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Supplementary material: Microbial-induced calcium carbonate precipitation: An experimental toolbox for in situ and real time investigation of micro-scale pH evolution†

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In this contribution we present two experimental methods to monitor pH changes in small volumes for microbial-induced calcium carbonate precipitation (MICP). The global pH monitoring method monitors pH changes in small volumes in real time using absorption spectroscopy. The monitored pH changes have been correlated with precipitation processes in the sample with help of optical microscopy. In addition, local pH changes have been monitored on a grain scale with confocal laser scanning microscopy (CLSM) in real time and *in situ*. The supplementary information contains, schematic drawings, calibration curves and signal processing information for the presented methods, as well as supplementary microscope images to the experiments shown in the main document.

1 Methods

1.1 Global pH monitoring

Absorption spectroscopy was used to monitor pH changes in small volumes (200 μL). Absorption measurements were performed with a spectrophotometer (SpectraMax® i3 Platform). The pH sensitive dye Phenol Red (0.4 μM) was used to make pH changes optically detectable. Phenol Red shows a strong pH dependence at 558 nm and 434 nm, leading to a continuous color change from yellow at low pH to pink at high pH values. Reaction solution and Phenol Red were added to a 96-well plate. After starting the reaction, the well plate was covered with transparent tape to minimize the gas exchange with the environment. During the reaction the temporal change of the absorption intensity at 558 nm was measured. In addition, to compensate for scattering due to the crystals in the solution, the background signal at the wavelength of 800 nm was measured, and the background corrected signal was used for pH calculations. A calibration curve was created by measuring the absorption intensity for the buffer solutions with well-known pH values (Figure S3, Figure S4). The background corrected absorption signal of the measurement can then be converted to pH values, and the average pH changes of the sample were detected (Figure S5).

1.2 Local pH monitoring

A confocal laser scanning microscope (Leica TCS SP5) (CLSM) was used to investigate local pH evolution on a single crystal level *in situ* and in real time. For that, a fluorescent pH-sensitive dye, R6G-EDA (N-(rhodamine 6G)-lactam-ethylenediamine), was added at a concentration of 0.18 mM to the reaction solution. In addition, a pH insensitive dye, Sulforhodamine 101 (SR101), was added with a final concentration of 15 μM. For measuring local differences of the pH, a flow-cell setup was used. For the flowcell Ibidi polymer slides and Ibidi sticky-Slide VI 0.4 were used (Ibidi GmbH, Gräfelfing, Germany).

A CLSM is used to scan the area surrounding crystallization or dissolution processes. The two fluorescent dyes were excited with an argon laser (excitation wavelength: 514 nm) and a diode pumped solid state laser (excitation wavelength: 561 nm). For spatiotemporal pH measurements the images were scanned sequentially line-by-line to avoid cross-talk between the two fluorescent dyes. The fluorescent signal was detected with two Leica HyD detectors in photon counting mode. R6G-EDA and SR101 were detected separately by setting the emission filter to 525 nm to 554 nm and 578 nm to 625 nm, respectively. The scan speed was set to 100 Hz, and image resolution was 512×512 pixel. The bit depth was set to 12 bit. All measurements were performed with a pinhole size of 1 Airy. After the measurements, images were processed using a 3×3 median filter, and the intensity ratio between R6G-EDA and SR101 was calculated pixel-by-pixel. Two standard curves were generated in order to convert the ratio of fluorescent intensities to pH values. For the standard curve, images were taken for 13 pH values in a range from 3.1 to 9.5. The intensity

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ratios were calculated and fitted with a Sigmoid function:

$$I = A_2 + \frac{(A_1 - A_2)}{(1 + \exp((pH - x_0)/dx))} \quad (1)$$

with the fitting parameters A_1: inital value; A_2: final value; x_0: center; dx: slope. The curves were used to convert intensity ratios into pH values. For precipitation processes the ratio between SR101 to R6G-EDA was used (further referred to as ratio 1, Figure S6). For dissolution the ratio of fluorescent intensities of R6G-EDA to SR101 was used (in the following referred to as ratio 2, Figure S7).

