters* 14, 135–148.
- Roberts, H.H., Carney, R.S., 1997. Evidence of episodic fluid, gas, and sediment venting on the northern Gulf of Mexico continental slope. *Economic Geology* 92, 863–879.
- Sassen, R., Roberts, R.R., Carney, C., Milkov, A.V., DeFreitas, D.A., Lanoil, B., Zhang, C., 2004. Free hydrocarbon gas, gas hydrate, and authigenic minerals in chemosynthetic communities of the northern Gulf of Mexico continental slope: relation to microbial processes. *Chemical Geology* 205, 195–217.
- Song, Z.G., Wang, C.P., 2006. The biomarkers of 2,6,10,15,19-pentamethylcosenes and their carbon isotopic composition in the sediments from the Gulf of Mexico. *Chinese Science Bulletin* 51, 1736–1740.
- Stadnitskaia, A., Muyzer, G., Abbas, B., Coolen, M.J.L., Hopmans, E.C., Baas, M., van Weering, T.C.E., Ivanov, M.K., Poludetkina, E., Sinninghe Damsté, J.S., 2005. Biomarker and 16S rDNA evidence for anaerobic oxidation of methane and related carbonate precipitation in deep-sea mud volcanoes of the Sorokin Trough, Black Sea. *Marine Geology* 217, 67–96.
- Stakes, D.S., Orange, D., Paduan, J.B., Salamy, K.A., Maher, N., 1999. Cold-seeps and authigenic carbonate formation in Monterey Bay, California. *Marine Geology* 159, 93–109.
- Thiel, V., Blumenberg, M., Pape, T., Seifert, R., Michaelis, W., 2003. Unexpected occurrence of hopanoids at gas seeps in the Black Sea. *Organic Geochemistry* 34, 81–87.
- Valentine, D.L., Reeburgh, W.S., 2000. New perspectives on anaerobic methane oxidation. *Environmental Microbiology* 2, 477–484.
- Zhang, C.L., Pancost, R.D., Sassen, R., Qian, Y., Macko, S.A., 2003. Archaeal lipid biomarkers and isotopic evidence of anaerobic methane oxidation associated with gas hydrates in the Gulf of Mexico. *Organic Geochemistry* 34, 827–836.