

## Interactive comment on "Inter- and intra-specific responses of coccolithophores to CO<sub>2</sub>-induced ocean acidification" by D. S. Wang et al.

## **Anonymous Referee #2**

Received and published: 6 March 2015

Unfortunately, the experiment system of authors seems to be incomplete for it of the ocean acidification study. However, these data have physiologic significance. I recommend that a word of "Ocean acidification" is rearranged to others (e.g., influence of the CO2 increase).

## General comments

1. The authors studied the response of calcified and non-calcified strains of Coccolithales for ocean acidification. They tried mimicry of ocean acidification by CO2 bubbling of various concentrations. As a result, the data about provided growth, photosynthesis and the nitrogen uptake are very interesting. However, the experiment system of authors cannot completely control pH. The pH fluctuations during the experiment are up to  $\pm$ 0.5 or more, and adversely affect the authenticity of the data. Perhaps, it would

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be due to the cell concentration is too high. This will be because the CO2 consumption caused by the photosynthesis is faster than CO2 supply. Furthermore, they have been added phosphorus and nitrogen in order to maintain the growth of cells. Unfortunately, this work is incomplete as the study on ocean acidification.

- 2. The authors continued the cultivation by the flask for 30 days. It is thought that it is a plan to observe long-term influence, but it will be difficult for plankton to be cultured for a long term if phosphorus is not added in the middle. Kayano et al. show that phosphorus of 30  $\mu$ M is exhausted in 3 days in the cultivation on Emiliania huxleyi (kayano et al. 2009). In fact Fv/Fm and Y(II) sharply decrease after the 7days (Figure 2). These results are the evidence that the cells were damaged. The micrographs at the experiment latter half should be shown.
- 3. The impact of CO2 increase and pH decrease are should be shown with distinction. CO2 increase is positive, but the pH decline has negative influence for growth on E.huxleyi. (Fukuda et al. 2014)

Specific Comments

p7 L18 Not "fv/Fm", but "Fv/Fm"

p6 L6 Phosphorus and nitrogen are added?

p6 L9 Please show bubbling speed.

p9 L15 The pH values fluctuates greatly during the experiment. Because the cell concentration is too high, may pH not be controlled?

p10 L5 Are you add phosphate during the experiment? Phosphorus in the medium as shown kayano et al (2009) is consumed very quickly. I consider that what continue being cultured for 30 days if phosphoric acid is not added on the way is difficult.

P10 L6 Why do Fv/Fm and Y(II) greatly decrease after the seventh day? I suspect that in the cells are died. Was the cells observed with a microscope?

P14 L12 The possibility of a high CO2 concentration will have a negative impact on the N-E?

p14 L15 What is the genetic difference?ãĂĂYou should describe the difference.

p17 L15 Why does each PON increase? Will this be because broken pieces of the plankton increase?

p18 L26 Yamamoto-kawai et al. (2009) has already reported the example which  $\Omega$ -cal becomes with less than 1 in the Arctic Ocean. I think that it is difficult to relate the lab experiment to ocean acidification.

p19 L10 Coccolith may decline by deficiency of the HCO3- (Fukuda et al. 2014) Reference

Kayano et al. (2009) Plant Cell Physiol 50 (8): 1522-1531. yamamoto-Kawai et al. (2009) Science 326 1098-1100. Fukuda et al. (2014) Photosynth Res 121:299–309

Thank vou

Interactive comment on Biogeosciences Discuss., 12, 675, 2015.