2 Supplementary Material

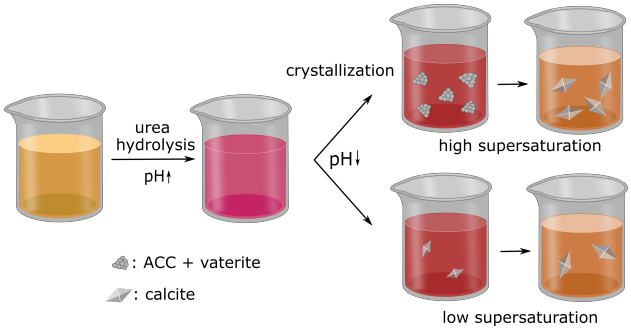

Fig. S1 Schematic illustration of the precipitation process: The pH is increasing due to urea hydrolysis. leading to supersaturation and precipitation. The pathway depends on the level of supersaturation. For high supersaturation ACC and vaterite precipitate first, transforming into calcite over time. For lower supersaturation, calcite is the favoured nucleating polymorph. Crystallization decreases the pH, indicated with a color-change of the pH indicator Phenol Red.

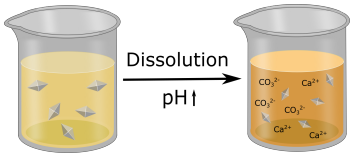

Fig. S2 Schematic illustration of the dissolution process: $CaCO_3$ crystals are dissolved with a lactic acid solution. The pH increases over time, leading to a color change of the pH indicator Phenol Red. Ca^{2+} and CO_3^{2-} are released into the solution, where CO_3^{2-} in aqueous solution reacts to bicarbonate and hydroxide ions according to Equation 9.

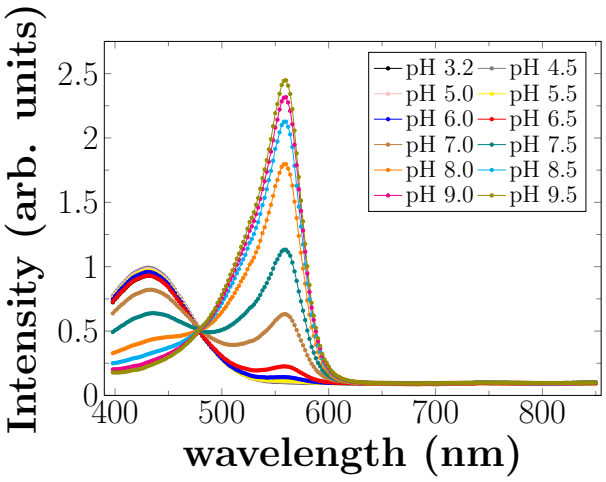

Fig. S3 Absorption spectra of pH sensitive dye Phenol Red. The pH sensitive dye Phenol Red shows two pH dependent absorption maxima at 558 nm and 434 nm. The background of measurements can be detected at wavelengths in the range of 650 nm to 850 nm since in this areas, there is no signal from the dye itself.

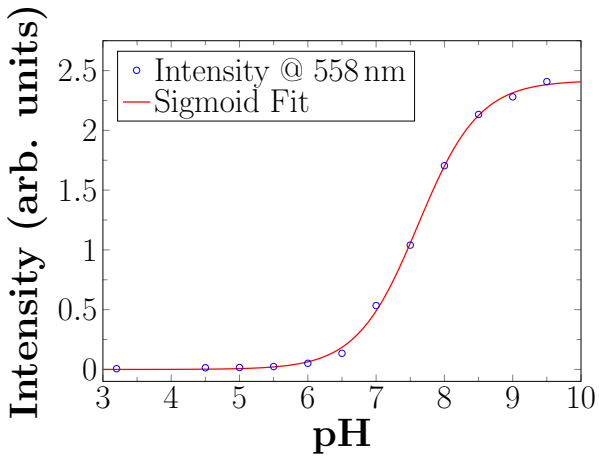

Fig. S4 Calibration curve for global pH measurements. The intensity values of the background corrected absorption maxima at 558 nm in dependence of the pH are fitted with a sigmoid function.

