

Maria Dittrich^a,
Beat Müller^a,
Denis Mavrocordatos^b,
Bernhard Wehrli^a

Induced Calcite Precipitation by Cyanobacterium *Synechococcus*

^a Swiss Federal Institute for Environmental Science and Technology, EAWAG, Limnological Research Center, 6047 Kastanienbaum, Switzerland

^b Swiss Federal Institute for Environmental Science and Technology, EAWAG, 8600 Dübendorf, Switzerland

This study investigated the role of the solution composition on calcite precipitation induced by cyanobacteria. The precipitation of calcium carbonate was induced by addition of cyanobacterium cells *Synechococcus* strain PCC 7942 in two artificial solutions with a different composition at similar saturation states in respect to calcite. Ion-selective electrodes for pH, Ca²⁺, and CO₃²⁻ monitored the experiments, and the morphology of precipitated crystals was analysed by scanning electron microscopy.

The calcite precipitation was observed in all experiments after the addition of the cells. The composition of solution (the ratio of dissolved inorganic carbon to dissolved calcium) strongly influenced the calcite precipitation. Based on laboratory experiment results, a possible mechanism for precipitation induced by *Synechococcus* is proposed linking precipitation with the conditions near to cell walls rather than with the saturation conditions in the bulk solution

Durch das Cyanobakterium *Synechococcus* induzierte Calcitfällung

Die durch Zugabe von Zellen des Cyanobakteriums *Synechococcus* (Stamm PCC 7942) induzierte Ausfällung von Calciumcarbonat wurde an zwei ähnlich stark übersättigten wässrigen Calcitlösungen unterschiedlicher Zusammensetzung untersucht. Während des Experiments wurden mit ionenselektiven Elektroden der pH-Wert sowie die Konzentrationen von Ca²⁺ und CO₃²⁻ verfolgt. Außerdem wurde die Morphologie der ausgefällten Kristalle mittels Rasterelektronenmikroskopie untersucht.

In allen Versuchen wurde nach Zugabe der Zellen Calcitfällung beobachtet, dabei erwies sich das Verhältnis des gelösten anorganisch gebundenen Kohlenstoffs zum gelösten Calcium als die entscheidende Einflussgröße für die Calcitfällung. Aufgrund der erhaltenen Ergebnisse wird ein Mechanismus vorgeschlagen, bei dem die induzierte Calcitfällung eher von den Verhältnissen nahe der Zellwand als vom Sättigungszustand der Lösung abhängt.

Keywords: Bacterium, Biomineralization, Ion-selective Electrode, Laboratory Experiment, Scanning Electron Microscopy

Schlagwörter: Bakterium, Biomineralisierung, Ionenselektive Elektrode, Rasterelektronenmikroskopie

Correspondence: M. Dittrich, E-mail: maria.dittrich@eawag.ch

1 Introduction

Both laboratory experiments [1–3] and field observations [4–6] suggest that cyanobacteria play an important role in calcite precipitation in both freshwater and marine systems. Calcite precipitation induced by cyanobacteria is not a mere side effect of photosynthesis in carbonate-rich water [7]. Active calcification has a beneficial effect on organisms and there are several possible positive consequences caused by carbonate precipitation [7]. These include that precipitation might serve as an effective buffer against a pH rise in alkaline environments. This mechanism has also been proposed for calcifying algae [8]. Another effect of precipitation could be the protection of the bicarbonate pump, which is possibly inhibited by carbonate ions [9, 10]. As cyanobacteria are low-light organisms that frequently inhabit environments of high light radiation, a light-shading function of the calcified sheath has been proposed for cyanobacteria [11].

The major physicochemical factor controlling calcite precipitation is the saturation state of the water with respect to calcium carbonate minerals. As calcite formation by cyanobacteria is extracellular, the ambient water has to be supersaturated with respect to calcium carbonate [2]. Calcite precipitation, however, is not always observed in supersaturated hard water lakes [12]. On one hand, different dissolved substances can inhibit the precipitation. On the other hand, calcite nucleation may start in the supersaturated microenvironment that we cannot measure in field experiments. Factors leading to calcite precipitation have to be determined under controlled conditions in order to monitor and separate their importance. This study investigates the role of the solution composition on calcite precipitation using solutions with different compositions, but at similar saturation state. Calcite precipitations were mediated by the addition of cyanobacteria *Synechococcus* strain PCC 7942 in a solution of calcium bicarbonate at different concentrations of inorganic carbon and dissolved calcium.

2 Materials and methods

2.1 Experiments

The precipitation experiments were carried out in a 750 mL five-necked flask placed in a transparent water bath in front of a fluorescent tube (Osram L36W/12-950) emitting a total radiance of $20 \mu\text{E m}^{-2} \text{s}^{-1}$. The water bath was connected to a thermostat (Colora K3DS) to keep the water temperature at 20°C , which is a typical water temperature in hard water lakes in the summer when the calcite precipitation was often observed. Solutions were stirred with a Heidolph MR2002 magnetic stirrer.

