

## ***Interactive comment on “Organic exudates promote Fe(II) oxidation in Fe limited cultures of *Trichodesmium erythraeum*” by Hanieh T. Farid et al.***

### **Anonymous Referee #1**

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This manuscript describes a (minor) experiment testing the effect of exudes from *Trichodesmium* cultures on Fe(II) oxidation rate, followed by a (minor) modeling attempt. The most interesting finding/suggestion is that *Trichodesmium* produces some organic ligands that can bind Fe(II) and hence (slightly) accelerate Fe(II) oxidation rate. However, this suggestion is not pursued further: no attempt is made to quantify these ligands, start characterize them (even basic features), and most importantly conduct speciation measurements (electrochemistry) to demonstrate that they exists, assess the complex strength with Fe(II) and even quantify them. Some basic characterization of the ligands is easy, while some requires special equipment and knowhow. However, there are plenty of collaborators that the authors can approach to make this scientific

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contribution more robust for a future submission. In addition, I have various issues with different aspects of the study as described below. One example is that since no Fe replete culture was tested with regards to Fe(II) oxidation, it is not even clear if these presumed ligands are released in respond to Fe limitation. All in all, in my opinion the study is of mediocre quality, too small of a scope, and adds very little to the scientific literature in terms of new concepts, methods or data.

Specific comments: 1. Replication- I did not see any mention of replication in the paper and hence assume that only 2 cultures were examined (2 levels of Fe') with regard to Fe(II) oxidation rates. This is problematic by all means, as we are dealing with a very complex and sensitive organism, which growth rate is hard to control. So the lack of replication makes the comparison between the cultures questionable (see below) and even cast some shadow on the observed trends with time of Fe(II) oxidation rates (will the next growth curve look the same?, will similar amount of presumed ligands be produced?)

2. Trichodesmium growth rates under different Fe' conditions- It is unclear why the authors chose to alter EDTA concentrations and not Fe in order to change Fe'. This would have prevented precipitation of Fe, and ease the modeling and the Fe(II) oxidation experimental part (less abiotic background).

I think too much effort was put into counting cells (which is very time consuming), instead of following in vivo chlorophyll a (Chl) concentrations. Once the cells are acclimated to a certain level of Fe, the in vivo chl, measured in a fluorometer is a good, fast and easy marker for growth rate. I do not see how the tedious cell counts contributed to this study. Maybe the authors can think of using these for estimating cell surface area, carbon biomass or anything that can contribute to the discussion of a modified manuscript.

On the other hand, not enough effort was invested into establishing the consistency of growth rates (= replicates) between the different conditions. Based on the data in

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Figure 1, I am not convinced that there are meaningful differences in the growth rates of both cultures. Note, growth rates for exponentially growing cells should be calculated from the slope of  $\ln(\text{cell})$  vs time and not the final-initial equation which is given in the appendix. However, since growth rates are not the only way to show limitation by Fe, I think the authors should have attempted probing for Fe limitation by some other means (examples -  $F_v/F_m$ ;  $\text{Chl}/\text{cell}$ ; marked drop in  $\text{Fe}/\text{cell}$ ; accelerated Fe uptake rate, growth enhancement after Fe addition, etc).

Maybe the differences between the cultures in terms of Fe limitation are not that important, but then a better explanation of why these are chosen is at place.

Most importantly, why no high Fe culture was tested?, How can we know that Trichodesmium effect Fe(II) oxidation only when it is Fe limited? Why 2 levels of low Fe' and no high Fe?

3. Data/figures- In Figure 2 the pseudo first order constant for abiotic conditions is about 3, but in figure 4&5 it is 0.0008 or so. This makes no sense... Figure 5 may be misleading as it is unclear for which sections in time these growth estimates refer. It is best to divide into exponential and stationary phase. In fact this whole figure is probably redundant as it shows exactly the same data as in figure 4 (are these indeed the exact same experiment?). Moreover, when the culture is dying (days 14-20 of the low EDTA culture), things get messy. What is exactly being released from dying cells, can we treat these as exudes? (not much was found but still... ). 322-325 Bad phrasing – It reads as if nothing is known, until you get to the end of the sentence. “Since both formation and oxidation rate constants for the Fe(II)EDTA complex were unknown and could not be independently constrained under our experimental conditions due to use of relatively low EDTA concentrations, the oxidation rate constant value was obtained from the literature”.

4. Suggestions to improve future manuscript I think that the suggestion that Trichodesmium produces ligands that presumably bind Fe(II) is novel point but is not

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developed enough. Here are few ideas that can be pursued. Ligand stability with time- In the methods it is mentioned that a biotic background for Fe(II) oxidation was established after keeping the filtrate for 5 hrs in the dark. If this is the case, it is very interesting. Are the exudes themselves short lived? Are they heat stable/sensitive? (proteins), is it really ligands? Can you show that there are ligands and that they are gone/stop binding (by collaborating with some electrochemistry speciation person?) Ligand concentrations – The authors claim that the concentrations of the exudes are unknown, but they can go after it and make a more meaningful story. Can they saturate them with Fe(II) and see that the Fe(II) oxidation rates reach those of abiotic Fe(II)? Can they dilute the culture and see that it approaches the abiotic background (and then refine the model). Again, can the actual concentrations be evaluated using metal speciation methods (electrochemistry)?

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