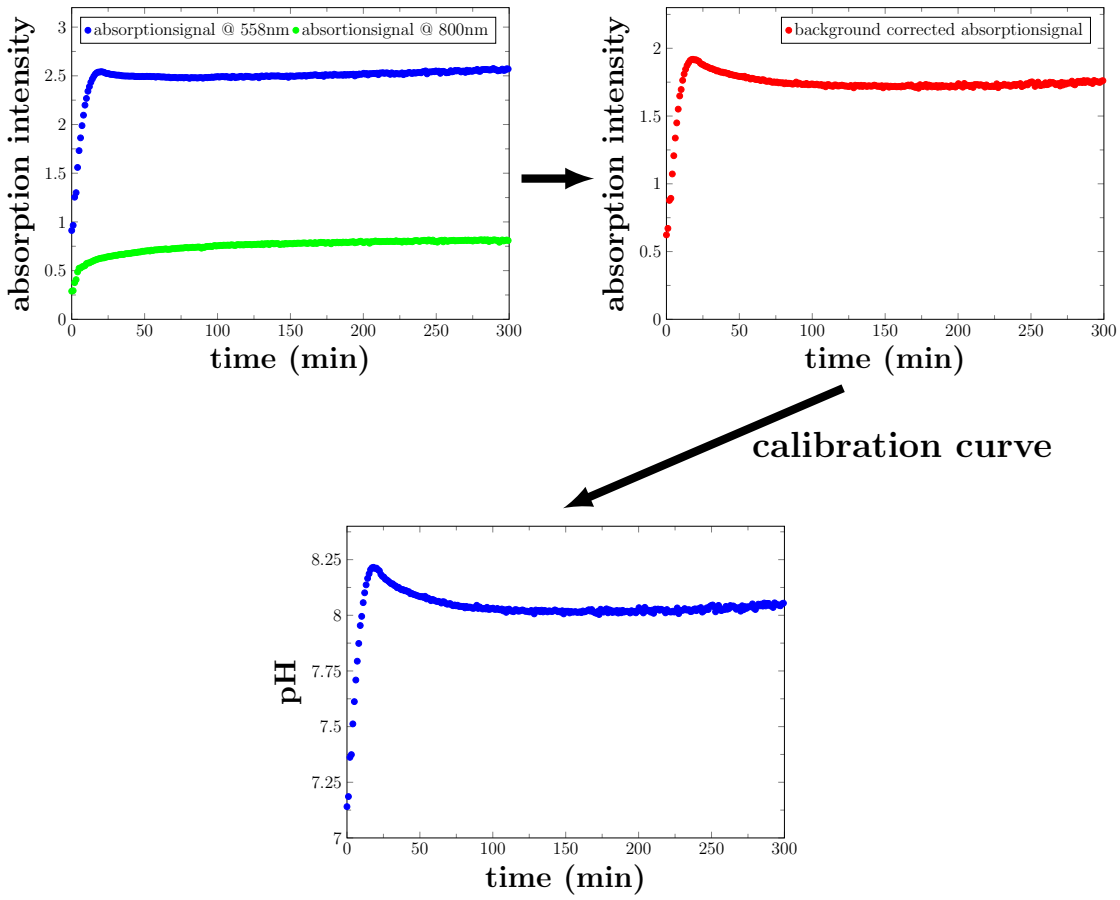

Fig. S5 Signal processing during global pH monitoring measurements: Absorption intensity at 558 nm and background signal at 800 nm are recorded over time. Substracting the two signals results in the background corrected signal. The background corrected signal can be converted to pH values with the help of the calibration curve (Figure S4).

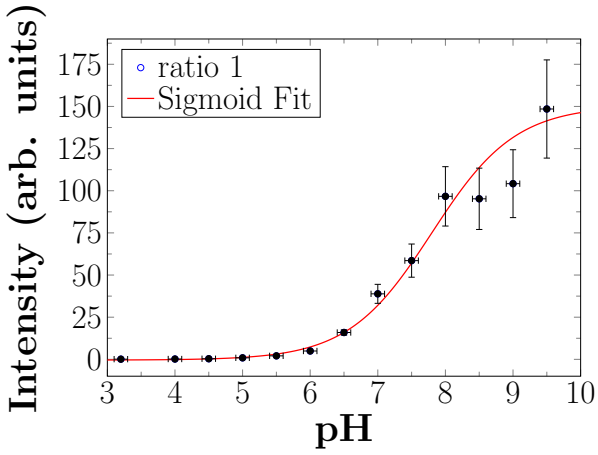

Fig. S6 Calibration curve (ratio 1) for local pH monitoring in precipitation experiments. The calibration values were fitted with a sigmoid function.

