**Response to Reviewers**

Ms. Ref. No.:  W8822

*Distributed microbially- and chemically-mediated redox processes controlling arsenic dynamics within Mn-/Fe-oxide constructed aggregates*

Samantha C. Ying, Yoko Masue-Slowey, Benjamin D. Kocar, Sarah D. Griffis, Samuel Webbb, Matthew A. Marcus, Christopher A. Francis, and Scott Fendorf

For Geochimica et Cosmochimica Acta

**EDITOR**

**\*\*SCOTT the editor's comments are just summaries of what the reviewers have to say, do we just cut and paste our responses form the reviewers here? Or just group all these comments together and say that we've addressed them and tell him to "refer to below responses"? THE LATTER. YOU DO NOT NEED TO PROVIDE AN ITEMIZED RESPONSE TO THE AE IE IF HE/SHE IS JUST REPEATING THE REVIEWS. YOU SHOULD JUST SAY THAT WE HAVE RESPONDED TO ALL OF THE REVIEWER'S COMMENTS. SPECIFICALLY MENTION THAT THE POINTS FROM THE AE WERE ADDRESSED.**

\*\* Write something here about how all the points from the AE were addressed

**REVIEWER 1**

**Comment 1-1**

In general, the manuscript leaves the impression of having been submitted in immature state and

that more time should have been spent on data interpretation and manuscript writing/polishing.

In many places, the text is hard to read. Information is packed into overly long sentences, where

beginning and end seem not always to fit logically or grammatically.

**Response 1-1 DONE**

We have re-written portions of the manuscript following the specific comments provided by the reviewers as described below.

**Changes 1-1**

Please refer to changes made in response to related comments (below) regarding writing style and editing from both reviewers.

**Comment 1-2**

The format of the bibliographic list is far from GCA requirements.

**Response 1-2**

Agreed.

**Changes 1-2 DONE**

We have edited the bibliography list and corrected all formatting mistakes following GCA guidelines.

**Comment 1-3**

The most critical point in this manuscript is that the authors do not provide a more quantitative

discussion and interpretation of their results based on imposed solution chemistry, mass transport

constraints, and microbial transformation rates (maybe even in a reactive transport model) - and

implications for the generalization of their results to different types of redox-dynamic soils

(gleys, pseudogleys, etc).

**Response 1-3**

We agree that providing more quantitative discussion helps clarify the explanation of chemical mechanisms within the aggregate system. We now calculated electron flow within the reactors using a mass balance and electron balance approach, thus deciphering the amount of lactate used for O2 respiration versus Fe/Mn oxide and As(V) respiration.

**Changes 1-3**

We've added a small discussion describing the electron balance within the reactors using lactate/acetate, O2, Fe(II), Mn(II), and As(III) concentrations over time. Details of the calculations and justifications are provided below based on specific reviewer comments regarding this topic.

**Comment 1-4**

Notably, in all the experiments with aerated solution, the dissolved O2 concentration was not sufficient for degradation of the added lactate (3 mM). Since both O2 and lactate have to diffuse into the aggregates, the experimental setup was thus biased towards the development of reductive conditions in the interior of aggregates, irrespective of whether O2 was present in solution or not. Drastically different system behavior might have been observed at lower (and more realistic?) lactate concentrations, where a larger fraction (or even all) lactate could have been respired aerobically. This point is not discussed at all.

**Response 1-4 DONE**

The objective of the experiment was to examine the redox gradient that is induced by microbial respiration within a physically complex environment. Therefore, we did not "bias" the experimental setup to produce a reducing core within the aggregate; that outcome resulted from the diffusion limited transport of oxygen due to the physical complexity of the aggregate. Furthermore, the Reviewer incorrectly interprets our experimental system; O2 was continuously bubbled into the reactor (i.e., O2 concentration within the solution was constantly saturated throughout the experimental run) whereas lactate is present (along with DO) only in the feed solution. Therefore, it is effectively an infinite source of O2 that could have been utilized by the bacteria in the exterior of the reactor, contradictory to the Reviewer's comment that O2 was not sufficient for degradation of the 3 mM of lactate. Instead, reducing conditions within the aggregate are formed from slow transport of O2 into the aggregate due to small pore size (diffusion limited transport).

**Changes 1-4**

No changes made.