The solutions were prepared to represent two different saturation states with respect to calcite. All chemicals were p.a. grade from Fluka Inc., Switzerland, unless otherwise stated. Solutions were obtained by dropwise addition of 350 mL NaHCO_3 to 350 mL $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Thus, solution 1 consisted of $3 \text{ mmol L}^{-1} \text{NaHCO}_3$ and $3 \text{ mmol L}^{-1} \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, whereas solution 2 was $1.5 \text{ mmol L}^{-1} \text{NaHCO}_3$ and $6 \text{ mmol L}^{-1} \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Ion-selective electrodes for pH, Ca^{2+} , and CO_3^{2-} were inserted through the 45 mm central opening and the set-up was equilibrated for 1 h.

The saturation index was calculated as ¹

$$\Omega = [\text{Ca}^{2+}] [\text{CO}_3^{2-}] / K_{s0} \quad (1)$$

where $[\text{Ca}^{2+}]$ and $[\text{CO}_3^{2-}]$ are the concentrations of calcium and carbonate measured with ion-selective electrodes, K_{s0} represents the solubility of calcite $4.047 \cdot 10^{-9} \text{ mol}^2 \text{L}^{-2}$ at 20°C [13].

Liquid membrane ion-selective electrodes were prepared using neutral carries for pH (Ionophore I), Ca^{2+} (Ionophore II, ETH 129) and CO_3^{2-} (Ionophore II, ETH 6019) available from Fluka Inc., Switzerland. PVC-based membranes were cast and mounted into commercially available electrode bodies (Fluka Inc.). The sensors were conditioned overnight and calibrated as described in [14]. Activities were calculated with the Güntelberg approximation [13]. From the signal instabilities and drifts observed, the error of the measurements can be estimated at ± 0.05 for the pH, $\pm 1 \cdot 10^{-5} \text{ mol L}^{-1}$ for CO_3^{2-} , and $\pm 1.2 \cdot 10^{-7} \text{ mol L}^{-1}$ for Ca^{2+} .

The blank experiments were run without the addition of bacteria during approximately 4 h, in order to compare the beginning of the precipitation. Additionally, experiments with seed crystals were also performed. These experiments involved the addition of calcite crystals, which were prepared in a suspension of 60 mg L^{-1} by suspending 9 g CaCO_3 (Merck Suprapure) in 150 mL H_2O . The suspension was left to equilibrate at room temperature for over two weeks before use. Different amounts of seeded crystal (0.5, 1, and 2 mL suspension) were added to the calcium bicarbonate solution of $3 \text{ mmol L}^{-1} \text{NaHCO}_3$ and $3 \text{ mmol L}^{-1} \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

Synechococcus cells suspensions, an unicellular freshwater strain of approximately $0.5 \mu\text{m}$ diameter (PCC 7942 Pasteur Institute, Paris, France), were grown in a culture medium BG11 as a batch culture [15]. The cells were then recovered by centrifugation (2500/min for 20 min). The supernatant was removed leaving approximately 5 mL of concentrated cells in suspension. The cells were washed three times in water. Afterwards, the living cells (as shown through microscopic in-

¹ This terminology deviates from the one in drinking water treatment, where saturation index is defined as $\lg \Omega$.

vestigation) were added to the solution to start the precipitation experiment.

Two experiments with different numbers of cells were performed in solutions. The cell abundance was $5.3 \cdot 10^6$ cells L^{-1} and $4.7 \cdot 10^7$ cell L^{-1} , in the first and second experiment with solutions 1 respectively. We added $7.4 \cdot 10^6$ cells L^{-1} in the first experiment and $1.1 \cdot 10^7$ cells L^{-1} in the second experiment with solution 2.

Immediately after the beginning of each experiment, the number of cells in the reaction vessels were estimated as follows; an aliquot of approximately 1 mL of the solution was passed through a $0.2 \mu\text{m}$ polycarbonate black Nucleopore filter. Cyanobacteria were enumerated following published procedures [16, 17]. Filters were examined with an epifluorescence microscope Zeiss Axiolab, mounted with a Neofluar100x objective, Optovar 1.6x and E-PI 10x ocular with a total magnification of 1600x. 300 to 400 cells were counted on each filter.

At the end of the experiments the samples were filtered through $0.2 \mu\text{m}$ polycarbonate filters. The filters were dried overnight at 50°C and stored in a desiccator. Dry material was then deposited onto SEM stub with carbon tabs. The carbon layer underneath the particles allowed us to analyze the uncoated specimens. The morphology of the precipitates was characterized by scanning electron microscopy (SEM, Philips XL30, LaB₆ filament).

2.2 Balance analysis

Total dissolved inorganic carbon $[C]_T$, was calculated using equations from Stumm and Morgan [13], as follows:

$$[C]_T = [\text{CO}_3^{2-}] / ([\text{H}^+]^2 / (K_1 K_2) + [\text{H}^+] / K_2 + 1)^{-1} \quad (2)$$

with K_1 and K_2 are equilibrium constants.

