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Optical Sensing of pH and O₂ in the Evaluation of Bioactive Self-Healing Cement

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ABSTRACT: Leakage from cementitious structures with a retaining function can have devastating environmental consequences. Leaks can originate from cracks within the hardened cementitious material that is supposed to seal the structure off from the surrounding environment. Bioactive self-healing concretes containing bacteria capable of microbially inducing CaCO₃ precipitation have been suggested to mitigate the healing of such cracks before leakage occurs. An important parameter determining the biocompatibility of concretes and cements is the pH environment. Therefore, a novel ratiometric pH optode imaging system based on an inexpensive single-lens reflex (SLR) camera was used to characterize the pH of porewater within cracks of submerged hydrated oil and gas well cement. This enabled the imaging of pH with a spatial distribution in high resolution (50 μm per pixel) and a gradient of 1.4 pH units per 1 mm. The effect of fly ash substitution and hydration time on the pH of the cement surface was evaluated by this approach. The results show that pH is significantly reduced from pH >11 to below 10 with increasing fly ash content as well as hydration time. The assessment of bioactivity in the cement was evaluated by introducing superabsorbent polymers with encapsulated Bacillus alkalinitrilicus endospores into the cracks. The bacterial activity was measured using oxygen optodes, which showed the highest bacterial activity with increasing amounts of fly ash substitution in the cement, correlating with the decrease in the pH. Overall, our results demonstrate that the pH of well cements can be reliably measured and modified to sustain the microbial activity.

INTRODUCTION

Crack formation can severely reduce the lifetime of cementitious materials. Even small cracks are penetrated by water with erosive and corrosive ions that further widen the cracks and corrode steel reinforcements. In installations designed with a retaining function, cracks may compromise the tightness, allowing either dangerous substances to reach the environment or loss of pressure in a pressurized system. One such cement application where tightness is highly important is the cementing of wells used in the oil and gas industry. Here, cementing serves to position steel casings in the borehole and to seal off and isolate different sections of the well. Cracks in oil and gas well cement installations can lead to the loss of zonal isolation in the well, resulting in a significant loss in well productivity with a corresponding negative economic impact. Another concern is “plug and abandonment” of oil and gas wells, where decommissioned wells are plugged with cement to contain the remnant gas and oil inside the abandoned well. Loss of zonal isolation in active oil and gas wells and fluid leakage due to cracks in cement plugs can lead to serious environmental issues. Strategies to potentially mitigate these cracks in the cement are self-“healing” or “sealing” of the cracks by either chemical or biological agents mixed into the wet cementitious mixture. Chemical self-healing agents typically consist of encapsulated precursors of polymers, such as epoxy resins or cyanoacrylates. Recently, biologically based healing of concrete has been proposed as an alternative self-healing technique, utilizing bacterial activity to precipitate CaCO₃ inside the cracks of concrete in above-ground structures. Products of chemical healing agents designed for well cement are commercially available; however, a bioactive self-healing well cement is yet to be engineered. The limitation on biological activity in well cement lies in the nature of the cement composition. Contrary to concretes, well cement does not contain sand or other large aggregates; instead, it is a pure cement paste with various

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additives. Cement produces alkalinity during hydration due to the dissolution of alkali ions and portlandite \((\text{Ca(OH)}_2)\) from the cement phases. As pH influences the bacterial activity, it is paramount to investigate the pH within well cements to further the potential development of a bioactive self-healing cement for use in oil and gas wells.

The pH of concretes has previously been measured with potentiometric or fiber optic sensors, and recently with optical sensing using planar optodes with an advanced lifetime camera, generating high-resolution images that show a detailed spatial distribution of the pH. Based on measurements with potentiometric sensors, the pH of concrete can be as high as 12.5; however, these values often represent the pH of milled concrete suspensions in water and not as such the pH of the porewater within the cementitious matrix. Fiber optic sensors can be cast inside the cementitious matrix and record the pH over time (pH 12.6); however, this technique does not take the spatial resolution of the matrix into consideration.

The pH of well cements has previously only been determined for slurry mixes (pH > 11.8), however, no high-resolution data of the spatial distribution of pH in the porewater or inside cracks exists for hardened well cements used in the oil and gas industry. Microbial life occurs in natural alkaline environments; however, a pH above 11 is generally not suitable for bacterial activity. Thus, such high pH values would hardly allow any bacteria-induced precipitation of CaCO\(_3\) to occur.

