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Interactive comment on "Influence of increasing dissolved inorganic carbon concentrations and decreasing pH on chemolithoautrophic bacteria from oxic-sulfidic interfaces" *by* K. Mammitzsch et al.

K. Mammitzsch et al.

klaus.juergens@io-warnemuende.de

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Comments to the review of Referee #2

We thank the Reviewer for the constructive and helpful comments, which have improved this manuscript. In the following we outline the changes we have made in response to the comments. Page and line numbers are according to the discussion paper. As similar comments were also raised by the first reviewer, please see our general comment in the reply of reviewer #1, before the response to the reviewer's

comments.

Reviewer comment: The connection between these laboratory data on a single bacterial strain and the natural system should be improved. Concentration levels are far beyond those in nature (mM rather than microM).

Response: Compared to environmental conditions, the substrate concentrations in the batch culture experiments are indeed high. However, our aim was not to mimic the natural situation in the redoxcline (in that case we would have used seawater as medium) but to use this model system to reveal the principal mechanisms regulating the growth of “S. gotlandica” str. GD1. A certain cell density and growth are currently required to examine those mechanisms. Similar substrate concentrations were used successfully in previous experiments to examine the utilization of different electron donors and acceptors, including organic carbon (Grote et al. 2012), and the stoichiometry of chemoautotrophic denitrification (Bruckner et al. 2013). Although conditions more closely resembling the environmental ones is desirable, there is currently no evidence that the results obtained at higher substrate concentrations cannot be applied to in situ conditions. However, as this is a critical point that should be considered in future experiments, we have added the following sentence in the Discussion (page 18384, line 20): “Since substrate utilization in the batch culture experiments is significantly higher than under environmental conditions, the results rather reflect the carrying capacity of “S. gotlandica” str. GD1 at the given conditions. Future studies should aim to more accurately simulate in situ conditions (e.g., with chemostat cultures). However, at present we have no evidence that the results concerning either substrate utilization or the impact of pH and the DIC concentration are not relevant to in situ conditions.”

Reviewer comment: Hydrogen sulfide is likely the major substrate used in nature, while for experimental convenience thiosulfate is used here. These experimental conditions complicate extrapolation of laboratory findings to natural systems. This should be clearly communicated to the reader.

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Response: We are aware that hydrogen sulfide is the major substrate in nature and “S. gotlandica” str. GD1 seems to be primarily responsible for hydrogen sulfide detoxification in the Baltic Sea (Grote et al., 2012). However, at the oxic-anoxic interface and the upper sulfidic zone thiosulfate concentrations are in a similar range as H2S concentrations (Bruckner et al, 2013), and substrate concentrations in experiments can be much better controlled when using thiosulfate instead of H2S. Nevertheless, we feel that our observations of the influence of increasing DIC and decreasing pH can be transferred to environmental conditions. To make this clearer to the reader, we added the following sentences to the Discussion (page 18382, line 26): “Hydrogen sulfide is the major substrate in nature and “S. gotlandica” str. GD1 seems to be primarily responsible for hydrogen sulfide detoxification in the Baltic Sea (Grote et al., 2012). However, at the oxic-anoxic interface and the upper sulfidic zone thiosulfate concentrations are in a similar range as H2S concentrations (Bruckner et al, 2013), and substrate concentrations in experiments can be much better controlled when using thiosulfate instead of H2S. Thus, as substrate we added thiosulfate, which could also serve as an alternative substrate under environmental conditions.”

Reviewer comment: The carbonate system description needs more attention: it does not follow the best practices for ocean acidification research (e.g. pH scale, methodology). pH changes during the incubations are rather large and it is sometimes not clear whether initial, mean or final concentrations/activities are reported.