Assuming a 1:1 relationship between Ca^{2+} concentrations and precipitated CaCO_3 , the total inorganic carbon change during the calcification was estimated at

$$\Delta[C] = [\text{Ca}^{2+}]_{\text{final}} - [\text{Ca}^{2+}]_{\text{initial}} \quad (3)$$

One mole of calcium ion and one mole of total inorganic carbon are released or removed together during the calcium carbonate precipitation. The negative value of $\Delta[C]$ indicated the calcite precipitation, while the positive value reflected the dissolution of calcite.

Consequently, the inorganic carbon that is uptaken by photosynthesis, net photosynthesis, $[P]$ can be calculated as follows:

$$[P] = \Delta[C]_T - \Delta[C]. \quad (4)$$

As photosynthesis calculation represents net photosynthesis, the negative value of photosynthesis indicated uptake of the total inorganic carbon.

3 Results

3.1 Blank and seed crystal experiments

We did not observe any precipitation during the blank experiments that lacked cells. During the seed crystal experiments a decrease of pH values was observed immediately after the

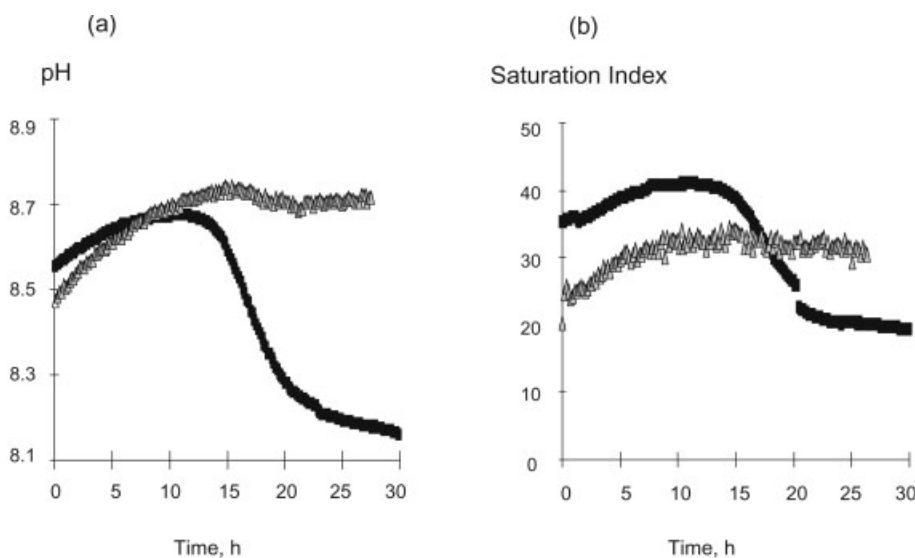


Fig. 1: Changes in pH (a), saturation index (b) during the experiments in the solution 1. Black squares indicate the experiment with the lower abundance of cyanobacteria (experiment 1) and grey triangles indicate the experiment with the higher cell abundance (experiment 2).

Änderungen des pH-Wertes (a) und des Sättigungsindex (b) bei den Versuchen mit Lösung 1. Schwarz: Experiment 1, geringere Zelldichte der Cyanobakterien. Grau: Experiment 2, höhere Zelldichte der Cyanobakterien.

addition of the seed suspension to the solution in the vessel, indicating the onset of precipitation. In all experiments the initial saturations were similar, close to 16, so the differences due to variations in the initial supersaturation are eliminated. A faster decrease of pH was observed in solutions with higher amounts of seed calcite.

3.2 Experiments in solution 1

When the *Synechococcus* cells were added to the vessel, there was a constant increase in pH over a period of 10 to 15 h. Once a critical pH level of approximately 8.7 was reached, calcite precipitation stopped and the pH dropped. A distinct drop in pH was observed during the first experiment (Fig. 1a) over a period of 5 to 6 h. The pH decrease in the second experiment was not as sharp with the pH changing from only 8.75 to 8.70 (Fig. 1a). The pH increase in the first phase occurred simultaneously with the saturation indexes rising up to 40 and 33 in the two experiments, respectively (Fig. 1b).

The SEM images showed that the precipitated crystals smaller than 5 μm were associated with cells. These crystals formed clusters up to 100 μm in diameter. Imprints of the shape of the *Synechococcus* cells sized approximately 1 μm were observed on their surfaces (Fig. 2). Additionally, we observed threads between the crystals which appeared to be very similar to the extracellular polysaccharides described in previous publications [18, 19]. We did not investigate the composition of these threads and so it is still unclear how they were formed.

