Biogeosciences Discuss., 8, C1050–C1054, 2011
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Biogeosciences Discussions

Interactive comment on "Effects of copper mineralogy and methanobactin on cell growth and sMMO activity in Methylosinus trichosporium OB3b" *by* E. Chi Fru et al.

Anonymous Referee #2

Received and published: 16 May 2011

General Comments

This study tackles a difficult and topical question regarding how the micronutrient Cu is accessed from solid sources by methanotrophic bacteria. The manuscript presents new data and concepts, which may be of value to the community if presented more clearly. However, much of the methodology is described with insufficient detail to adequately evaluate the significance of the data presented. Imprecise writing with many grammatical errors and improper or awkward word choice compounds this problem. Much of the data presented appears to have large errors (e.g. Figure 1, Figure 3B), which don't allow conclusions without more sophisticated statistical analyses of signif-

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Interactive Discussion
Discussion Paper

icance. The geochemical modeling and its interpretation with respect to the speciation of Cu and dissolution of Cu bearing minerals are not thorough, and, in many cases, not strictly correct. In order for this study to achieve its full potential, I would recommend reformulating the paper and clarifying the issues raised below. In light of these criticisms, I would argue that the two obvious findings of this study are that restricting physical contact to poorly soluble Cu minerals enhances sMMO activity and that supplying relatively soluble Cu minerals inhibits sMMO activity. How these two findings relate to other aspects of methanotroph physiology and capacity to metabolize methane remain unclear in the paper.

Specific comments and technical corrections

Abstract

Ln 1 where specifically does 'in situ' refer to?

Ln 11 the phrasing that the culture was grown 'on' Cu-minerals is misleading. I guess it was grown on CH4 and O2? Perhaps rephrase to 'grown with' or 'in the presence' of Cu minerals. The same applies to several other instances in the paper.

Ln 12 'influences' should be 'influence'

Ln 18 see comment below on the relationship between pMMO and sMMO. As far as I can tell, you haven't measured pMMO activity, and from the manuscript it isn't clear how pMMO and sMMO relate. So, what can you say about pMMO from your data?

Introduction

Pg 2853

Ln 6 add 'the' before 'Cu-to-cell'

Lns 10-11 please clarify what you mean by activity. Is this methane oxidation rates, methanotroph growth rates?

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Pg 2854

Ln 2-5 awkward sentence please rephrase. What do you mean 'the influence of mb for. . .Cu minerals'?

Ln 6 Presumably you mean 'addresses' not 'intersects'?

Lns 12-16 Does sMMO activity exclude pMMO activity? If not, what is the exact relationship between pMMO activity and sMMO activity? If pMMO activity is partially or wholly independent of sMMO activity, then many of your conclusions may not be justified. Please clarify.

Materials and Methods

Pg 2855

Lns 13-14 Minerals, by definition, do not have an aqueous phase. I think you mean that the minerals chosen represent a range in solubility.

Ln 14 also awkward language. I guess you mean that the table summarizes calculated aqueous Cu concentrations at equilibrium with the respective mineral phases.

Pg 2856

Lns 6-9 Please provide analytical figures of merit for your Cu analyses here. Detection limit, accuracy, and precision.

Lns 16-17 How was elevated sMMO activity defined, with respect to what? Why were elevated activities required for the experiments?

Lns 23-27 Please provide analytical figures of merit for your CH4 analyses here. Detection limit, accuracy, and precision.

Pg 2857

Lns 1-2 Please also provide information on the analytical figures of merit for your sMMO activity assay. This is critical for evaluating the significance of many of your results.

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Ln 11 “cultures’ should be ‘culture’

Ln 22-23 please reference this statement.

Results and Discussion

Section 3.1 I am not convinced by the data shown. There is typically a large difference in duplicate measurements, which makes it hard to tell whether or not there are any statistically significant differences between the different treatments. Also, please report the absolute concentrations in the different treatments and consider whether or not the differences are significant based on your analytical figures of merit.

Lns 11 12 What does this mean? Please be explicit. Please also compare the observed Cu dissolution level to that predicted by your thermodynamic calculations. At equilibrium, the free ion concentration will be set by the mineral solubility, whereas the amount of aqueous Cu complexed by mb set by the stability of the complex. At equilibrium then, the total dissolved Cu concentration is the sum of the free ion concentration and that complexed by mb. Please report these values and discuss how they relate to the observed aqueous Cu concentrations. I believe that any differences found between your measurements of aqueous Cu concentrations and these predicted values should be attributed either to kinetic effects or sorption of Cu and mb (e.g. through ternary complexes) to the mineral surfaces. I am not sure your experimental design will allow you discriminate between these two possibilities, but perhaps running a longer set of experiments with more temporal resolution and higher precision will allow you to ascertain if equilibrium is being approached.

Pg 2859

Section 3.2 Please compare initial growth rates here (from the linear portions of the growth curves). Are there significant differences between CuCO3-CU(OH)-2, CuO, and Cu free media. If so, why? Comparing growth rates here may allow you to discern as to whether or not there is a physiological response to Cu mineralogy in addition to

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the regulation of sMMO activity.

Section 3.3

Pg 2860

Lns 7-10 strictly speaking this isn't correct. See comments above regarding mineral dissolution and free ion activity.

Pg 2861

The data presented in Fig. 3 is not very clear, with the exception of the disappearance of sMMO activity in the CuCO3-Cu(OH)2 experiments.

Ln 16-18 Why should mineral contact elicit sMMO activity if contact enhances Cu availability to the organism. Shouldn't increased Cu availability lead to pMMO activity?

Ln 19 I think 'grow' should be 'growth' here.

Section 3.5

Pg. 2862

Is this because of enhanced dissolution rates due to the maintenance of low free ion activity levels under disequilibrium conditions? Is there any information on the association kinetics of Cu-methanobactin complexes that could be used to compare to the dissolution kinetics of the Cu-minerals?

Section 3.6

This section is unclear and I have a hard time trying to figure out what you mean here. For example, pg 2862, Lns 22-24, I fail to understand what noting other mbs has to do with their role(s) or the Cu sources. Again, Pg. 2863, Ln 9, what is a 'central' role? Lns 19-21

Interactive comment on Biogeosciences Discuss., 8, 2851, 2011.