Biogeosciences Discuss., 11, C5329–C5333, 2014
www.biogeosciences-discuss.net/11/C5329/2014/

Biogeosciences
Discussions
Open Access

Interactive comment on "Role of extracellular polymeric substances (EPS) from *Pseudomonas putida* strain MnB1 in dissolution of natural rhodochrosite" *by* H. Wang and X. Pan

H. Wang and X. Pan

xlpan@ms.xjb.ac.cn

Received and published: 23 September 2014

Dear editor and referees, Thank you very much for your kind comments on our manuscript "Role of extracellular polymeric substances (EPS) from Pseudomonas putida strain MnB1 in dissolution of natural rhodochrosite". We would like to express our sincere thanks to the reviewers as well. Those insightful comments are very helpful for improving the paper. The manuscript has received substantial revisions based on the three referees' comments. Every section of the manuscript, including Introduction, Materials and Methods, Results and Discussion, Conclusion and References have been revised carefully. The manuscript has been edited by a native English-speaking

expert from UK, and particular attention is paid to syntax and grammar. We hope the revised manuscript can reach the publication standards of Biogeosciences (BG). Thank you in advance for your consideration. Detailed responses to the comments are listed as follows.

Anonymous Referee #1: "The authors present an interesting laboratory study on the influence of EPS on rhodochrosite stability. The presented results clearly show that the presence of microbial EPS enhanced mineral dissolution, increasing the amount of Mn(II) required for microbial Mn(II) oxidation. Furthermore, Wang and Pan identified functional groups of the EPS primarily responsible for mineral dissolution. The study contributes to the, to date, fragmentary knowledge about microbial extracellular molecules, elucidating their potential impact on mineral stability. The presentation of the data is mostly adequate and appropriate literature citing is provided. The manuscript is reasonably short and does not require substantial shortening. The included figures are sufficient to illustrate the results. Unfortunately, in its current form the manuscript is not acceptable for publication. The general spelling and grammar requires substantial improvement. In the Material and Methods section several flaws are present (see specific comments) and it is not explained on what basis the dissolution rate of rhodochrosite was calculated. In addition it also not mentioned how the used MnO2 was synthesized. Furthermore, no information is provided about the statistical tests, which were." Reply: We have revised the manuscript accordingly. First of all, the general spelling and grammar was corrected by an English editing service and an expert from the UK. Secondly, in the Material and Methods section, the information was provided including the statistical tests, the formula used to calculate the dissolution rate of rhodochrosite and the reference used to synthesize δ-MnO2. In addition, the crystallographic characters of biogenic Mn oxides and δ-MnO2 performed by powder XRD and SEM-EDS were provided. Specific comments: 7274 l. 5 do not abbreviate strain name here. Reply: We have corrected. 7274 l. 7 rephrase, rhodochrosite is not oxidized, it is only dissolved. Once available, the Mn(II) ions are oxidized. Reply: We agree with the comments, and made revisions accordingly.