3.3 Experiments in solution 2

In the beginning, the pH values were 8.4 and increased in both experiments at the same rate during the first 2 h. The pH

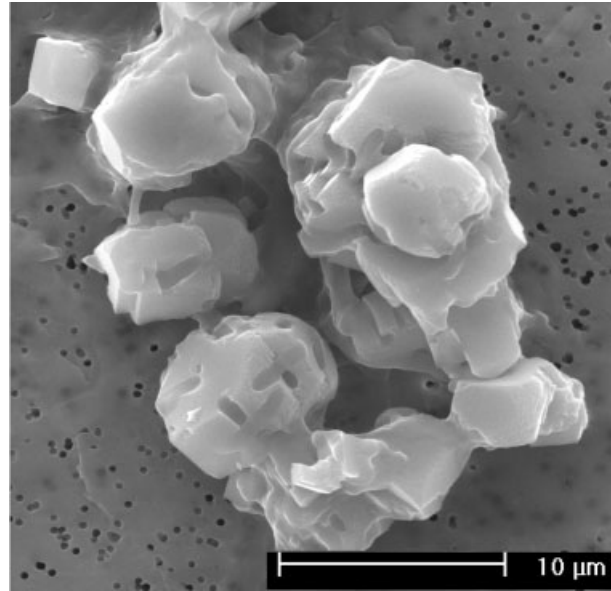


Fig. 2: SEM pictures of the precipitated calcite crystals from the experiments with *Synechococcus* in the solution 1.

Rasterelektronenmikroskopische Aufnahme der aus Lösung 1 in den Versuchen mit *Synechococcus* ausgefallenen Calcitkristalle.

value in the first experiment reached its maximum (8.6) after 4 h. It then dropped to pH = 7.9 and subsequently increased again slowly (Fig. 3a). The second experiment with a higher number of cells lead to a higher pH maximum (8.8), which was reached after about 7 h (Fig. 3a).

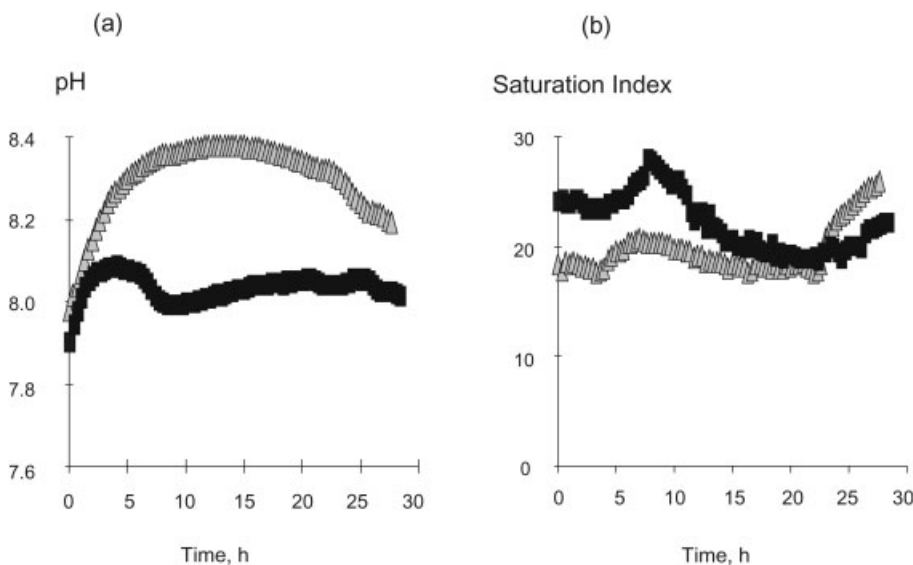


Fig. 3: Changes in pH (a), saturation index (b) during the experiments in the solution 2. Black squares indicate the experiment with the lower abundance of cyanobacteria (experiment 1) and grey triangles indicate the experiment with the higher cell abundance (experiment 2).

Änderungen des pH-Wertes (a) und des Sättigungsindex (b) bei den Versuchen mit Lösung 2. Schwarz: Experiment 1, geringere Zelldichte der Cyanobakterien. Grau: Experiment 2, höhere Zelldichte der Cyanobakterien.

