Influence of acid treatment on the calcite exoskeleton of algae via impedance flow cytometry

Douwe S. de Bruijn[1*], Dedmer B. Van de Waal[2], Wouter Olthuis[1], Albert van den Berg[1]
[1]BIOS Lab-on-a-Chip group, MESA+ Institute, Max Planck – University of Twente Center for Complex Fluid Dynamics, University of Twente, The Netherlands,
[2]Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), The Netherlands, *d.s.debruijn@utwente.nl

1. Introduction

The coccolithophore species *Emiliania huxleyi* is a globally distributed species of calcifying algae, which creates a calcium carbonate exoskeleton. The algae produce particulate inorganic carbon (PIC) via calcification and particulate organic carbon (POC) via photosynthesis.[1] The relative production of PIC and POC (PIC:POC) is an important parameter indicating the calcification state of cells and as such for ocean carbon cycle modeling.

2. Research goal

In previous research we have differentiated cells with and without exoskeleton (calcified vs decalcified) based on their electrical impedance phase response.[2] In this research we gradually modify the PIC:POC ratio using a series of acid treatments (see Figure 1). The acid treatments will dissolve the exoskeleton (PIC) to a certain extent, while the organic cell (POC) stays unchanged. We investigate the influence on both the electrical volume and the phase. We propose a classification system (Figure 3a), based on small to large (electrical volume) and calcified to decalcified (phase) coccolithophore cells.

3. Sample preparation

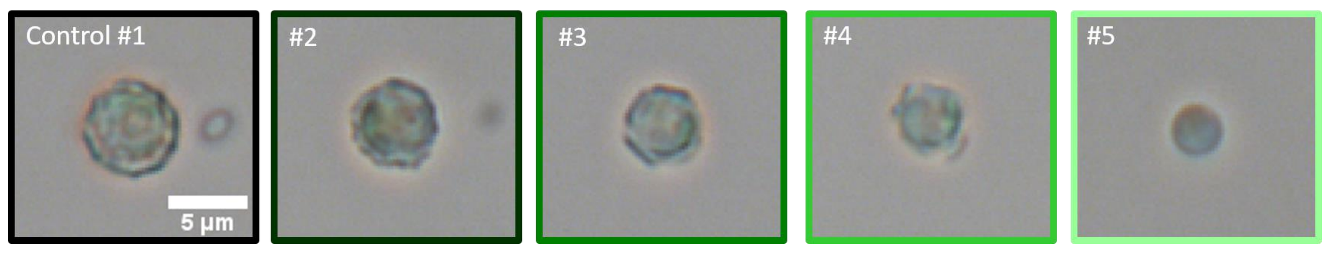

Figure 1: Optical images showing changes in the exoskeleton, due to different acid treatments. The amount of added acid increases from left to right, creating treatments with a varying range of calcification levels.

CO_2

Seawater carbonate buffer
$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$

Photosynthesis
Cell growth
Particulate organic carbon (POC)
$CO_2 + H_2O \rightarrow CH_2O + O_2$

Calcification
Calcite shell formation
Particulate inorganic carbon (PIC)
$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2$

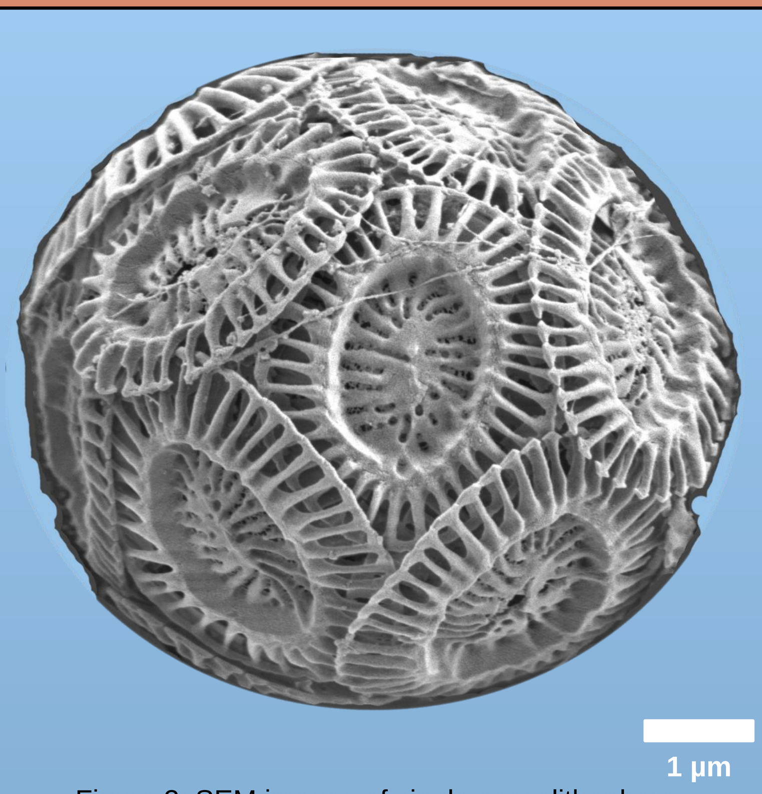

Figure 2: SEM images of single coccolithophore Emiliania Huxleyi.

