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-MnO_2. In addition, the crystallographic characters of biogenic Mn oxides and δ-MnO_2 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)

By anonymous Referee #2:

“Wang & Pan submitted a manuscript describing results from an experimental study on the role of EPS on the oxidative dissolution of natural rhodochrosite ((MnX)CO3). The presented topic is of clear fundamental international interest and fits into the scope of BG. However, as outlined below, in its concept and present shape it is not suitable for publication. First of all, the presented study contains numerous linguistic and grammatial errors that make a detailed evaluation of the short ms not easy. The referencing is clearly not complete and up to date. In particular, the experimental results show that EPS is impacting the oxidative dissolution of natural rhodochrosite, which is interesting. However, having sayd this, it remains questionalbe, why natural rhodochrosite and not pure MnCO3 was for used for the study! Based on the very limited chemical analysis of the initial and final solid as well as the aqueous solution, more then 50% of the used solid seems to be actually SiO2. The powder XRD makes clear that the carbonate fraction is not homogeneous in its chemical composition but likely contains foreign ions as Ca, Mg and Fe. These components significantly impacts the dissolution kinetics of MnXCO3 solid-solutions (e.g., Boettcher & Dietzel, EMU notes 2010). The Impact of foreign ions on the reactions at the solid-water interface may be enormous. This is not considered in this study at all and since a complete chemical analysis of the solid is lacking, can also not be evaluated for comparative later use. ”

Reply: We appreciate the reviewer’s constructive comments and made substantial revisions based on the referees’ comments. First of all, the general spelling and grammar was corrected by an English editing service. Some relevant references were supplemented (Böttcher and Dietzel, 2010; Prieto et al., 2013; Putnis and Ruiz-Agudo, 2013). Moreover, in the revised manuscript, Powder XRD and SEM-EDS for natural and synthetic rhodochrosite were obtained to explain mineral information. XRD results showed that the raw mineral mainly contains rhodochrosite (83.4%) and some quartz (16.6%). SEM-EDS results show that the content of elemental Si was 12.91% (wt). These results indicated that the content of quartz in natural rhodochrosite was not as high as the reviewer’s guess (>50%). Moreover, the effect of foreign ions on the reactions of natural rhodochrosite was considered in the revised manuscript. We have investigated the differences of bacterial oxidative dissolution of natural and synthetic rhodochrosite. We have found that the oxidative dissolution kinetics of natural rhodochrosite was slower than that of synthetic rhodochrosite. The results might be mainly attributed to the differences of crystal structure or the incorporation of foreign ions such as Al, Mg and Si.

Supplemental references:

(1) Böttcher, M. E., and Dietzel, M.: Metal-ion partitioning during low-temperature precipitation and dissolution of anhydrous carbonates and sulphates ion partitioning in ambient-temperature aqueous systems. EMU Notes in Mineralogy, 10. Mineralogical Society, Twickenham, UK, pp. 139-187, doi: 10.1180/EMU-notes.10.4, 2010.

(2) Prieto, M., Astilleros, J. M., and Fernandez-Diaz, L.: Environmental Remediation by Crystallization of Solid Solutions, Elements, 9, 195-201, doi: 10.2113/gselements.9.3.195, 2013.

(3) Putnis, C. V., and Ruiz-Agudo, E.: The Mineral-Water Interface: Where Minerals React with the Environment, Elements, 9, 177-182, doi: 10.2113/gselements.9.3.177, 2013.

By L. Wang (Referee #3):

"In this manuscript, an interesting laboratory study was carried out to investigate role of extracellular polymeric substances (EPS) from P. putida strain MnB1 in enhancing dissolution of natural rhodochrosite. The results showed that EPS were found to play an important role in increasing dissolution of natural rhodochrosite. To my opinion, the study was innovative and the manuscript should be accepted for publication after minor revision. Some of my suggestions are as follows: "

1. In the part of introduction in pages 7274 and 7275, this study aimed to investigate the role of EPS in oxidative dissolution of natural rhodochrosite using a Mn oxidizing bacterium. What is the purpose of investigating the effect of EPS on Oxidative dissolution of rhodochrosite? Is it for the resource utilization of Mn or the removal of Mn contaminants?

Reply: Microbially mediated oxidation of Mn(II) to Mn oxides have been demonstrated in previous studies, however, the mechanisms of bacteria how to dissolve and oxidize using a solid Mn(II) substrate are poorly understood. So, the purpose of this work is to highlight the importance of Mn(II) oxidizing bacteria and its EPS in the dissolution and oxidation of natural rhodochrosite. This study is helpful for understanding the biogeochemical processes of the formation of biogenic Mn oxides from a solid Mn(II) origin.

2. In the part of introduction in pages 7274 and 7275, the author mentioned that "Oxidative dissolution of rhodochrosite leads to produce dissociative Mn(II) and Mn oxides". What kinds of Mn oxides are formed in the process of the oxidative dissolution of rhodochrosite? And what kinds of Mn oxides were formed through the dissolution of microorganisms?

Reply: These questions were resolved in the revised manuscript. "XRD and SEM results indicated that biogenic Mn oxides were composed of poorly order, poorly crystalline phyllomanganate, similar to δ-MnO_2, but varied in morphology (Figs. 2 and 3). The SEM graphs showed that a number of cells (the arrows) were adhered to the surface of biogenic Mn oxides (Fig. 2a-d). EDS analysis showed that the biogenic and synthetic Mn oxides were mainly composed of O and Mn, and other elements such as Fe, P and Mg."

3. The role of EPS in oxidative dissolution of natural rhodochrosite was investigated using a Mn oxidizing bacterium, Pseudomonas putida MnB1. Dose the bacteria itself have an effect on the oxidative dissolution of natural rhodochrosite?

Reply: In this work, *Pseudomonas putida* MnB1 played an important role in the oxidative dissolution of natural and rhodochrosite. First of all, living cells can effectively dissolve natural and synthetic rhodochrosite and subsequently oxidize liberated Mn(II) ions to form bacterial Mn oxides. Moreover, bacterial EPS from *P. putida* strain MnB1 played an important role in enhancing the dissolution of natural rhodochrosite.

4. In the part of discussion in page 7281 line 14 to 16, these results suggested that the functional groups of N-H in proteins, C=O in COOH or amide I and C-H or C-O-C in polysaccharides were directly involved in the dissolution of natural rhodochrosite. Why these functional groups are considered to play an important effect on the dissolution of natural rhodochrosite?

Reply: In this study, functional groups of bacterial EPS involved in dissolution of natural rhodochrosite were investigated by FTIR analysis. As shown from Fig. 6, we compared with FTIR spectra of EPS before and after reaction with natural rhodochrosite. Several absorption peaks (e.g., 2391 cm^{-1}, 1400 cm^{-1} and 1083 cm^{-1}) were changed significantly after the reaction between EPS and natural rhodochrosite, and these results were mainly attributed to the involvement of functional groups in bacterial EPS.