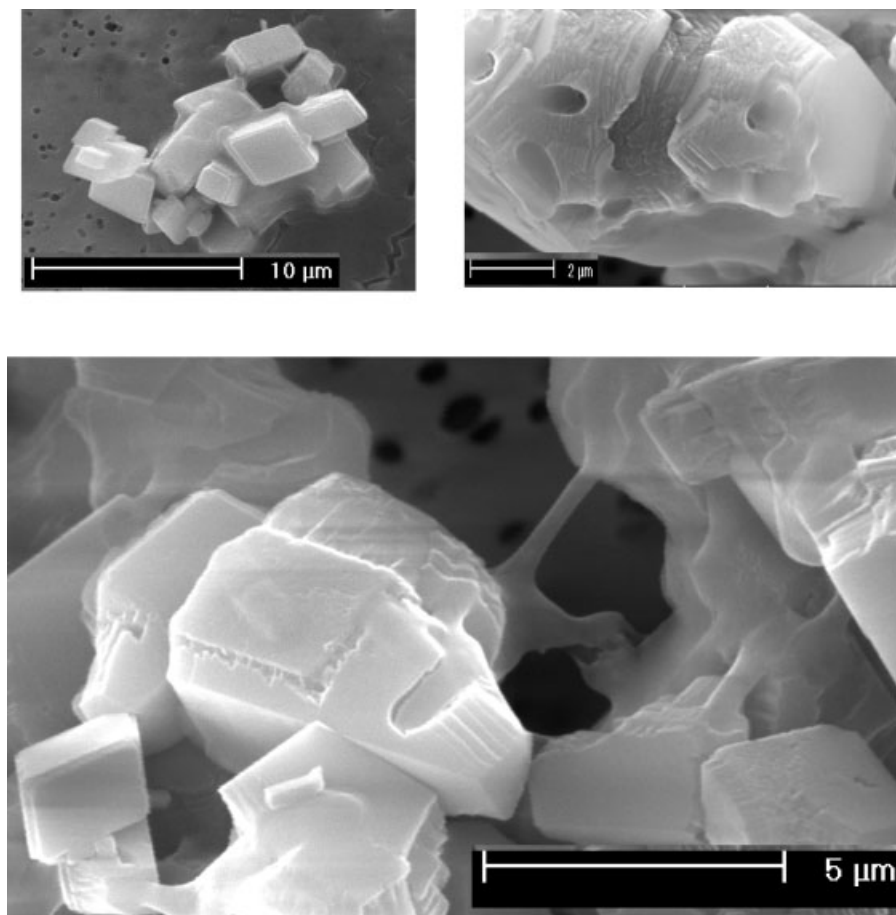


Fig. 4: SEM pictures of the precipitated calcite crystals from the experiments in the solution 2.

Rasterelektronenmikroskopische Aufnahme der aus Lösung 2 in den Versuchen mit *Synechococcus* ausgefallten Calcitkristalle.

As for the experiments with solution 1, the pH did not drop after reaching a maximum level, but remained constant for 10 h. Finally the pH dropped towards a probable steady state. The saturation indexes increased immediately after the addition of the cyanobacteria cells and reached maximums of 28 and 20 (Fig. 3b). We observed calcite crystals of 1 to 5 µm in size. Some crystals were formed around the cells, which are recognized as holes in the crystals (Fig. 4). Similar threads were present between the crystals like in experiment 1.

4 Discussion

Calcite precipitation in hard water lakes is extracellular, therefore lake water has to be supersaturated in respect to calcite. However no calcite precipitation was observed by the high calcite supersaturation [12]. The experimental conditions in this study were close to those of the natural supersaturated hard water lakes where the precipitation was observed, for example Lake Constance, where the mean calcium concentration was approximately 1.5 mmol L⁻¹ and alkalinity

changed from 2.5 to 1.7 mmol L⁻¹ [12]. The laboratory experiments using solutions of the same saturation in respect to calcite allowed us to prove the role of the solution composition in a precipitation process.

The use of the pH, CO₃²⁻, and Ca²⁺ ion-selective electrodes during the experiments was an effective way to monitor the precipitation reactions induced by the growth of cyanobacteria. In addition to pH and Ca²⁺ ion-selective electrodes applied in the study of calcite precipitation upon algal biofilms [18], we used an electrode for carbonate (CO₃²⁻) and were able to calculate the saturation index Ω .

4.1 Solution 1

Experiments performed in solutions with higher levels of inorganic carbon concentration and of lower calcium concentration showed that the increase of pH led to calcite precipitation (Fig. 5). The negative changes of calcium indicated that calcite precipitated during this period.

