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Biogeosciences Discussions

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

B. Orcutt and C. Meile

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We would like to thank all 3 reviewers for their thoughtful and engaging comments which helped improve the manuscript. Aside from the necessary clarifications we have modified the presentation of Figures 3 and 4 in order to make the information contained in them more accessible (see new Figure 3). The most significant changes were made in the comparison between model simulations and the laboratory data from Nauhaus et al., by moving away from an extreme endmember comparison to a more realistic one that takes into account aggregate size classes. This was done by adding a new figure (Figure 4) which replaces and expands upon the data originally contained in Table 6

(which has been removed). We now more clearly argue that while acetate is the most favorable candidate of the 3 metabolites investigated, none of them is likely to be used as intermediate. Our detailed responses to each point raised by the reviewers follow below.

Reviewer #1 (Andy Dale)

1) model setup - Reply: The physical model domain is spherical, but computationally is reflected by a quadrant of a circle by exploiting symmetries. Using symmetries, we had indeed captured the spherical 3D nature of the problem - a key feature because of the change in volume with respect to the radial coordinate, whose importance is correctly implied in the reviewer's comment. To avoid possible misunderstanding, we have clarified the description of the model domain. Instead of "Making use of symmetries, the computational domain is defined as a quadrant of a circle, which when rotated about a vertical axis and mirrored horizontally approximates the spherical physical domain.", it now reads: "In the computational model, the physical three-dimensional spherical setting is represented by a quadrant of a circle (Fig. 1) and by imposing rotational symmetry at the vertical coordinate axis, and mirroring the resulting half sphere on the horizontal plane."

2) transport through cells - Reply: We agree that differences exist in the permeability of cells to the metabolites considered, but did not address the issue at the cellular level for the following reasons. First, diffusional transport is only one way metabolites can enter the cell and it can be complemented by a variety of active - but in our particular setting largely unknown - uptake mechanisms. Second, we are not resolving intracellular concentration levels, and hence would have to base flux estimates on guesses of concentration gradients. Given the metabolites' use as substrates to central metabolic cycles, we also suspect that even if differentially permeable walls were explicitly accounted for, a correction for that effect on transport across cells would be masked by source/sink terms in the cells themselves. Because of these reasons, we decided not to include an additional and poorly constrained adjustment factor in the model descrip-

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tion (see also comment 1 of reviewer 3). However, we agree that there is significant uncertainty in transport parameters at the scale of our study. To address that, we examined the impact of some of our assumptions regarding the diffusion coefficient. We focused on the extracellular medium by considering the differential effect of EPS on the diffusion coefficients of dissolved gases and charged substances. Removing the influence of EPS on diffusion, however, did not significantly impact the calculated activity of the consortia (see first paragraph of Section 3.1). Thus, we address the valid reviewer comment regarding the uncertainties in transport coefficients and their differences between metabolites in an extracellular context that we feel is better constrained and show that the effect is second order compared to other factors studied. To clarify the issue, we added the following to the text when introducing the effective diffusion coefficient: "Without explicit knowledge of intracellular metabolite levels and cross-membrane transport, metabolite transport in the model is restricted to the extracellular aqueous phase."

3) comparison with Nauhaus experiment - Reply: In the original manuscript, we focused on maximum endmember scenarios, assuming that all cells were operating at the rate observed for the smallest sized aggregates, and formulated our conclusions based on these rates. As this has clear limitations in the comparison to the experimental work in which a wide range of aggregate sizes has been observed, we decided to follow the reviewer's suggestion and provide rate estimates for a mixed-size community, which represents a more realistic comparison. As a consequence, we significantly revised section 3.3. Specifically, we now show simulation results that reflect the observed aggregate size-class distribution (see new Figure 4). The results show that none of the intermediates considered leads to volumetric rates that approach the observed ones, a finding now clearly stated in both section 3.3 and the conclusions (see also reply to next item). To make the manuscript more accessible, we have also augmented Figure 1 with a visual illustration of the variation in the thermodynamic factor and the distribution of the exchangeable species.