**Comment 1-5**

Also, the authors do not provide any data on the temporal evolution of dissolved O2, lactate and acetate in the effluent from the different reactors (although lactate and acetate measurements are mentioned in the materials section) - data that would be essential for a thorough interpretation of the results. It would also have been helpful to compare the amounts of O2 and lactate consumed in the reactors with the initial amounts of As, Fe and Mn in aggregates, and with the amounts leached from the system during the experiments in order to check for electron balance.

**Response 1-5 DONE (1-3)**

Agreed. Please refer to **Response 1-3**, which addresses the same concern.

**Changes 1-5**

Please refer to **Changes 1-3** provide above.

**Comment 1-7**

As a major finding from this study, the authors highlight the possibility that As accumulation at

the exterior of aggregates during oxic solution conditions may be followed by a pulse As release

when conditions shift to anoxic (in the porewater). Although this can be seen in the respective

experiment (Fig. 4), the As pulse is not that drastic. Considering further that the extent of this As

pulse may strongly depend on lactate and O2 concentrations in the influent solution, this

observation should be generalized more carefully.

**Response 1-7 DONE (1-3)**

We agree with the reviewer that decreasing the lactate concentration would decrease the total amount of metal reduction in the aggregate system (see Pallud et al., 2010); if lactate concentrations were lower, a less pronounced pulse would result. The pulse was caused by a shift to completely anoxic conditions; therefore O2 concentrations should not impact this result. However, our goal was not to observe the effects of carbon source limitations. Instead we demonstrated that when carbon source is not limited, arsenic release from aggregate systems is dependent upon the location of the reducing front. This reducing front was shown to be dependent upon the aeration status of the entire system, controlling the iron oxide concentration available on the exterior of the aggregate.

**Changes 1-7**

With the addition of electron balance calculations and reference to the impact of decreased lactate concentrations on metal reduction within aggregate systems (Pallud et al., 2010) we believe the impact of lactate and O2 on the reduction of As, Fe, and Mn within the aggregate has been clarified.

**Comment 1-8**

More generally, it seem that the authors should better describe the effective novelty of their work, without overemphasizing a single observation that may be highly dependent on imposed solution chemistry. Considering the aforementioned points (and further details mentioned below), major revision of this manuscript and re-review of the resubmitted manuscript is recommended. Most importantly, data on lactate, acetate and O2 in the reactor effluent should be provided, and the experimental results should be discussed on a quantitative basis (also considering factors such as solution flow rate, solution and aggregate volume, limiting conditions for solute diffusion into and out of aggregates etc). The effect of selected lactate and O2 concentrations on experimental outcome, and consequent limitations for generalization of observed trends for different types of soils should be discussed as well.

**Response 1-8 DONE (1-3)**

We have rewritten statements as suggested; details are provided below with the Reviewer's detailed comments. Please refer to responses above for comments (e.g., 1-3) regarding lactate, acetate, and O2.

**Changes 1-8**

Please refer to changes listed with item 1-3, and details under "minor comments" below.

**Minor Comment 1-10**

L47-51: Long sentence, hard to read (exemplary for many sentences in this manuscript). Shorter

and more concise sentences would facilitate reading.

**Response 1-10 DONE**

The sentence is grammatically correct. Thus, this becomes a matter of taste, and one in which we disagree with the Reviewer (and the Reviewer should note that not all readers would agree with his/her comments that a simple sentence structure is always superior). We have nevertheless altered this sentence (and others) to provide an overall shorter sentence structure.

**Changes 1-10**

This sentence along with others within the manuscript have been edited for wording and grammar to be shorter in structure.

**Minor Comment 1-11 DONE**

L53: "anoxic" should be used for "O2-free" solution conditions, and "anaerobic" for

microorganisms respiring lactate by using electron acceptors other than O2.

**Response 1-11**

Agreed.

**Changes 1-11**

We changed "anaerobic" to "anoxic."

**Minor Comment 1-12**

L55: "less selective" is misleading, since As(V) generally adsorbs much more strongly than

As(III)

**Response 1-12**

The Reviewer appears to be confusing "less selective," with adsorption strength.

**Changes 1-12**

No changes made.

**Minor Comment 1-13 ADD references to Review Response if Scott approves them**

L61-63: Split into several sentences. How can Fe(II) and Mn(II) be "reproduced"? What are the

"oxidized counterparts" of Mn(II) and Fe(II)? Aqueous Fe3+ and Mn4+; or which types of

minerals specifically? Be more specific.