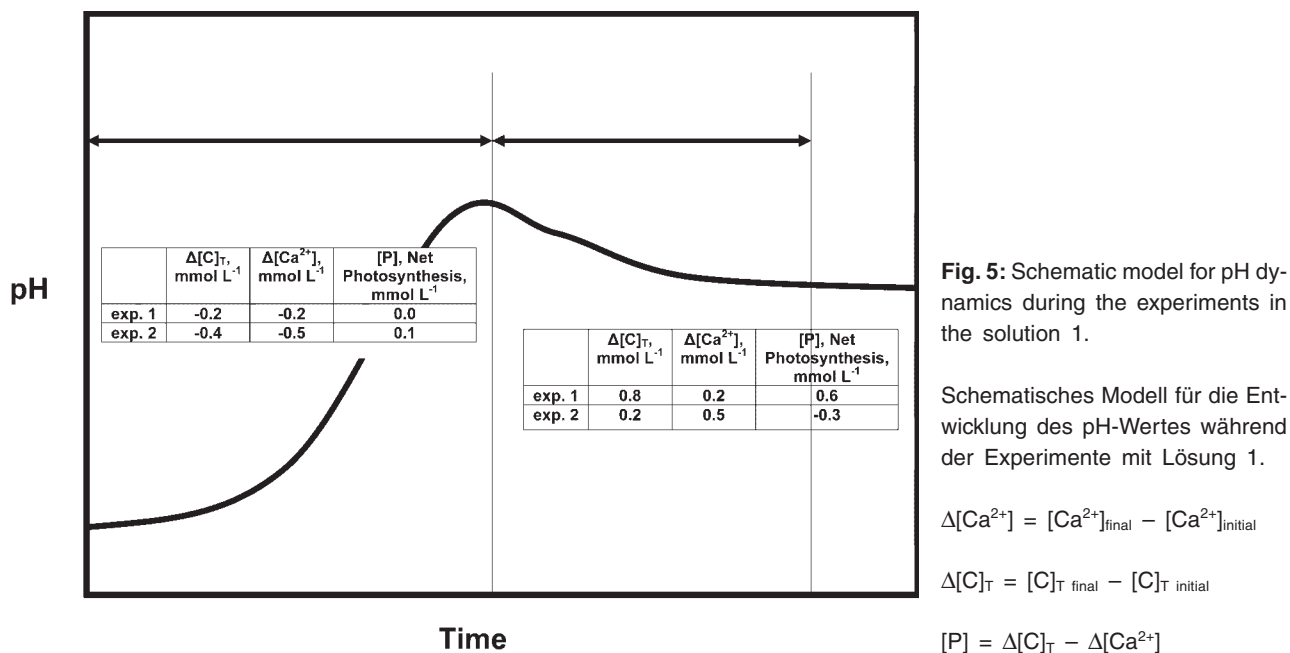


Fig. 5: Schematic model for pH dynamics during the experiments in the solution 1.

Schematisches Modell für die Entwicklung des pH-Wertes während der Experimente mit Lösung 1.

$$\Delta[Ca^{2+}] = [Ca^{2+}]_{final} - [Ca^{2+}]_{initial}$$

$$\Delta[C]_T = [C]_{T\ final} - [C]_{T\ initial}$$

$$[P] = \Delta[C]_T - \Delta[Ca^{2+}]$$

The comparison of the experiments with seed crystals indicated that the added cells caused the increase of pH due to photosynthetic activity. The same time pattern of pH increase over a period of 10 to 15 h and the following drop of pH (as in experiment 1) was observed in the laboratory experiments with unicellular alga *Chlorococcum* [20]. Additionally, the absence of precipitation in the blank experiments supported the assumption that the cells were responsible for the calcite precipitation.

The higher number of cells that were added to the solution can explain the absence of the rapid drop of pH in the second experiment (Fig. 1a). The other reason may be the different metabolic state of the cells, which were grown as a batch culture and were not synchronized in respect of growth stage. The fluorescence microscopic investigations allowed us to calculate the number of metabolic active cells. However, we did not have any information to indicate as to what metabolic state they were in.

The net photosynthesis was positive during the second phase of both experiments indicating that the respiration was higher than photosynthesis. This effect may be caused by the presence of some heterotrophic organisms, as the cultures were not axenic (Fig. 5).

The positive value of the net photosynthesis for the whole first experiment ($(0.0 + 0.6)$ mmol L⁻¹ = 0.6 mmol L⁻¹) indicated the release of dissolved carbon and the overcoming of the respiration over the photosynthesis during the experiments (Fig. 5).

In contrast to the first experiment the total net photosynthesis during the second experiment was negative ($(0.1 - 0.3)$ mmol L⁻¹ = -0.2 mmol L⁻¹) reflecting the dominance of photosynthesis (Fig. 5).

Although we found calcite crystals at the end of the experiments, the total change of calcium concentrations was zero. Due to the sensitivity of the calcium sensor, slight variation in the calcium concentration could not be observed.

4.2 Solution 2

The results from the experiments with solution 2 (performed with lower concentration levels of inorganic carbon and higher levels of calcium concentration), showed the calcite precipitation during the first 5...10 h as the change of dissolved calcium was negative. At this time the net photosynthesis was negative, indicating the uptake of inorganic carbon (Fig. 6). Looking at the whole experiment time, calcite precipitation was dominant over dissolution.

The total net photosynthesis was positive during the first experiment ($(-0.31 - 0.64 + 1.80)$ mmol L⁻¹ = 0.65 mmol L⁻¹) reflecting the dominance of respiration, while the negative value ($(-0.5 - 0.6 + 0.5)$ mmol L⁻¹ = -0.6 mmol L⁻¹) of the second experiment indicated that photosynthesis prevailed.