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4) acetate as intermediate - Reply: Given the de-emphasis of the previous endmember bulk rates (see above), we have significantly revised this section to point out that all three modeled intermediates do not match the rates measured in the Nauhaus experiment. With the modeled rates lower than the observed ones, our results are qualitatively consistent with the findings presented by Wegener et al. 2008 in the Environmental Microbiology September issue. We have revised section 3.3 and the conclusions accordingly.

5) most likely intermediate - Reply: Our revisions (see reply to #3 and 4 above) clarify the likelihood for the 3 metabolites to be the intermediate species and lead to a more objective comparison to the results of the Nauhaus experiment. The new figure 4 replacing Table 6 shows the corresponding model derived estimates for the relative contribution of the different size-classes to the overall bulk rate. Model simulations indeed suggest that thermodynamic limitations can limit the activity, and that this limitation is more pronounced as the aggregate grows in size. However, independent experimental data will be needed to settle the question of the extent of spatial variability of metabolic activity in the aggregate. While the model suggests that for the 3 intermediates investigated higher bulk rates are obtainable with rate parameters that lead to partial thermodynamic limitations, we cannot conclude that methane oxidation is indeed limited to a thin layer at the outside of the ANME subdomain. However, data elucidating the spatial activity pattern (e.g. by using nanoSIMS nitrogen and carbon incorporation data) in a well-characterized setting will provide useful constraints on reaction rate constants.

6) units - Reply: fixed

7) complex Figures 3 and 4 - Reply: In the light of this comment we decided to present Figures 3 and 4 in a different way, that better illustrates the dependence of the aggregates oxidation rate on methane concentrations and the energetic threshold value. Specifically, we condense the many simulations shown as individual circles in the original Figs 3 and 4 into a single figure. In this new Figure 3, we now show the

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maximum rates that can be achieved for a given methane concentration and value of m*deltaGATP, which vastly simplifies the presentation without losing much of the original substance. Note that since AOM rates tend to plateau with increasing kSR (at a given KmEX-value; see Figure 2 and discussion below), we only show the results for a sufficiently high kSR (at a value of kAOM that leads to maximum rates) rather than for a range of kSR values. Aside from making the presentation more accessible, moving away from showing the dependence of rates on parameters was also motivated by the fact that several parameters can balance each other out (e.g. kSR and KmEX, which resulted in the diagonal pattern in the large circles in the original figure 3), so that their individual value is not a unique identifying feature. Figure 4 now summarizes the model results in a way that matches the Nauhaus experiments closely. We also clarify the statement regarding upper limits of rate constants, which we associated with a collapse of the spatial extent of the zone of reaction (both AOM and SR). In the first paragraph in section 3, we now state that: "In the absence of rigorous constraints on reaction rate constants, we only consider model parameterizations that allow for AOM in a section extending beyond one cell diameter away from the SRB subdomain, and for SR within more than one cell diameter distance from the ANME zone, so that results characterized by drastic changes at or below the scale of individual cells are not included in the analysis."

8) insensitivity to methane - Reply: This statement is a slight oversimplification of our findings. The maximum cell specific rate of AOM for acetate is stated to be sensitive to methane concentration, and the resulting rate of AOM also reflects this sensitivity. In the case of hydrogen, the value of the maximum cell specific rate leading to the maximum RAOM (at a given KmCH4, KmEX and sufficiently large kSR) was found to be insensitive to the methane concentrations examined. The methane dependence through the kinetic Monod term is indeed minimal, because we assumed the KmCH4 to be small compared to the environmental concentration in all cases, and because there is only very little methane drawdown in the aggregate itself. However, the above findings can be explained by differences in diffusion coefficients and reaction stoichiometries,

