

***Interactive comment on “Short-term changes in anaerobic oxidation of methane in response to varying methane and sulfate fluxes” by G. Wegener and A. Boetius***

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General remarks and recommendations:

Improve description of experimental setup: What is the source of methane in the reservoir?

AC: As a methane source we have used commercially available methane:CO<sub>2</sub>-mixture, (90:10) kept in the reservoir headspace at 1.5 atmospheres. The details are now given in the materials and methods section (line 171 ff).and in table 1

The authors should analyze the composition with respect CO and higher hydrocarbons instead of speculating about an additional electron donor for sulfate reduction (p. 3076,

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I. 20-22). Can sulphate reduction of sediment substrates really be excluded?

AC: The H<sub>2</sub> and higher hydrocarbon content of the commercial gas used as methane source was <500ppmv and <3000ppmv, respectively. Taking solubilities into account, both potential electron donors for SR together could explain 0.012  $\mu\text{mol (g dw)}^{-1}$  or ca 8-10 % of the observed sulfide production in the experiment. The background sulfide production in these sediments was around 6-8% of the total sulfide production; hence both background sulfide production terms together could explain ca. 15-20% of the total sulfide production. We also calculated the mass balance between sulfide produced and methane consumed based on measurement of the reservoir medium at the beginning and end of the experiment 1a, (constant conditions) and found the same pattern, of 10% higher SR compared to AOM. Hence, we conclude that the background SR from sediments and impurities in the gas explain the observed offset, but that the main variation observed and discussed in the MS is due to the methane driven sulfide production. We have revised the manuscript accordingly.

e.g. 237ff: alternative electron acceptors in gas phase; line 348ff: high offset in the methane experiment,

There are no starting conditions reported for the experiments (Fig. 2). At which temperature were the experiments conducted? A table summarizing general parameters of all experiments (flow rate, temp, reservoir concentrations, sampling period, etc.) should be provided.

The table with the desired information on the sampling sites was added (table 1). The term '&#8220;flow rates&#8221;'; was changed to '&#8220;flow velocities&#8221;'; if it was meter / time (compared to volume / time). Additionally more information on starting conditions is given in the text. Gullfaks sediments were percolated for 120 days with constant methane concentrations of around 1.6 mM and starting sulfide concentration of 0.5 mM. The flow rate was 36 ml d<sup>-1</sup> (velocity of 32 m yr<sup>-1</sup> (GF, porosity 35%); 14 m yr<sup>-1</sup> (BS, HR, porosity ~80%), (Line 219 to 225)

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Methane concentrations used in the experimental setup roughly correspond to methane solubility at atmospheric pressure. Although it is quite clear that operating the system at higher pressures would drastically increase the operational costs, it would be good to mention that methane concentrations cold seeps in submarine environments are much higher and hence comparability is limited. I suppose that all sediments used in this study were sampled from water depths  $>100$  m.

AC: This is now clarified in the text (lines 120ff, 140ff)). We now refer to future research needs for kM-measurements in high-pressure incubations (end of results; line 386 to 387))

I doubt that the system setup is useful to answer the (highly demanded) question of the efficiency of ANME communities in consuming methane at high fluid flow rates. First of all, I wonder why the flow rate was chosen to be so high. Although the authors give a few references the chosen rate is clearly above the average flow rate usually observed in cold seep environments

AC: The flow rate was chosen to be that high a) to obtain an efficiency measure at a high end member of flow velocities, as estimated for the Haakon Mosby mud volcano, and at the bubbling Gullfaks site and b) to avoid gradients in methane and sulfate in the columns, to be able to allow for replicate sampling in each column in a follow up experiment (Wegener et al. 2008), so that the whole passage time is equal to the contact time (36 h). The link between flow velocities and methane oxidation rates as well as efficiencies of the microbial filter in our experiment vs. other sites is now clarified (introduction; line 80 to 88)