Chemical	Concentration
Peptone from casein	$15\,\mathrm{g\,l^{-1}}$
peptone from soy meal	$5\,\mathrm{g\,l^{-1}}$
NaCl	$5\,\mathrm{g\,l^{-1}}$
urea	$20\,\mathrm{g\,l^{-1}}$

Table S1 DSMZ medium 220

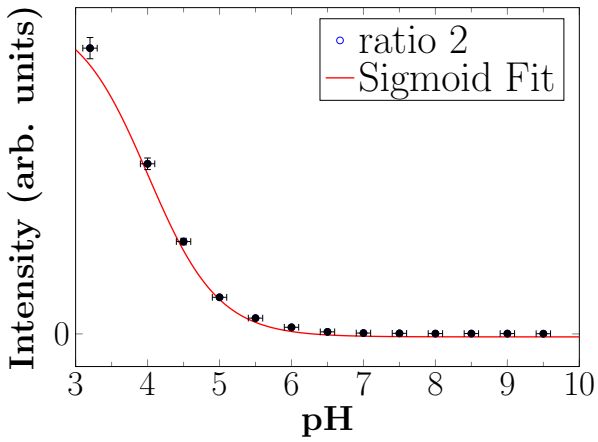

Fig. S7 Calibration curve (ratio2) for local pH monitoring in dissolution experiments. The calibration values were fitted with a sigmoid function.

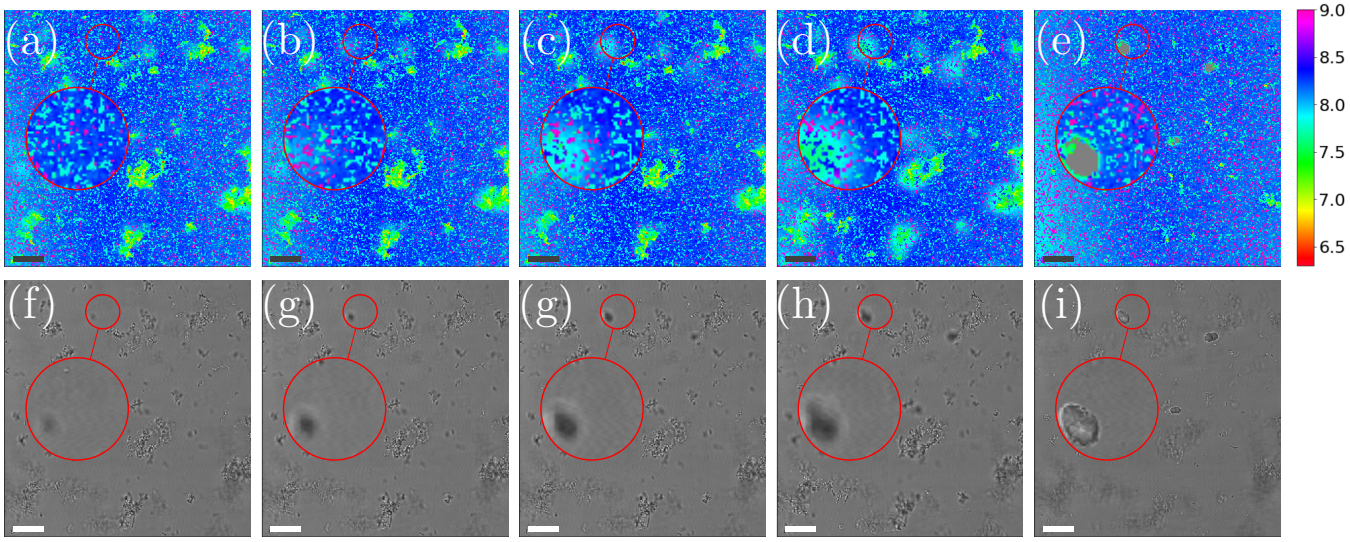

Fig. S8 Spatial pH map during the crystallization process (a)-(e) and corresponding brightfield images (f)-(i). The shown time-points are 12 min, 13 min, 14 min, 15 min, 16 min after the start of the experiment. Calcite growth starts out of focus after 11 min. The crystal increases in size and appears in the focus after 14 min. Spatial pH distribution shows lower pH during the crystallization (b,c,d). The pH is color-coded and can be identified with the color-bar on the side. The scale-bar in all images is 25 μm.

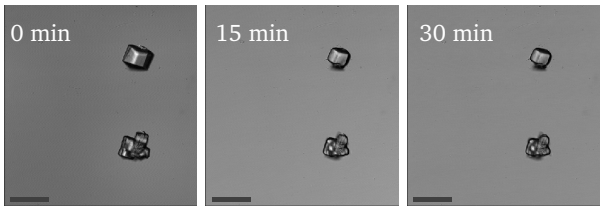

Fig. S9 Dissolution of calcite crystals with lactic acid right after adding the acid to the flow cell (=0 min), after 15 min and 30 min reaction time. The scale-bar corresponds to 75 μm.