**Response 1-13**

Rapidoxidation of Fe(II) generally produces ferrihydrite initially, which can then transform into more crystalline phases over time (Schwertmann and Murad, 1983; Hansel et al., 2003). Oxidation of Mn(II) can form Mn oxide phases of low crystallinity such as birnessite (Tebo et al., 2004).

**Changes 1-13 DONE**

We have re-written the sentence to include examples of Fe- and Mn-oxides that can form when Mn(II) and Fe(II) are oxidized in soil environments.

**Minor Comment 1-14 DONE**

L66: Not very precise statement.

**Response 1-14**

Agreed.

**Changes 1-14**

The sentence was replaced with "Hence, the relative flux of Mn and Fe out of soils and sediments varies depending upon redox conditions."

**Minor Comment 1-15 DONE**

L81-83: check sentence.

**Response 1-15**

We've re-written the sentence.

**Changes 1-15**

The sentence was replaced with "Anaerobic respiration upon As and Fe and Mn oxides is initiated when anoxic conditions develop. The resulting reductive transformation of Fe(III) oxides decreases oxide surface area, resulting in the release of adsorbed As into the aqueous phase."

**Minor Comment 1-16 DONE**

L83-86: Split into several sentences. Clearly describe under which specific conditions formation

of magnetite and concomitant As(III) sequestration may play an important role.

**Response 1-16**

To be clear, the present sentence is fine grammatically and would not need to be split. However, we are happy to provide more detail on the conditions under which magnetite formation occurs and As(III) is sequestered.

**Changes 1-16**

We've added a description of the specific conditions under which As sequestration occurs as magnetite forms as noted by Coker et al., (2006), Herbel and Fendorf (2006), and Kocar et al., (2006), and conditions for its reductive dissolution as presented by Tufano et al. 2008.

**Minor Comment 1-17 DONE**

L100-111: Matter of taste, but is it really necessary to provide this summary at the end of the

introduction?

**Response 1-17**

Providing a brief summary is useful to highlight the key findings; we found the summary to be a bit detailed and therefore have revised it to state only the main conclusions.

**Changes 1-17**

We have re-written the brief summary of our findings at the end of the introduction to be more succinct.

**Minor Comment 1-18 DONE**

L106-107: Check sentence.

**Response 1-18**

Agreed.

**Changes 1-18**

We've removed this sentence and re-written the second portion of the paragraph to present the conclusions in a more succinct manner.

**Minor Comment 1-19 DONE**

L117-118: Check wording. Did the reaction really cool before filtering?

**Response 1-19**

Yes, the solution was cooled before filtering to not damage the filtering device. The solution was stirred to homogenize the suspension of oxides during the cooling period.

**Changes 1-19**

No changes made.

**Minor Comment 1-20 DONE**

L122: confirm (not confirmed)

**Response 1-20**

Agreed.

**Changes 1-20**

"confirmed" replaced with "confirm."

**Minor Comment 1-21 DONE**

L124-135: Be more precise: How much ferriyhdrite and birnessite with how much sand, at what

solid to solution ratio. What were Fe and Mn concentrations of final mixed sand. What amount

of sand was reacted with phosphate containing solution. How much phosphate was sorbed to

coated sand? Was there any P loss during reaction with arsenate?

**Response 1-21**

We have revised the methods to provide greater detail on the reaction conditions. Specifically, ferrihydrite and birnessite slurries were mixed with 250 g of sand to produce 0.061 mmol Mn/g sand and 0.1353 mmol Fe/g sand. The entire 250 g of sand mixture was incubated in 0.26 μM of sodium phosphate for a final adsorbed concentration of approximately 0.78 nmol phosphate/g sand. Concentration of phosphate in effluent was below detection level after flow was initiated in the aggregate reactors.

**Changes 1-21**

We have added the information requested by the Reviewer in the Methods section Aggregate construction and reactor setup.

**Minor Comment 1-22 DONE**

L135: Considering that the molar Mn/Fe ratio of the sand should be 1:10, these As/Fe and

As/Mn molar ratios must be wrong??