7274 l. 21 move reference directly behind ..reactivity and:::existence, if references are specific. Reply: Done. 7274 l. 25 this sentence is very lost in this paragraph and thus confusing Reply: the sentence was corrected. 7275 l. 5 add reference Reply: We made corrections accordingly. 7275 l. 10 has it been demonstrated or not? Reply: the sentence was rewritten. 7275 l. 26 change to ..oxides were analyzed by scanning:::. Reply: Yes, we have corrected. 7276 l. 5 do not abbreviate strain name here. Reply: We have corrected. 7276 l. 15 MilliQ is a trademark. Rather write "ultra-purified water" Reply: We made revisions according to reviewer's suggestions. 7276 l. 16 add preparation of Mn oxides Reply: According to your suggestion, we added the experimental details in sections 2.5 in our revised manuscript. 7276 l. 21 did you powder the mineral for XRD-Analysis? Add information Reply: Before experiments, the mineral samples were dried at ambient temperature and sieved through a 200 mesh nylon screen after grinding. New data about powder XRD was provided in the new manuscript (see Fig. 1). 7276 l. 21 inaccurate. Give a percentage values of rhodochrosite & quartz Reply: the percentage values of rhodochrosite and quartz contents were calculated (see 2.2 Section). 7277 l. 5 how much suspension? Reply: the information was provided (see 2.3 Section). 7277 l. 5 add space after:::cells Reply: Done. 7277 l. 9 how many ml of aliquot per sampling? Reply: We have corrected (see 2.6 Section). 7277 l. 9 do not give RPM values for centrifugations as. Use the g value for better comparison Reply: the g values were provided in the revised manuscript. 7277 l. 12 how did you calculate the dissolution rate of Rhodo? Needs to be mentioned in the M&M section Some where you need to mention that you analyzed EPS prior and after reaction with Rhodo. How did you treat EPS after the reaction? Purification, etc..? Reply: We added all these details in the revision (see 2.4 Section). 7277 l. 19 how long each time? Reply: the missing information was provided (see 2.4 Section). 7278 l. 8 add method accuracies for Mn(II) and Mn oxides concentration analyses Reply: We made corrections (see 2.6 Section) 7278 l. 18 what about the cleaning procedures of the minerals from the bacteria treatments? Did you clean them? Otherwise the EDS spectra are rather useless. Reply: the pretreatment

process used in this study was provided (2.4 Section) 7278 l. 20 which software was used for XRD spectra analyses? Reply: the related information was provided (see 2.6 Section). 7279 l. 2 where do the SEM graphs show the presence of cells? Add this information to the figure caption. Reply: We made further explanations (See Fig. 2) 7279 l. 2 how can you deduce a crystallographic information from an SEM picture, please clarify. Reply: The powder XRD data were provided to explain crystal structure of biogenic Mn oxides (see Fig. 3) 7279 l. 13 delete "For example" Reply: Done. 7279 l. 22-23 this sentence is very confusing, please rephrase Reply: The sentence was deleted. 7279 l. 26 rhodochrosite was not oxidized, but only dissolved Reply: We have revised according to reviewer's comments. 7280 l. 5 give pH values in the text Reply: Done. 7280 l. 6 the pH did not decrease, you started at different pH values, clarify Reply: We have revised. 7280 l. 8 this sentence is confusing and your statement needs further explanation Reply: The sentence was deleted. 7280 l. 17 why do you not show these data? I think it is important so see that EPS only enhanced dissolution, while the oxidation than has to be attributed to the bacteria. Reply: We have provided these data in the new manuscript (see Fig. 4). 7281 l. 10 do you mean "...reacting with..."? Reply: We have revised "reacting of" to "reacting with". 7281 l. 14-16 speculate on the mechanism. How could these functional groups interact with the crystal? Complexation of Mn ions? Decrease of hydrophobicity at crystal surface, surface charge...? Reply: It is difficult to distinguish the pathway of natural rhodochrosite dissolution involved by EPS based on this study. In our future work, we plan to further study the mechanism of EPS in the dissolution of natural rhodochrosite at water-mineral interfaces. 7281 l. 17 "... dissolve Rhodochrosite and subsequently oxidize liberated Mn(II) ions to form Mn oxides." Again, you can not state that Rhodochrosite was oxidized, it was only dissolved. The resulting Mn(II) ions were oxidized. Reply: We have revised. Table 1 re-organize table so that you can delete the first row first row. Do you mean Rhodochrosite dosage? Reply: We have revised (see Table 1). Fig. 1 this a spectrum of the mineral you used for your experiments, right? please add this information. Reply: Done. Fig. 2 the EDS spectra of "biogenic

Mn oxides" and the synthetic one seem to have very little in common. Shortly explain differences in figure caption. Reply: We provided XRD data of biogenic Mn oxides in new manuscript. Fig. 3 flip a and b as this order is more logic as dissolution happes prior to precipitation. Reply: We have corrected (see Fig. 4). Fig. 4 write out "arbitrary units“ in Y-axis label Reply: the unit was provided (see Fig. 6)

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/11/C5329/2014/bgd-11-C5329-2014-supplement.zip

---

Interactive comment on Biogeosciences Discuss., 11, 7273, 2014.