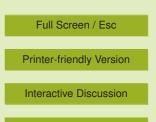**Biogeosciences
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Interactive comment on "Influence of increasing dissolved inorganic carbon concentrations and decreasing pH on chemolithoautrophic bacteria from oxic-sulfidic interfaces" *by* K. Mammitzsch et al.

Anonymous Referee #1

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Mammitzsch and co-workers present data from a set of culture experiments investigating the effect of projected future ocean acidification on the anaerobic denitrifying sulphur oxidising bacterium Sulfurimonas gothlandica strain GD1. The relevant physiological question whether future decreased pH or future increased inorganic carbon (DIC) concentrations are affecting growth and substrate uptake of GD1 is addressed. Therefore, a time series batch culture experiment sampled on daily intervals as well as several batch culture experiments sampled after 14 days of incubation were conducted. Experimental manipulations included gradients of a natural combination of pH

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and DIC, as well as experiments were pH and DIC are manipulated independent from each other using the buffer HEPES. Physiological data on the anthropogenic influence on important biogeochemical processes such as denitrification are urgently needed. pH treatment levels and DIC concentrations applied were reasonably chosen. The manuscript is fairly well organised and conclusions made are relevant and reasonably well discussed with reference to current literature. However, the produced dataset suffers several methodological problems, impeding an unambiguous interpretation of most of the data. 1. Growth rates are not presented within the manuscript and cannot be calculated from batch cultures that were sampled after 14 days. Figures are only showing cell counts mostly measured after an incubation time of 14 days. This incubation period was chosen with reference to Grote et al. (2012) and Bruckner et al. (2012) who performed similar experiments on GD1. Figure 4 in the manuscript is showing bacteria to reach substrate (thiosulphate) limitation already after 8 days of incubation in the given experimental setup with cell numbers around $15*10^6$ cells ml^{-1}. Cultures examined for results of DIC and pH optima shown in Figure 1-3 ($\sim$10-30$*10^6$ cells ml^{-1}) can be expected to be in a stationary phase for several days before sampling, thus steady state growth rates cannot be calculated. On page 18381 line 9-12 the authors state exponential growth to be occurring between day 6 and 9 (Fig. 4). Actually, the culture should have been growing exponentially between the start of the experiment until about day 8, when thiosulfate became limiting. Steady state growth rates could have been calculated with additional data on cell counts before the incubation as well as before growth limitation (e.g. day 6). 2. Substrate and treatment concentrations are insufficiently determined by measurements during and after incubations. While sulphur oxidation is over-determined with measurements of thiosulphate and sulphate, inorganic carbon species are hardly documented. Only potentiometric pH data (NBS-scale) is shown, which is not calibrated to any seawater scale. Those pH measurements are only reported for starting conditions of the experiments. Changes in the buffered experiments were classified as negligible. A relatively substantial pH decrease of 0.45 ± 0.1 is mentioned as a consequence of sulphur oxidation in the non-buffered setup (page 18379

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line 19). It remains unclear how pH developed in the single treatments. This pH shift is discussed as if it naturally accompanies sulphur oxidising denitrification (page 18382 line 4-11). Substrate concentrations appear unrealistically high. References indicating whether a conversion of ∼1mM of nitrate with 1mM of thiosulphate, leading to this pH shift; is a realistic scenario for Baltic Sea deep water are missing. Direct measurements of DIC and phosphate are lacking completely. 3. High cell densities are in these experiments combined with large nutrient resources (9μM PO4, 91μM NH4, 1mM NO3, 1mM thiosulphate) so that substantial consumption of DIC can be expected before substrate limitation is reached. Cells grown at various DIC concentrations presented in Figure 2 can rather be interpreted for carrying capacities of the growth media than used to infer on cellular growth rates. Here, DIC below 500 μM might as well be ultimately limiting the amount of biomass produced, while thiosulphate might have been limiting at DIC above 800μM (page 18381 line 1-2). The only data presented within this manuscript that could be used to infer on growth and substrate utilisation are the ones presented in Figure 4, within the first four to fife days, when DIC concentration and pH can be assumed to be close to initial values. The results are therefore not sufficient to support conclusions made within this manuscript.

Due to shortages in the experimental setup, presented data within this manuscript cannot be compared to cited literature dealing with the influence of environmental factors on growth or carbon fixation in bacteria. The publication in Biogeosciences is therefore not recommended.

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

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