Nevertheless, calcite was precipitated during both experiments. The total negative value of calcium change ($-0.2 + 0.9 - 2.0$) mmol L⁻¹ = -1.3 mmol L⁻¹ and $(-0.2 + 0.5 - 0.5)$

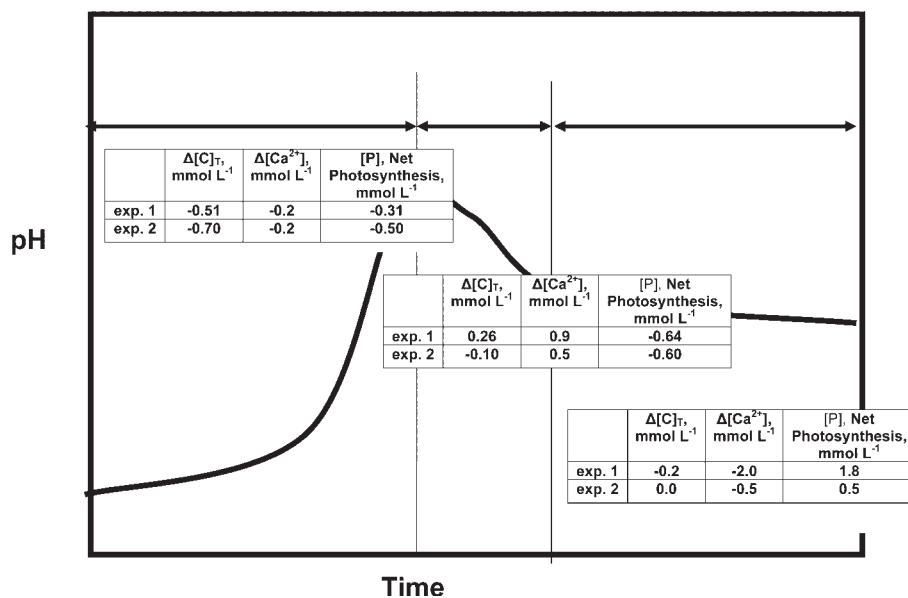


Fig. 6: Schematic model for pH dynamics during the experiments in the solution 2.

Schematisches Modell für die Entwicklung des pH-Wertes während der Experimente mit Lösung 2.

$$\Delta[Ca^{2+}] = [Ca^{2+}]_{\text{final}} - [Ca^{2+}]_{\text{initial}}$$

$$\Delta[C]_T = [C]_{T \text{ final}} - [C]_{T \text{ initial}}$$

$$[P] = \Delta[C]_T - \Delta[Ca^{2+}]$$

mmol L⁻¹ = -0.2 mmol L⁻¹ reflected remarkably more calcite precipitation during the first experiment than during the second experiment.

The results from the experiments with both solutions showed that the intensity of calcite precipitation induced by cyanobacteria *Synechococcus* depends on the composition of the solution, rather than the saturation state. Although the saturation index of the solutions were the same, the addition of cyanobacteria led to different calcium changes in solutions 1 and 2.

The former laboratory experiments with filamentous cyanobacteria *Scytonema* and *Schizothrix* from freshwater mats, demonstrated that two factors can be of major importance: the bicarbonate uptake and the suitability of the sheath for carbonate nucleation [7]. Our experiments however, indicated that it was the ratio between dissolved carbon and calcium that influenced the calcite precipitation, rather than the bicarbonate uptake.

The occurrence of a different intensity of precipitation by the same saturation state provided the first evidence that the microenvironment and physiological state of cells may be responsible for the calcite nucleation. The model for precipitation remains still unclear. Either calcium ions were adsorbed on the sheath and precipitated with carbonate ions, or alkaline microenvironment of cells provides an environment that is conducive to calcite precipitation in the presence of Ca.

The morphology of the calcite crystals indicates that the cell walls of the cyanobacteria acted as a substrate of nucleation. The precipitated crystals contain marks and holes of the same shape and size as the *Synechococcus*. Crystals were

also found with the same imprints in experiments involving field and laboratory studies [12, 20]. In former experiments with *Synechococcus* hexagonal mineral morphologies had been found [21] on calcified membranes.

The next step will be to provide an in-situ observation of the precipitation using different ratios of calcium and inorganic dissolved carbon. Such kinds of experiments have already been performed studying abiotic calcite precipitation by applying the atomic forced microscopy (AFM) [22]. A tool to measure chemical parameters and to observe the reaction under the microscope simultaneously is now under construction. This method will allow an in-situ observation of the calcite nucleation of the cell and consequently the details of the precipitation mechanism.

5 Conclusion

The results of these initial experiments are encouraging and demonstrate by direct measurements the potential of cyanobacteria *Synechococcus* to precipitate calcite. The amount of the precipitated calcite varied in experiments using solutions with a different ratio of dissolved inorganic carbon and calcium. Remarkably more calcite precipitated in the solution with higher carbon but lower calcium concentrations. The results of the experiments provide some evidence that the cell walls of the cyanobacteria acted as a substrate of nucleation of CaCO₃. The unicellular cyanobacteria *Synechococcus* induced the precipitation of the rhombohedral calcite crystals under both conditions. Ion-selective electrodes were shown

to be a useful tool for precipitation experiments. The precipitated crystals contain marks and holes of the same shape and size as the *Synechococcus* cells.

Acknowledgements

We would like to thank Ruth Stierli for her help with the ion-selective electrodes and Christina Schnock for the technical assistance. Critical and constructive comments from two reviewers helped to improve this paper.