(p. 3074, l. 18-19). Secondly, in natural seep environments methane and sulfate are coming from different reservoirs and only react in a narrow sediment layer close to the sediment surface. If methane passes this horizon (because of high flow rates) it usually reaches the seawater. Knowing methane fluxes below and above the AOM zone then allows the calculation of a methane oxidation rate (as well as their efficiency). In case

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of the experiment fluid flow rate determines the reaction time depending on the length of the sediment column. I find it very difficult to transfer this situation to the natural environment. How can the contact time between reservoir water (methane enriched artificial seawater) and sediment be related to the residence time of methane in an AOM zone (of x-cm thickness) at given upward directed flow velocity. This seems to me to be the only way to make the results of this study being comparable to results obtained from field observations. Although flow rates may be very high (as commented above) the choice of the experimental setup seems to be not appropriate to derive quantitative aspects on AOM efficiency directly related to fluid flow. In order to determine rate constants for quantitative use of the experiments it would be good to see how the systems reacts as a function of temperature. Did the authors think about modifying their experiments in this respect or is there any preliminary data available?

AC: There are many natural seeps where the contact zone of methane and sulfate at concentrations of a few mM is 5-15 cm thick, e.g. at the gassy Gullfaks sands, in the Guaymas sedimentary vents, the E. Med mud volcanoes, the Costa Rica seeps and at Hydrate Ridge in the Calyptogena beds. It is not well understood which processes lead to the reflux of sulfate &#8211; but it was speculated that bottom water inflow cells can develop by the degassing of sediments, or that the bioturbation and bioirrigation by megafauna can cause the sulfate supply. We have clarified this in the text (line 65 ff). .

Page 3076, line 23 ff: I think that the results of the short column experiments should be documented the same way as in fig. 2 in order to enable comparability of the results. In addition, it would be helpful to find compilations of results (rates, concentrations) in tables rather than being listed in the text.

AC: A compilation of the results from the different inoculated sediments is given in Table 3

In addition, why not showing comparative data from the field (Page 3077, line 2)

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AC: A table with AOM and SR rates achieved from ex situ measurements and our own data was added (table 2)

Pages 3079-3080: I have doubts that the experiments are suitable to derive half saturation constants. There is much scatter in the data and the range is quite low. At least a more thorough discussion and comparison to other experimental studies would be appropriate.

AC: Real half saturation constants were not provided; we proposed a possible range of apparent half saturation for AOM. This is now revised. Additionally we now outlined methods and advances in achieving better half-saturation constants. For methane 354 ff; for sulfate Line 371 ff))

Specific remarks:

Page 3071, line 13-14: Unfortunately, the biochemical...; this is what frequently can be read in manuscripts related to this topic. I would be very much interested to read a brief overview on what is known / needs to be found out on biochemical pathways and regulating factors of AOM instead of a simple statement for the purpose of supporting research on AOM consortia.

AC: The following text was added &#8220;Metagenomic and proteomic studies support the hypothesis that some pathways in AOM are based on a reversal of methanogenesis, since several key enzymes of the anaerobic methanotrophs are closely related to those of methanogens (Hallam at al., 2004; Krüger et al., 2005; Meyerdierks et al., in press). It was recently shown that the assimilation of carbon by both consortia partners is directly coupled to AOM (Wegener et al., 2008), however the metabolic link between the consortia partners remains unknown.&#8221; (line 57 to 66))

Page 3071, line 26: Flow rates of 0.1-2.5 m/yr span over an order of magnitude. I wonder if this difference shouldn&#8217;t have an effect on methane oxidation efficiency. Is it constantly 50%? AC: This paragraph was revised adding additional references,

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and discussing the differences between methane consumption efficiency and different types of diffusive and advective flow. However, where sulfate-depleted subsurface fluids are transported upwards at high velocity  $> 0.4 \text{ m yr}^{-1}$  AOM becomes sulfate-limited, and the efficiency of the microbial filter shrinks to  $< 25\%$  (Niemann et al. 2006). Flow velocities above  $2.5 \text{ m yr}^{-1}$  of sulfate-depleted geofluids can even prevent the flux of sulfate into the sediment and completely inhibit AOM activity (De Beer et al. 2006), with the consequence of high methane efflux to the hydrosphere. (line 81 to 87f)

Page 3077, line 6: what is a "substantial amount"?

