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***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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The archaea-SRB aggregates found in methane-seep sediments remain somewhat of a mystery. What processes are operating within these aggregates? How is it possible that the rate of syntrophic sulfate-reduction/methane-oxidation (SR/MO) – which is severely limited by thermodynamic and physical constraints – is among the highest sulfate reduction rates measured in marine sediments?

Orcutt and Meile present an aggregate-scale reaction-transport model to investigate

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the thermodynamic feasibility and kinetics of coupled SR/MO. Their main conclusions are: (1) syntrophic SR/MO is thermodynamically and physically possible for a variety of intermediates; (2) methane oxidation becomes less energetically favorable as the aggregate increases in size; (3) interspecies acetate transfer allows for the highest rate of syntrophic SR/MO; (4) methane oxidation is thermodynamically inhibited (and methanogenesis is energetically favored) if bulk concentrations of hydrogen, acetate, or formate exceed 100 nM, 0.1 μM , and 0.3 μM , respectively; and (5) syntrophic SR/MO via interspecies acetate transfer might account for rates of sulfate reduction measured in a laboratory enrichment experiment (Nauhaus et al. 2007). The first four conclusions are well-supported (although the implications of (4) are not fully developed). Conclusion (5) may be overstated: the highest model-derived sulfate-reduction rate (85 $\mu\text{mol gws}^{-1} \text{d}^{-1}$) is only 1/3 of the measured rate (250 $\mu\text{mol gws}^{-1} \text{d}^{-1}$), and assumes that microorganisms are able to utilize (conserve) an energy yield of only 1 kJ mol^{-1} .

This model is a logical advance from the 1-dimensional model presented in Sorensen et al. (2001) and represents a significant contribution. However, there are a few points that the authors should address prior to publication.

1. Modeling of diffusive transport of dissolved species from the bulk fluid into the aggregate.

Setting the porosity within the aggregate to 0.3 (the interstitial space between closely-packed cells) implies that cells are impermeable to diffusing solutes. That is, the model assumes (unrealistically) that diffusing solutes travel a tortuous path around the SRB and archaea cells.

Is it proper to treat all dissolved species the same with respect to transport across the cell membrane? Small lipophilic molecules such as hydrogen and methane readily diffuse across the cell membrane whereas ions such as formate and acetate cannot, and must rely on membrane channels for uptake. I suggest that the authors add another factor to equation (3) to account for differential transport across the semi-permeable

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cell membrane.

2. Threshold free-energy for energy conservation.

The threshold ΔG value (1 kJ/mol, used to demonstrate that the model-derived rate of syntrophic SR/MO can be as high as 1/3 the observed rate in the laboratory enrichment) is well below observed and predicted threshold energy values (see Hoehler, 2004; Geobiology, 2:205-215). Cells functioning at such low energy yields have very slow growth rates. For example, if the average ΔG for methane oxidation to acetate within the archaeal cell is -2 kJ mol⁻¹ (2x the threshold value) and the optimal cell-specific rate of methane oxidation is 0.1 fmol cell⁻¹ d⁻¹ (pg 1948, line 27), the cell doubling time is estimated as follows:

$(2 \text{ kJ mol}^{-1})(0.1 \times 10^{-15} \text{ mol cell}^{-1} \text{ d}^{-1})(\text{mol ATP}/60 \text{ kJ})(10 \text{ g biomass/mol ATP}) = 3.3 \times 10^{-17} \text{ g biomass cell}^{-1} \text{ d}^{-1}$; if the cells weigh $4.3 \times 10^{-14} \text{ g}$, the biomass doubling time is 3.5 years.

The cell-doubling time measured for the laboratory enrichment is 7 months.

3. What is the exchangeable species?

The authors conclude that syntrophic SR/MO with hydrogen or formate as intermediate is not fast enough to support measured rates of sulfate reduction, but that acetate exchange might support measured rates. The model also predicts that methane oxidation is not thermodynamically favorable if environmental concentrations of acetate exceed 0.1 μM . Acetate concentrations were not reported for the laboratory enrichment experiment, but have been measured in Hydrate Ridge sediments from which the enrichment cultures were isolated. Valentine et al. (2005; J. Geophys. Res., 110) measured porewater acetate concentrations of $\sim 100 \mu\text{M}$, suggesting that syntrophic SR/MO via interspecies acetate transfer is not thermodynamically feasible in Hydrate Ridge sediment. This merits discussion.

4. Energetics become less favorable as the consortia grow in size.

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The authors focus their simulations on the smallest size class (3 μm diameter) since these are the most abundant in the laboratory enrichment. But the more interesting size class is the "mega-aggregates"; (25 μm od) containing 140,000 cells per aggregate (Table 2). Most of the exponential growth in biomass occurred in these large aggregates (Table 5), yet the model predicts that the energetics of methane oxidation becomes less favorable as the aggregates grow. This also merits discussion.

Editorial suggestions:

1935:6 - the DNA- and lipid-based investigations do not necessarily indicate that consumption of sulfate and methane is mediated via a syntrophic relationship between SRB and archaea. Rather, the DNA-based studies reveal aggregates composed of methanogen-like archaea and SRB, while the lipid-based studies show that archaea (and SRB to a lesser extent) contain cell carbon that is highly depleted in ^{13}C . A single organism capable of non-syntrophic SR coupled to MO cannot be ruled out on the basis of the DNA and lipid data.

1938:28 - "... the concentrations are imposed to reflect those measured in field samples (Table 3)." I think the authors mean "... reflect those measured during the in vitro experiment."

1945: 12 - The definition of ΔG_{ATP} is not consistent. On page 1941, the product of m and ΔG_{ATP} is used to refer to the threshold free energy change; the m is dropped in later references.

1946: 22 - change "of" to "on".

1947: 13 - The text states that the cell-specific rate of methane oxidation for inter-species hydrogen transfer not affected by changes in methane concentration. Why is this so?

1949: 6 - "modeled rates are slightly lower than those observed." Change to &"modeled rates are a factor of three lower than those observed."

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1949: 9 - "Experimental data allows ... ". Change to "Experimental data allow ...".

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