Response: It was not possible to calculate the pH via the alkalinity because of the artificial brackish water. Thus, we instead used a pH meter. However, the NBS scale is currently the most commonly used one (see e.g., Krause et al. 2012, PLosOne; Wannicke et al, 2012, BG, Frommel et al., 2010, BG; Franke und Clemmensen, 2011, BG). Accordingly, its further use is the best way to allow comparisons between our data and those from other, similar experiments, including studies of other bacterial taxa (Takai et al., 2006; Inagaki et al., 2003; Scott and Cavanaugh, 2007). Although not all publications mention the method used for measuring pH, the NBS scale seems to be

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the most frequently chosen one. Because of the anoxic medium, it was not possible to open the cultivation bottle and measure the pH directly. Moreover, we could not have worked sterilely if the measurements had been made directly. For these reasons, we decided to take sub-samples. In the revised manuscript, we distinguish between the pH measured at the beginning (marked as pHs) and end (marked as pHe) of the incubation time. It is now clear whether we are referring to the initial or final pH.

Reviewer comment: Terminology needs attention: hydrogen carbonate vs. bicarbonate, it is also not clear whether bicarbonate or total inorganic carbon concentrations are mentioned. The availability of substrates during incubations requires better documentation.

Response: We changed “bicarbonate” and now consistently use “hydrogen carbonate”. We have also clarified whether we mean hydrogen carbonate or DIC. In addition, we calculated the theoretical DIC speciation (with the CO2sys program after Lewis and Wallace, 1998) and added to the manuscript (page 18376, line 1): “The distribution of the DIC speciation at pH 6.5 is 70.96 % hydrogen carbonate, 28.96 % carbon dioxide and 0.07 % carbonate, whereas at pH 8.0 the distribution of the DIC speciation is 95.59 % hydrogen carbonate, 1.23 % carbon dioxide and 3.17% carbonate.”

Reviewer comment: How sure are the authors that substrate limitations (either DIC, nitrate or thiosulfate) did not affect final cell yield and its pH dependency.

Response: Although thiosulfate seemed to be entirely consumed at the end of the experiment (see Fig. 4), this did not interfere with our conclusions regarding the role of suboptimal DIC concentrations. Furthermore, based on calculations using the stoichiometry for chemoautotrophic denitrification given in Bruckner et al. (2013), the carrying capacity with the substrate concentrations used in this experiment would be approximately 5 $\times$ 108, 6 $\times$ 107, and 3.5 $\times$ 107 cells mL-1 for DIC, thiosulfate, and nitrate, respectively. According to this stoichiometry, nitrate would be the first limiting factor. Earlier experiments with this strain did not produce higher cell numbers with higher

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thiosulfate concentrations (Bruckner et al., 2013; unpublished data). Therefore, other potentially limiting factors, related to cell concentration, have to be considered, such as the accumulation of inhibitory metabolic products. For this reason in the revised manuscript we have defined maximal cell numbers also as the carrying capacity (page 18382, line 26): "Although thiosulfate seemed to be entirely consumed at the end of the experiment (see Fig. 4), earlier experiments with this strain did not produce higher cell numbers with higher thiosulfate concentrations (Bruckner et al., 2013; unpublished data). Therefore, other potentially limiting factors, related to cell concentration, have to be considered, such as the accumulation of inhibitory metabolic products. For this reason, we defined maximal cell numbers also as the carrying capacity."

Reviewer comment: Clearly the experimental conditions need better documentation, this to be a lasting paper. Perhaps the authors have these detailed data; then please provide this to the reader, including growth rates.

Response: The experimental conditions are now better documented (see above). The growth rate for the experiment shown in Figure 4 has been added.

Reviewer comment: Finally, some of the conclusions are not supported (e.g., p. 18379, l. 23).

Response: We are not sure what the Reviewer specifically meant by this comment. The result described on page 18179, line 23 shows that a decrease in pH of 0.45 units in the unbuffered medium had no influence on maximal cell numbers ($2.57 \times 107 \pm 4.62 \times 106$ cells mL-1) when the starting pHs was 7.1 (i.e., the current pH value in the redox zones of the Baltic Sea). These data are supported by the controls, in which the medium was buffered with HEPES (thus, the pH was kept stable) and maximal cell numbers were $2.33 \times 107 \pm 1.76 \times 106$ cells mL-1. Thus, in both treatments (unbuffered and buffered medium) the same maximal cell number was reached.

Interactive comment on Biogeosciences Discuss., 9, 18371, 2012.