

***Interactive comment on* “Constraints on mechanisms and rates of anaerobic oxidation of methane by microbial consortia: process-based modeling of ANME-2 archaea and sulfate reducing bacteria interactions” by B. Orcutt and C. Meile**

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Received and published: 17 June 2008

General comments

In this paper, the authors take an interesting modeling approach to examine in detail the role of micro-scale transport on the feasibility that anaerobic oxidation of methane (AOM) can occur through several intermediate species in consortia of anaerobic methanotrophs (ANME) and sulfate reducing bacteria (SRB). The elusive identification of the intermediate electron shuttle has challenged the AOM community for

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quite some time. The chemical intermediates tested are hydrogen, formate and acetate - the 3 most commonly-proposed. A comparison of model and experimental data by Nauhaus et al. (2007) (hereafter Nauhaus et al.) leads to the conclusion that acetate is the most likely candidate. The model is novel and timely, and provides valuable additional information about constraints on AOM in microbial consortia. The manuscript is generally well-prepared and concise but I have some constructive criticisms which I hope will improve the paper. I recommend the MS for publication in Biogeosciences with minor corrections. It is a good piece of work and I look forward to seeing it published.

I can be contacted directly for comments or questions on dale@geo.uu.nl (Andy Dale)

Specific comments

1) The set-up of the model requires more explanation. The continuity equation is described in equation (2), and the computational model domain is shown schematically in Fig. 1 as a single quadrant of a circle. One can thus deduce that the model is described by polar coordinates in 2-D, although this is not explicitly stated in the MS. It would be interesting to know how the choice of the domain area affects the outcome of the model. For example, would it not be simpler and arguably more realistic to assume a completely circular domain? This would eliminate problems in defining the boundary conditions along the straight edges of the quadrant defined (at 0 and 90 degrees). How are these boundaries defined? Does the symmetry of the quadrant allow some sort of wrap-around boundary along the edges? The choice of modeling a single quadrant rather than a whole circle should be explained.

2) The diffusion coefficients (eq 3) are calculated by treating the microbial cells as hard spheres which are impenetrable to solutes. How realistic is this assumption, particularly with regards to the dissolved gases? Presumably, a faster transport of hydrogen by trans-cellular diffusion would allow for higher cell specific rates of AOM.

3) On page 1944, L7-9, the authors state: As the smaller sized consortia are nu-

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merically more abundant in experimental observations (Table 5), the remainder of the simulations focused on the smallest size class (i.e. 3 μm diameter aggregates). However, Table 5 further shows that the 3×10^{-6} m size class, whilst accounting for 75% of the aggregates, represents roughly only 3% of the total cells in the sediment. On the other hand, the 25×10^{-6} m size class, whilst accounting for only 3% of the aggregates, contains 60–70% of the cells. Ignoring the larger aggregates is only valid if it can be shown that the smallest ones are responsible for oxidizing the bulk of the methane. Whilst the model might suggest that this is the case, the authors should show that this is backed-up by experimental data. I guess that the large aggregates are not considered for further modeling for expediency, the authors hint that AOM does not occur all the way inside these large consortia under the assumptions of their model. I think this an important result which should be given more emphasis since it provides a broader framing of the performance and applicability of the model. Why not add an extra column in Table 5 showing the size-class model rate of AOM? I think the MS would greatly benefit from an extra figure explaining these results in more detail, perhaps using color images of the model domain showing the spatial extent of AOM and SR under a range of maximum cell specific rates, minimum energies etc?

4) The authors conclude that acetate is the likely intermediate (from the 3 tested) which can support the measured rates of AOM in experiments by Nauhaus et al. Is this result consistent with compound-specific isotope data reported in the literature? This is a fast-moving field, and plenty of isotope labeling studies provide compelling evidence that methane-derived carbon is assimilated directly into ANME biomass, but I am not immediately aware of any studies where this is also true for the SRB. In fact, based on lipid ^{13}C isotope analysis, Wegener et al. (2008) suggest that acetate and formate are not intermediates in these types of consortia. These discrepancies deserve discussion.

5) In continuation, the authors arrive at their conclusion that acetate is the most probable intermediate based on a comparison of the Nauhaus et al. maximum rate (230 $\mu\text{mol g dry_sediment}^{-1} \text{ d}^{-1}$) and the higher model-derived rate for acetate at a

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methane concentration of 19 mM (85, 12 and 13 micromol gwet_sediment-1 d-1, for acetate, formate and hydrogen, respectively [page 1949, line 2]). All 3 model derived rates are an order of magnitude lower than the observed rate, which could be construed as an indicator that none of the 3 species is the intermediate. Given the fact that much of the ANME deep inside the larger consortia are probably not oxidizing methane (this is what I suspect), it seems that their conclusion may only be somewhat true for the smaller consortia, which does not explain how the consortia can grow to such large sizes. The comparison of the model results with the experimental results of Nauhaus et al. should be examined more objectively.

6) There appears some confusion between the units in the MS; (1) page 1936 line 28 and page 1937 line 9 the authors assess the process rates per aggregate. (2) The AOM rate in Fig. 2 is given in nmol CH₄ gdw⁻¹ d⁻¹. (3) The AOM rate in Fig. 3 and Fig. 4 are given in nmol CH₄ cm⁻³ d⁻¹ (per cm³ of what?). (4) In section 3.3 and Table 5, AOM is reported in units of nmol CH₄ per gram of wet sediment (gws) d⁻¹, whilst referring to Figs. 3 & 4 which have different units. (5) There is a mismatch between the reported AOM rates (gws per day) and those of Nauhaus et al. which are originally reported in grams dry sediment per day, not wet sediment. If my calculations are correct, a correction factor of around 2 to 5 must be applied to convert this rate to a wet sediment basis, for a porosity of 0.7 and 0.9, respectively. It would help the reader to report the relevant porosities, densities and unit conversions between the systems in a small table or else in the methodology section.

7) Fig. 3 (and 4) is quite complex, and more care is needed to introduce this figure and integrate it in the Discussion. For example, where is there so much complexity to the size and distribution of the circles (AOM rates), with small circles apparently intermingled with larger ones? In Section 3.2 (page 1945, line 11-14) I do not see the corresponding maximum specific rates of SR on Fig. 3 or 4. I also find it hard to follow the logic or the figure caption for Fig. 3k and the transition from light to dark circles. Why should the zone of SR collapse because the cell specific SR rate is too high? On

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line 20 (page 1946) the authors state that this is because the zone where substrate for SR is available becomes smaller than a cell diameter, which is considered a lower limit. Please clarify what exactly is meant here.

8) Page 1947, line 6-26. The model indicates that the AOM rate and the maximum cell specific AOM rates are insensitive to methane concentration and Gibbs energy threshold. No explanation is provided for this remarkable result.

Minor comments/suggestions (P = page, L = line)

P1943, L1-4: Please rephrase what is meant by: the reaction zone collapses to the thickness of a cell.

P1944, L11-12: A reference is needed for this sentence.

Figure 3 shows results for various methane concentrations. Please state explicitly in the figure caption that these methane concentrations correspond to the boundary condition.

References

Nauhaus, K., Albrecht, M., Elvert, M., Boetius, A., & Widdel, F. In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. *Environmental Microbiology* 9, 187-196. 2007.

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Interactive comment on Biogeosciences Discuss., 5, 1933, 2008.

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