**Response 1-22**

The values provided are correct. Each of the solids has a different sorption capacity; the As/Fe and As/Mn ratios reflect the combined effect of sorption and mass of Fe/Mn in the solid.

**Changes 1-22**

No changes made.

**Minor Comment 1-23 DONE**

L147-156: Be more precise. What was the volume of the reactor? What was the volume of the

aggregate? How much time did it take to replenish solution volume once based on the used flow

rate? This information is essential for better interpretation of results.

**Response 1-23**

The details are now provided in the manuscript.

**Changes 1-23**

We've include the dimensions and volume of the reactor, volume of aggregate, and time required to exchange one reactor volume of solution based on flow rate in methods section under section **Flow-through reactor experimental procedure**. Specifically, the volume of the reactor was 75.6 mL and the volume of the aggregate was 8.18 cm3 with a porosity of approximately 0.6. The solution flow rate was set at 1 mL per hour, therefore, it took approximately 71 hours to replenish the solution volume within the reactor.

**Minor Comment 1-24 DONE**

L160-165: Include short statement on working principle? Was As(III) purged to get

measurement of As total and As(V) (and As(III) by difference)?

**Response 1-24**

Yes, arsenite is reduced by sodium borohydride, generating arsine gas, which is purged out of solution by N2, leaving only As(V) in the remaining solution. Total As is measured in a separate untreated sample and As(III) is calculated as the difference between the untreated total sample and the treated, purged sample.

**Changes 1-23**

We've added two statements to clearly state that sodium borohydride acts as a reductant of As(III) to form arsine gas, and that As(III) is calculated by taking the difference between treated and untreated samples.

**Minor Comment 1-25 DONE**

L168-170: Did you ever test whether zonation was radially symmetric (i.e., same in all directions

from center of aggregate)? Was reactor solution stirred, such that there is no chemical gradient in

solution surrounding aggregate?

**Response 1-24**

Radial symmetry of the reducing zones is easily noted by the color transformation of the aggregate. We have conducted dozens of aggregates and the transformations are always highly symmetric. The aerated solution surrounding the aggregate was continuously mixed throughout the experiment. The solution in the anoxic reactors was not stirred, but maintained at a flow rate sufficient to diminish solution gradients in the simulated macropore.

**Changes 1-24**

No changes made.

**Minor Comment 1-26 DONE**

L175-176: Only report significant digits.

**Response 1-26**

Agreed.

**Changes 1-26**

We've changed the values to only display significant digits.

**Minor Comment 1-27 DONE**

L180-181: Samples were presumably not "sonicated anaerobically" but "sonicated in anoxic DDI

water"?

**Response 1-27**

Yes, the reviewer's presumption is correct.

**Change 1-27**

Changed "sonicated anaerobically" to "sonicated in anoxic DDI water."

**Minor Comment 1-28 DONE**

L194-199: Check singular/plural.

**Response 1-28**

Yes, the statement should be changed from plural to singular.

**Changes 1-28**

Changed "were" to "was" when referring to "arsenic speciation."

**Minor Comment 1-29 DONE**

L203: Glovebag is neither oxic nor anoxic, only the gas-phase in bag.

**Response 1-29**

Although we understand the reviewer's concern, the term "anaerobic glove-bag" is an accepted industry term that is used by manufacturers of glove-bags.

**Changes 1-29**

Included gas phase composition (95%N2:5%H2) following the term "anaerobic glove-bag."

**Minor Comment 1-30 DONE**

L214: Concentration does not "decay". What exactly do you mean by "pseudo-steady-state"?

When would it be a real steady state?

**Response 1-30**

We agree that concentrations do not "decay." Pseudo-steady state implies that we have reached a steady-state under the current conditions but that long-term changes masked by the limited duration of our experiment cannot be ruled out.

**Changes 1-30**

Changed "decay" to "decline".

**Minor Comment 1-31**

L213-L258: Very descriptive - would be more helpful to highlight most important trends over all

treatments - and include lactate, acetate and O2 data. Was there any O2 in effluent of aerated

reactors? How much? What about lactate and acetate?

**Response 1-31**

We have revised the writing within the results section to be more succinct. As mentioned above, we have also included lactate, acetate, and O2 data as mentioned above in response to similar requests from this reviewere. For the aerated reactors, the effluent O2 concentrations were at saturation with respect to air (maintained through continual air-sparging). There was both lactate and acetate in the effluent of all reactors.