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and their impact on the reaction energetics, in a similar argument as presented in the third paragraph of Section 3.2. At the relatively low maximum kAOM value possible for hydrogen at low methane concentrations, the relatively higher diffusion coefficient for hydrogen allows hydrogen to be removed from the ANME core fast enough to maintain a relatively high FTAOM, which explains why hydrogen can support a higher rate of AOM than formate at low methane, as shown in the new Figure 3A. As the concentration of methane is increased, our simulations showed that the cell specific rate of AOM can increase for acetate, due to improved thermodynamics by higher methane concentrations, but that was not the case for formate and hydrogen. This difference can be explained by the stoichiometries of the reactions, where one acetate molecule is produced per methane consumed versus four formate or hydrogen molecules. Thus, at higher cell specific rates of AOM, too much hydrogen or formate is generated and the inner core of the ANME experiences thermodynamic limitation, counterbalancing the favorable effect of increased methane concentrations. Since the rate of AOM is a function of the cell specific rate of AOM (which remains constant), the thermodynamic factor (which approaches one in these maximum rate scenarios), and the Monod term for methane (which is also nearly one since there is little variation in the methane concentration), the rate of AOM from hydrogen remains nearly constant across the range of methane concentrations considered. We now expand on this topic in the second to last paragraph in Section 3.2.

P1943, L1-4; P1944, L11-12; caption Fig.3: all fixed

Reviewer #2 (Bernie Boudreau)

1) aggregate growth - Reply: Growth dynamics are indeed not considered explicitly in the model. The motivation and justification for neglecting this aspect lies in the slow growth rates. Doubling times of 6 months have been observed in the Nauhaus experiments (Nauhaus et al., 2007), likely exceeding those applicable to our model as the computed metabolic rates are substantially lower than those observed. Doubling the number of cells would lead to roughly 1.26 larger aggregate radius, and an increase

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by one order of magnitude slightly more than doubles the aggregate radius. These changes are smaller than the differences in size classes we study, suggesting that this is of minor importance in our application. Hence, we did not assess the importance of growth explicitly - as pointed out correctly, this would require significant changes in the modeling approach - but in the revised manuscript, we now explicitly state that we do not consider growth when comparing model results to the Nauhaus experiments (last sentence added to section 2.1): "Due to slow observed growth rates, with doubling times on the order of 6 months (Nauhaus et al., 2007), the model does not take into account potential changes in aggregate size due to growth."

2) The consortia are assumed to be isolated from other aggregates, without any real analysis of this assumption. - Reply: The environmental context into which the aggregates are placed impacts their functioning, and we agree that the placement of aggregates with respect to their neighbors is one such environmental aspect. In our model, we set the model domain at approx. 5 times the aggregate radius away from the aggregate surface, which results in aggregate densities consistent with observations. Because of the uncertainty and variability of aggregate spacing, we had tested the sensitivity of our results towards the spacing and found that unless the aggregate are in very close proximity - a distance less than an aggregate size - the results don't show a strong sensitivity to this variable. While we understand the reviewer's concern, we feel that the brief explanations given in the manuscript (minimum spacing given on 1937/26, and the mentioning of our findings on 1944/10-18 of the original submission) address this issue sufficiently.

3) half saturation constants - Reply: In the original manuscript, Figures 3 and 4 showed the rates of AOM activity across a wide range of ratios of the exchangeable species half saturation constant (KmEX) to the average steady state concentration of the exchangeable species (EX) in the model domain. While this ratio does not show the magnitude of the half saturation constant relative to the concentration of the exchangeable species in the aggregate itself (which is relevant for the reaction), we verified that

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the values tested span the 0 and 1st order kinetics domain of Monod kinetics. As a consequence of making figures 3 and 4 more accessible, and by focusing on maximum rates, we no longer detail the variation of the rates with the KmEX/EX ratio. While our results indicated that RAOM was sensitive to the KmEX:EX ratio, high reaction rates could be achieved at most KmEX:EX ratios, by simultaneously adjusting the value of the cell specific rate of SR (kSR), as was evident from the original Figure 3. In the revised version, we show simulations for KmEX of 100nM, but state now explicitly in the text (section 3.2) that "similar maximum rates were achieved at higher (up to 1 mM) and lower (down to 0.1 nM) KmEX values by varying the cell specific rate of sulfate reduction (kSR). Similarly, varying kAOM allowed balancing the effect of changing KmCH4, which – as KmEX – is poorly constrained." Thus, we concede that the values we have chosen for half saturation constants are our best guesses in the absence of empirical data, but stress that our results suggest that for the range of methane concentrations and intermediates considered, the build up of EX is a more significant driver than methane concentrations, and that similar maximum rates can be obtained for a range of Km values in combination with the appropriate k-values.