Sinking flux

Carbon sequestration

4. Classification

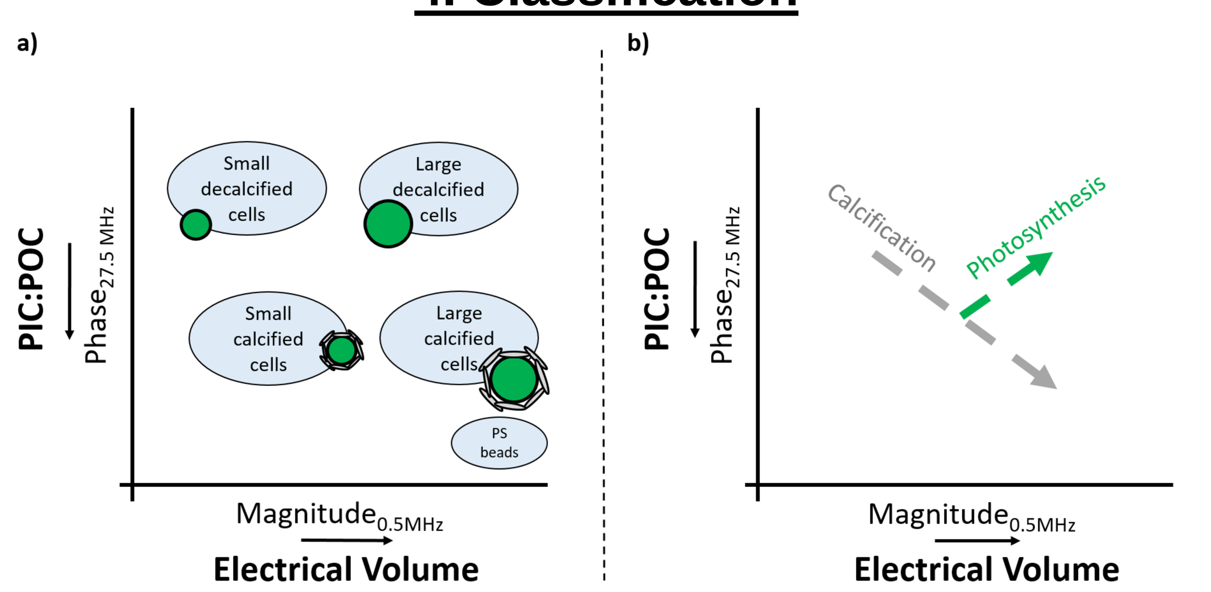

Figure 3: (a) Proposed classification of coccolithophores according to PIC:POC (via phase) and electrical volume (via magnitude). The magnitude and phase are normalized with respect to 5 µm polystyrene beads. (b) Expected transition, due to calcification or photosynthesis in coccolithophores.

5. Experimental setup

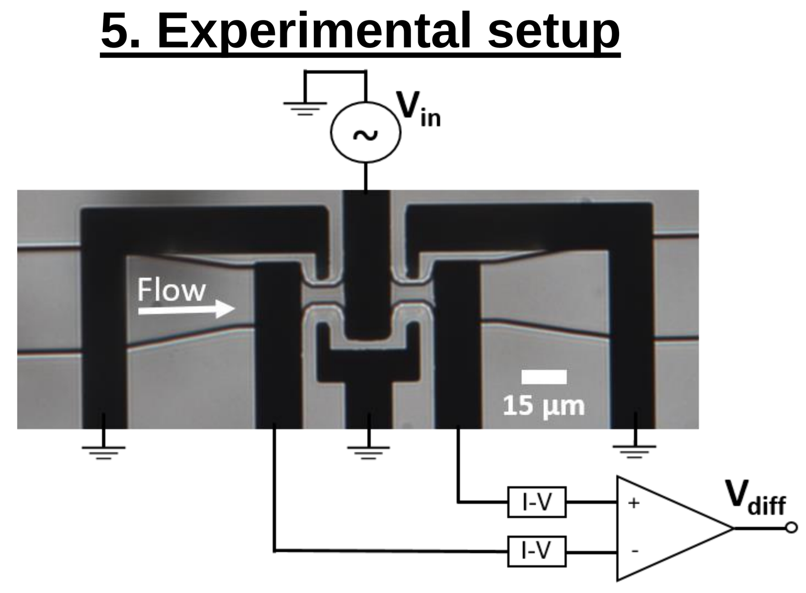

Figure 4: Coplanar electrode configuration for differential impedance measurements.[2] The chip is made of a channel-containing PDMS layer on top of glass with platinum electrodes. The impedance was simultaneously measured at 0.5 and 20 MHz using a lock-in amplifier and preamplifier (Zurich Instruments HF2LI and HF2TA).