**Change 1-31**

We have re-written portions of the results to be more succinct and included lactate, acetate, and O2 data as requested.

**Minor Comment 1-32**

L308-311: But this depends on lactate concentration as well. What if lactate would have been

adjusted to 30 uM rather than 3 mM? Maybe oxic conditions would have been maintained

throughout aggregate?

**Response 1-32**

Yes, we agree with the reviewer that if electron donor concentrations were limited, there would be less reduction in the system; however, again, our objective was not to observe the consequences of a carbon limited environment, rather we wanted to document that redox gradients that would ensue as aeration status of the system were shifted, and the concomitant elution of As, Fe, and Mn.

**Changes 1-32**

No changes made.

**Minor Comment 1-33**

L338-340: Independent of Fe reduction or Fe release? This is not the same. No Fe release does

not mean there is no Fe reduction.

**Response 1-33**

The manuscript currently states "independent of Fe reduction" which, as the reviewer points out does not mean Fe release. It seems the reviewer is in agreement with the current sentence.

**Changes 1-33**

No changes made.

**Minor Comment 1-34**

L348-352: It is fairly evident that Mn(III/IV)-oxide reduction prior to As(V) reduction leaves

little room for substantial Mn(III/IV)-oxide-induced As(III) oxidation...it is also not clear why

those results demonstrate that O2 is required for Mn(III/IV)-oxides to be an effective oxidant for

As(III). As long as Mn(III/IV)-oxides exist, they will react with Fe(II) and As(III) (in absence

and presence of O2).

**Response 1-34**

**\*\* This part confuses me too... \*\* I am speculating, but it appears that if Mn-oxide is undergoing reduction, even if still present, it does not serve as an oxidant. From other work, we notice that Fe-oxides don't tend to adsorb arsenic if their reduction is taking place even though the solid is present.**

**Changes 1-34**

**Minor Comment 1-35**

L361-381: When generalizing observations from this study, high lactate level used in these

experiments must be considered. What are typical concentrations of readily degradable organic C

in soil solution? Would same trends be observed at much lower lactate concentrations?

**Response 1-35**

**GO AHEAD AND SPECULATE. YOU HAVE A GOOD FEEL FOR HOW THE VARIABLE NOTED WILL INFLUENCE THESE SYSTEM SO GIVE IT A GO.**

**Changes 1-35**

**Minor Comment 1-36**

L384-404: Are those conclusions really supported by the data? In real soils, due to much lower

degradable C levels, microbial activity might even be limited to the exterior of aggregates - such

that interiors may remain unreduced even if soil solution turns anoxic (e.g., pseudo-gley patterns

in subsoil with reductively depleted flow channels and oxic "interiors").

RESPONSE

CHANGE

SOILS HAVE VARIABLE C LEVELS AND NOT ALL ARE C-STARVED AS THE REVIEWER INDICATES. IT IS EFFECTIVELY THE DIFFERENCE BETWEEN OLIGOTROPHIC AND EUTROPHIC SYSTEM. OR CONSIDER A SOIL AT THE DISH VERSUS YOKO'S FIELD SITE (OR THE SOILS IN CAMBODIA).

**Minor Comment 1-37**

L414: Bibliography is not really GCA style.

**Response 1-37**

Agreed.

**Changes 1-37**

We've revised the references list to reflect the GCA formatting guidelines.

**Figure Comment 1-38**

Fig.2 and Fig. 3: Why does Fe reach 200 uM after 20 d in Fig. 2, but 400 uM in Fig. 3?

**Response 1-38**

**\*\* SCOTT, THIS is one of the reasons we went "qualitative" on this paper... I don't actually know how to reason this one...\*\* MY GUESS IS THAT YOU HIT SIDERITE SATURATION. I CHECKED THE VALUES AND YOU ARE RIGHT ABOUT AT THAT POINT, AND THE Fe(II) TREND LOOKS LIKE IT HAS HIT A SATURATION WALL. Any data to support or refute that possibility?**

**Figure Comment 1-39**

Fig. 5 (and other figures): Maybe more prominently display treatment in panels (rather than just

in caption)

**Response 1-39**

We agree that more prominent display will be better.

**Changes 1-39**

We have labeled the lines directly on the figure rather than only defining the symbols in the legend.