References

- [1] Thompson, J. B., Ferris, F. G.: Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology* **18**, 995–998 (1990).
- [2] Merz-Preiss, M.: Calcification in cyanobacteria. In: *Riding, R. E., Awramik, S. M.* (Eds.): *Microbial Sediments*. Springer, Berlin, 2000, pp. 50–56.
- [3] Yates, K. K., Robbins, L. L.: Radioisotope tracer studies of organic carbon and calcium in microbially derived CaCO₃. *Geochim. Cosmochim. Acta* **1**, 129–136 (1999).
- [4] Hodell, D. A., Schleske, C. L., Fahnenstiel, G. L., Robbins, L. L.: Biologically induced calcite and its isotopic composition in lake Ontario. *Limnol. Oceanogr.* **43**, 187–199 (1998).
- [5] Robbins, L. L., Yates, K. K., Shinn, G., Blackwelder, P.: Whiting on the great Bahama bank: a microscopic solution to a macroscopic mystery. *Bahamas J. Sci.* **10**, 2–6 (1996).
- [6] Thompson, J. B., Schultze-Lam, S., Beveridge, T. J., Des Marais, D.: Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial picoplankton. *Limnol. Oceanogr.* **42**, 133–141 (1998).
- [7] Merz, M.: The biology of carbonate precipitation by cyanobacteria. *Facies* **26**, 81–102 (1992).
- [8] Raven, J. A., Smith, F. A., Walter, N. A.: Biomineralization in the Charophyceae sensu lato. In: *Leadbeater, S. C., Riding, R.* (Eds.): *Biomineralization in Lower Plants and Animals*. Clarendon Press, Oxford, 1986, pp. 125–139.
- [9] McConnaughey, T. A. in *Doumenge F, A. D., Toulemont A.* (Eds.): *Past and Present Biomineralization Processes*; Bulletin de l'Institut Océanographique, Monaco. Musée Océanographique, Monaco, 1994; Vol. Numero special **13**, pp. 137–162.
- [10] Lukas, W. J.: Photosynthetic assimilation of exogenous HCO₃⁻ by aquatic plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **34**, 71–104 (1983).
- [11] Rowland, S. M., Gangloff, R. A.: Structure and paleoecology of lower cambrian reefs. *Palaios* **3**, 111–135 (1988).
- [12] Stabel, H. H.: Calcite precipitation in Lake Constance: chemical equilibrium, sedimentation, and nucleation by algae. *Limnol. Oceanogr.* **31**, 1081–1093 (1986).
- [13] Stumm, W., Morgan, J.: *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters*. John Wiley & Sons, New York, 1996.
- [14] Müller, B., Bius, K., Stierli, R., Wehrli, B.: High spatial resolution measurements in lake with PVC based liquid membrane ion-selective electrodes. *Limnol. Oceanogr.* **7**, 1728–1733 (1998).
- [15] Rippka, R., Waterbury, J. B., Stanier, R. Y.: Isolation and purification of cyanobacteria: some general principles. In: *Staff, M. P., Stolp, H. G., Truper, H. G., Balows, A., Schlegel, H. G.* (Eds.): *The Prokaryotes*. Springer, Berlin, 1981, pp. 212–220.
- [16] Callieri, C., Pinolini, L. M.: Picoplankton in Lake Maggiore, Italy. *Int. Rev. Gesamten Hydrobiol.* **3**, 491–501 (1995).
- [17] MacIsaac, E. A., Stockner, J. G.: Enumeration of phototrophic picoplankton by autofluorescence microscopy. In: *Kemp, P. F., Sherr, B. F., Sherr, E. B., Cole, J. J.* (Eds.): *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, 1993, pp. 187–197.
- [18] Hartley, A. M., House, W. A., Leadbeater, S. C., Callow, M. E.: The use of microelectrodes to study the precipitation of calcite upon algal biofilms. *J. Colloid Interface Sci.* **183**, 498–505 (1996).
- [19] Beech, I. B., Tapper, R. C.: Exopolymers of sulphate-reducing bacteria. In: *Wingender, J., Neu, T. R., Flemming, H. C.* (Eds.): *Microbial Extracellular Polymeric Substances*. Springer, Berlin, 1999, pp. 119–127.
- [20] Hartley, A. M., House, W. A., Callow, M. E., Leadbeater, S. C.: The role of a green alga in the precipitation of calcite and the coprecipitation of phosphate in freshwater. *Int. Rev. Gesamten Hydrobiol.* **80**, 385–401 (1995).
- [21] Schultze-Lam, S., Harauz, G., Beveridge, T. J.: Participation of a cyanobacterial S layer in fine-grain mineral formation. *J. Bacteriol.* **174**, 7971–7981 (1992).
- [22] Dove, P. M., Hochella, M. F.: Calcite precipitation mechanisms and inhibition by orthophosphate: In situ observations by scanning force microscopy. *Geochim. Cosmochim. Acta* **57**, 705–714 (1993).

[Received: 11 December 2002; accepted: 30 May 2003]