6. Experimental results

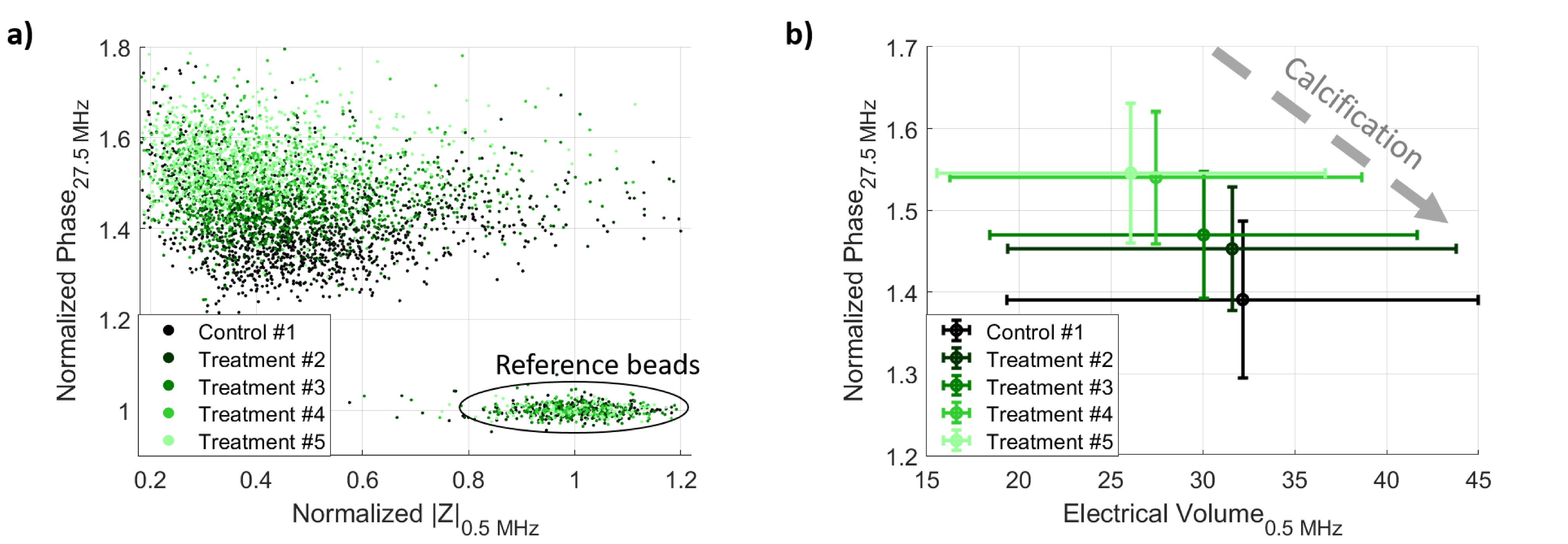

Figure 5: (a) Scatterplot of the normalized phase versus the normalized magnitude for all particles and all treatments (500-1000 cells per treatment). (b) The mean normalized phase as function of the mean electrical volume for each treatment. The transition from decalcified (#5) to calcified (#1) follows the same trend as hypothesized (Figure 3b).

7. Conclusion & Discussion

We can conclude that the electrical volume decreases with the amount of acid and the phase increases with the amount of acid.

This supports the expectation that the size of the exoskeleton decreases with the amount of acid, reducing the total volume of the cell and decreasing the PIC:POC ratio.

In future experiments we will calibrate the presented impedance sensor with mass spectroscopy based measurements of cellular PIC and POC.

Acknowledgements
This work is part of the research program of the Foundation for Fundamental Research on Matter (FOM), which is part of the Dutch Research Council (NWO). We would also like to thank the Max Planck – Center for Complex Fluid Dynamics for part of the funding.

References
[1] B. Rost, U. Riebesell, Coccolithophores and the biological pump: responses to environmental changes, Coccolithophores. (2004) 99–125.
[2] D.S. de Bruijn et al., Calcification state of algae studied with impedance flow cytometry, MicroTAS conference. (2021).