4) rate laws - Reply: We chose a Monod dependency for SRB on EX and performed simulations that ranged from effectively 1st order (RSR ˜ kSR/KmEx * EX for KmEX$>>$EX) to 0th order (RSR ˜ kSR for KmEX$<<$EX), which encompasses the well documented case highlighted in the review (see reply to 3 above). The Km value for sulfate was taken from Dale et al., which is based on experimental studies, and to our knowledge there is no evidence that would point to a significantly better choice. With the effect of the choice of KmEX related to fitting kSR, and KmSO4 reasonably well constrained, we set KmCH4 to be an order of magnitude lower than the environmental concentration, so that methane is not limiting. As our simulations show little methane drawdown, we found only weak spatial variation in methane. In such a setting, the effect of changes in KmCH4 on RAOM can be balanced by increasing kAOM which is now explicitly mentioned in the revised version (see above reply).

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5) thermodynamic potential factor - Reply: We agree with the reviewer that the formalism adopted in this manuscript has shortcomings in that it does not do justice to the complexity of the microbial machinery. However, it does add to the 'classic' kinetic-only description in that it approximates real energetic constraints. We agree that a cell may allocate resources such as to optimize its overall 'well being'. However, as resources become scarce, the flexibility to do so lessens. While by no means meant as a stringent argument, our deliberate choice of a very low minimum deltaG can be seen as reflecting a cell's flexibility. Thus, we do concede the point made but argue that it does not affect our work significantly, in particular if a cell is performing at the energetic edge. Because we find this issue to be outside the scope of our paper, we did not change the manuscript, even though we agree on the substance.

Reviewer #3 (Marc Alperin)

1. impermeable cells - See reply to comment #2 by Reviewer 1

2. Threshold free-energy for energy conservation. - Reply: We agree that our lowest choice for the threshold free-energy is unlikely to be relevant in the field, but find it useful in the formulation of endmember scenarios aiming at bracketing the potential of the 3 metabolites as intermediates. To account for the valid comment, we now explicitly state towards the end of section 2.3 that "In our model, a range of m*deltaGATP values from 1-10 kJ/mol H+ is considered. The lower end of this range in particular represents an end-member setting with minimal energetic constraints on cell functioning."

3. What is the exchangeable species? - Reply: Given the de-emphasis of the previous endmember bulk rates (see above), we have significantly revised this section to point out that all three modeled intermediates do not match the rates measured in the Nauhaus experiment. As we limit our manuscript to the comparison with the laboratory experiment which provides data, we decided against discussing the model results for the wide range of in situ environmental concentrations of acetate or other compounds at Hydrate Ridge or other cold seep systems where ANME-2 consortia have been

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found.

4. Energetics become less favorable as the consortia grow in size. - Reply: As discussed in our response to Reviewer 1's third comment, we now provide rate estimates from a mixed-size community which more closely represents the data in the Nauhaus experiment. As shown in section 3.3 and in our new Figure 4, at low cell specific rates of AOM activity, the larger sized consortia contribute the most to the calculated bulk rates of AOM due the larger number of ANME cells in these aggregates. As the cell specific rate of AOM increases, the concentration of the exchangeable species within the inner core of the large aggregates becomes too high and leads to thermodynamic limitation. Thus, at high cell specific rates of AOM, only the smaller sized aggregates can contribute to the bulk rate of AOM. While it is true that the larger sized aggregates appeared to have increased relatively more than the smaller aggregates in the Nauhaus experiment, as shown in Table 5, we chose not to comment on this because we do not account for aggregate growth in our model and our estimates of bulk rates are far lower than those measured in the Nauhaus experiment, making a comparison tenuous.

Editorial suggestions:

1935/6, 1938/2, 1946/22, 1949/9: reworded; 1945/12: changed to m*deltaG_ATP where appropriate; 1947/13 (sensitivity to methane): See response to comment #8 by Reviewer 1; 1949/6 (comparison to experimental data): This section has been significantly revised to point out that all intermediates produce rates of AOM that are much lower than those observed in the Nauhaus experiment.

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