AC: Sentence changed: We can exclude nutrient limitation in our systems since all potentially limiting trace elements were added at sufficient concentrations according to the standard recipe for the cultivation of anaerobes (Widdel and Bak) (line 260 to 263)

Page 3077, line 20-23: I can't detect a total mass balance here. Please be more precise.

AC: a detailed mass balance was added As an additional independent measure, the total sulfide production of the system (Exp. 1a) was calculated from the reservoir concentration  $[(\text{H}_2\text{S}_{\text{end}} - \text{H}_2\text{S}_{\text{start}}) \times \text{total media volume} = 3.2 \text{ mmol}]$ . This matches the decline in the integrated methane concentration  $[(\text{CH}_4_{\text{in}}(n) - \text{CH}_4_{\text{out}}(n)) \times \text{flow rate} \times \text{time} = 3.0 \text{ mmol methane oxidized}]$ , when taking into account the above described offset between sulfide production and methane oxidation (line 243 to 248))

Page 3077, line 25-27: It would be nice to see the reproducibility of the experiments. Why only showing one data set? Why not all, the average, etc.. Which column length has been used here?

AC: A table with the results from all experiments was added (table 1)

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Page 3079, line 13: What is meant by an adjustment time and what is meant by more than 8 days? How many days exactly? Is it different for both replicates? Again, which column length has been used? Be more precise! Please provide a table with all experiments and setting details (as mentioned above).

AC: Adjustment time; referred to the time the methane and sulfate flux was kept at one level, before the next change in concentrations. We have rephrased this sentence to be clearer. This was between 8 and 12 days for both replicate; a table specifying conditions and results is now provided.

Page 3079, line 24-25: The offset is clearly more than 20-30% in most cases. The explanation is not sufficient.

AC: The mass balance between total sulfide development in the medium to methane matches by  $\pm 10\%$ , but the individual measurements were unfortunately less precise. We have looked up the composition of the methane, which would support only minor (up to  $0.012 \mu\text{mol gdw}^{-1}$  (calculation line 237ff)) to the budget. The high offset in Exp. 3 is most likely due to contamination of the medium: The high excess of sulfide production (up to the double of methane consumption) in this experiment is most likely due to contamination with alternative organic electron acceptors for sulfate reduction (acetate etc.) after medium exchange. However, this contamination may not affect the methane budgets, which show linear relation between methane concentration and methane consumption in the whole tested methane range;

Page 3079, line 26-27: What is meant with a methane saturation effect here and the related  $K_m$  value? Please provide more extensive information.

AC: the paragraph was revised as follows. Hence we conclude that the apparent  $K_M$ -value for methane in AOM is above the tested range, i.e.  $> 2 \text{ mM}$ . This is in agreement with Nauhaus et al. (2002), who observed a linear relationship between methane oxidation and sulfide production at methane pressures below  $0.1 \text{ MPa}$  (about  $1.5 \text{ mM}$ ), and suggested a high methane  $K_M$  in the range of several  $\text{mM}$  for AOM and

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methane-fueled SR. (line 354 to 360) | Figure 2: Why are H<sub>2</sub>S concentration high from the beginning? Write hydrogen sulfide or H<sub>2</sub>S instead of sulfide (axis label).

AC: High H<sub>2</sub>S-concentrations (~0.5 mM) in the reservoir medium were chosen because we wanted to supply substantial reduction capacity against possible oxygen contamination. Sulfide was changed to H<sub>2</sub>S in the axis

Figure 3: Report H<sub>2</sub>S in mM; ml/l is confusing

AC: Changed as suggested

Minor editorial comments

Page 3070, line 1: A major role in regulation of global...

AC: changed as suggested

Page 3071, line 9: Due to... AC: changed as suggested

Page 3072, line 11: ...not possible to obtain... AC: changed as suggested

,Page 3075, line 22: delete according AC: changed as suggested

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**BGD**

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