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Biogeosciences Discussions

Interactive comment on "Integrative analysis of the interactions between *Geobacter* spp. and sulfate-reducing bacteria during uranium bioremediation" *by* M. Barlett et al.

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A. Is the title appropriate given the findings? The introduction states there is little direct interaction between Geobacter and SRB populations. Perhaps "Integrative modeling of Geobacter and SRB's during uranium bioremediation" would be more accurate.

- We agree with the reviewer's comment. We have changed the title of the manuscript to "Integrative analysis of the Geobacter *spp.* and Sulfate-Reducing Bacteria during uranium bioremediation".

B. In figure 1A there are 2 peaks in cell numbers for Geobacter and SRBs during the

first acetate addition. One peak is early in the experimental treatment (day 15) and corresponds to the increased acetate supply and decline. However, the second peak in cell numbers is around day 50 when most of the acetate has been utilized. Do the authors have any idea what may have caused this spike in SRBs and to a lesser extent the increase in Geobacter? What electron donor may be present in groundwater or their experimental system to account for this increase? It does not appear to be acetate.

- It's true that there is no clear increase in the acetate or any other measured electron donors that would account for the spike in cell numbers seen at the 50 day mark.
- There is a lot of heterogeneity in bacterial distributions, even within the sediment bottles, and, although bottles were mixed prior to sampling, it is likely that a pocket of high cellular density was picked up in the sampling process, thus producing a spike in numbers for both Geobacter and SRB. The numbers drop back into the expected patterns as of the next sampling date.
- We have added a paragraph discussing this point at the end of Section 3.1.

C. In figure 2, the chemical models seem to do a very good job reproducing the concentrations of acetate, sulfate, and Fe II to a lesser extent. However, the cell abundance models are not as robust, showing results that overestimate the Geobacter fraction initially and then underestimate the population. The SRB model seems to miss the first 2 peaks in cell abundance completely and only captures the final peak. Conversely, the Geobacter model is only sensitive to the first peak in cell numbers and does not catch the last 2 peaks.

- As mentioned in our response to the previous comment, the sediment bottle is highly heterogeneous; this heterogeneity likely contributed to the discrepancy between the model predictions and the experimental measurements.

- In addition, it is well known there are multiple Geobacter species as well as multiple SRB species in the Rifle sediment. We used the metabolic model of Geobacter sulfurreducens to represent all Geobacter species, and we used a stoichiometric model of SRB based on Desulfobacter postgatei data is used to represent all SRBs.
  - Since two representative models were used to simulate an environment containing a variety of Geobacter and SRBs, we were only able to predict the larger trends, but not the fine details.
  - In particular, it is known that Desulfobacter postgatei itself is not one of the SRB species in Rifle, though there are a number of similar and likely related species (Miletto et al. 2011). However, we were forced to base our model on the Desulfobacter postgatei kinetics because the SRB species in the Rifle sediment have not yet been isolated.
  - Given the circumstance described above, it is particularly difficult to predict the exact cell numbers because different strains of the same organism grow at different rates, have different yields, and respond to slightly different environmental cues.
  - However, we acknowledge the reviewers observations and have highlighted these points in a paragraph added to the end of Section 3.1.

D. In figure 3, the model results indicate the acetate concentration does not seem to change in the presence of 100% Geobacter, suggesting the entire population of Geobacter is being supported by $>$ 1 mM acetate. Is this a reasonable prediction for 10^9 cells per ml?

- The reviewer is correct. This is a typo.
- It should have been 10^9 cells per liter or 10^6 cells/ml.

E. Other studies at the Rifle site indicate there are multiple microorganisms that take up acetate beyond Geobacter and SRBs. These microbes would not be assayed by the in situ fluorescence methods being used. Could these other acetate utilizers explain the lower than expected Geobacter fraction in the early stages of the acetate amendment?

- It is true that there are other acetate users that may be active right at the beginning of the acetate injection, and could indeed explain the discrepancies between the model and the experimental data. However, characterizing all the acetate utilizers requires additional microbial physiology studies including isolation, sequencing, and genome-scale modeling studies to more clearly answer this question. Nonetheless, we included a discussion of this topic in the last paragraph of Section 3.1.
- There are other possible causes for the discrepancies: [1] The system is highly heterogeneous, introducing errors in our sampling. [2] Two representative models were used to represent a complex community containing multiple Geobacter species and multiple SRB species, making it difficult to predict the fine details.
- Specifically regarding the early over-prediction of Geobacter fraction: [1] The prediction of the Geobacter cell numbers is quite good (Fig 2C). On the other hand, the model missed the first peak of the SRB at day 20 (Figure 2B). Therefore, the SRB made the most impact on the different in the Geobacter fraction. [2] This discrepancy in the SRB dynamics is most likely due to the fact we used a simple stoichiometric model of SRB based on Desulfobacter postgatei, as we discussed above.

F. Prior research using 57-Fe-goethite amendments with Rifle sediments also indicated the iron reducers and sulfate reducers are simultaneously active when acetate is added in millimolar concentrations. However in that study, the goethite was utilized in the beginning of the acetate addition casting some doubt on the "Difficult to use Fe" vs. the

"Easy to use Fe" concept. Additionally, the goethite had little overall effect on Geobacter populations. This discrepancy in findings between the two studies could result from the different concentrations of the iron amendments. Can the authors comment?

- The reviewer brings up a most excellent point. We have just submitted another manuscript that addresses this issue to the journal Biotechnology and Bioengineering.
- Without digressing too much from this manuscript, we will give a brief summary of our results that will hopefully answers the reviewer's question:
  - In the present work, Fe(III) oxide was added after Fe(III) was depleted, which lead to a temporary rescue of Geobacter growth. (Figure 4, 5 in the present manuscript). Since the resuscitation took place after a period of Geobacter inactivity and decay, the effect of the Fe(III) addition was very obvious.
  - On the other hand, Moon et al. added Fe-Geothite at the beginning of their experiment. This will cause Geobacter to grow slightly faster in the initial phase, but the effect on the population would not be so obvious, especially not on a log-scale graph.
  - Since only a batch addition of Fe(III) is used, the added Fe(III) is used up in both cases and does not significantly prolong uranium reduction. In fact, our new simulations shows that adding Fe(III) early on can hasten the depletion of Fe(III), having a negative effect on uranium reduction. (Zhuang et al. Submitted) This also explains why the added Fe-geothite is used up early in the Moon experiment (See Supplementary Figure 4).
  - In order to maintain long-term uranium bioremediation effect, a continuous addition of Fe(III) is required. Simulation shows that either Fe(III) oxide (easy-to-use) or goethite (hard-to-use) can work fine. (Zhuang et al. Submitted)

Finally, efforts to model the response of microbial communities to field perturbations are tremendously important for predicting how bacterial populations react to bioremediation efforts. Ultimately, these discrepancies between field data and model results will help to elucidate where the models are inadequate and eventually lead to an improved understanding of how the microbiota respond to changing environments.

- We agree with the reviewer whole-heartedly and have indicated this point in end of Section 3.1.

References

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