Here, a basic single-lens reflex (SLR) camera was modified to image the pH and oxygen consumption in a highly alkaline cracked well cement. In an effort to reduce the pH of well cement specimens, pozzolanic fly ash was added, which reacts with calcium and hydroxyl ions in solutions, reducing the amount of formed portlandite and thereby the pH at high substitution levels. A novel pH planar optode was used to measure the pH of the porewater in the cement matrix and the water in induced cracks in hardened well cements. The experiments were conducted with various amounts of fly ash substitution to evaluate the effect on the pH. The bioactive potential of well cement was evaluated by measuring the oxygen consumption inside the cracks using oxygen planar optodes.

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**RESULTS AND DISCUSSION**

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**Two-Dimensional (2D) Determination of pH Inside of Cracks of Well Cement.** It is paramount to understand the abiotic factors affecting the metabolic activity and growth when implementing biology in a nonbiological system. For cementitious materials, the highly alkaline pH of the pore solution likely represents a key controlling factor. Imaging pH at highly alkaline conditions found within cement is not trivial and has only recently been demonstrated for concrete surfaces. While those studies used a lifetime-based readout, the present work is the first report of the ratiometric pH imaging of cement using a single-lens reflex camera that can be acquired easily and without large investments (approximately $1000). As the applied pH indicator emits light in the near-infrared (NIR) region of the electromagnetic spectrum, it was necessary to remove the NIR blocking filter from the camera prior to the experiments. A typical sigmoidal calibration curve was obtained with this equipment, and the pH indicator system enabled imaging pH within the range 8–11 (Figure 1). Measuring pH optically allowed the visualization of the spatial distribution of pH in the porewater of the cement matrix and in the induced cracks without destructive sampling. The pH of the well cement was measured in a high resolution of up to 50 μm per pixel with a steep gradient of 1.4 pH unit per 1 mm visually observed, equivalent to more expensive systems.

The optode design allowed measurements of pH at the cement surface when pressed against the pH-sensitive film of the optode (Figure 2). The measured values represent the pH of the water layer in contact with the optode and in direct equilibrium with the porewater in the outer layers of the cement specimens. In agreement, the substitution of fly ash caused the pH measured inside cracks to decrease (Figure 2). Cement hydration results in the formation of a calcium–silicate–hydrate (C–S–H) phase and portlandite as the main hydration products, where the solubility of portlandite buffers the pH of the pore solution. The reaction of fly ash consumes portlandite and forms C–S–H and calcium aluminate hydrate phases similar to those found in hydrated Portland cement. Thus, the degree of fly ash reaction depends on the available amount of portlandite and high substitution levels can thereby be used to reduce the pH of the pore solution in blended cements. The pH of the porewater in

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**Figure 1.** Calibration curve of pH optode and experimental setup. The colors of the calibration curve are the true colors of the raw RGB images at the respective pH. The calibration curve has its dynamic range between pH 8 and 11, which is thus the pH range of the optode. The cement specimen is pressed on the optode and kept in place by a distance spacer. The camera with removed NIR filter is coupled to the LED light source through a trigger box controlled by the image acquisition program on the computer.

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which is the biological agent used in self-healing concrete specimen to optode contact. The measurements were highly replicable but did depend on a good percentage of fly ash, the pH of the cement pore solution. For the 30 wt % fly ash substitution, the pH decreased from 10.5 to 9.75 with a suitable growth medium. The aerobic respiratory spores were added to cracks in cement specimens and hydrated with a suitable growth medium. The aerobic respiratory activity of the bacteria inside the cracks was subsequently monitored with oxygen optodes, which enables the reversible visualization of oxygen concentrations in 2D (similarly to the

Figure 2. pH imaging of well cement specimens with varying percentages of fly ash substitution. The optode measures pH in the range of 8–11, thus the white color in the images correspond to a pH value of more or equal to 11 and black less or equal to 8. The dotted line is the outline of the cement specimens with induced cracks. Substituting 10 wt % of the cement with fly ash decreases the pH within the pH range of the optode. Increasing the substitution of fly ash does not seem to influence the pH of the porewater in the cement itself; however, the induced crack becomes more and more visible due to a decrease in the cements alkaline buffering capacity. At 30–50 wt % fly ash substitution, the pH of the ingressed ASW is not nearly as affected by the cement as in 0–20 wt %. The 40 wt % specimen had a rougher surface and therefore not as good a contact to the optode as the others, which is why the pH fluctuates in this particular image. The measurements were highly replicable but did depend on a good specimen to optode contact.

Figure 3. pH imaging of well cement with 0 or 50 wt % fly ash substitution and 1 (top) or 2 (bottom) months of prehydration times. Extended prehydration time alone reduced the pH of the cement porewater from above 11 to ~9. Substituting 50 wt % of the cement with fly ash and extending the prehydration time decreased the pH of the cement porewater to below 10 and that inside the crack to ~9.

Decrease of pH by Increased Hydration Time to Potentially Sustain Bacterial Activity in Cracks of Well Cement. Portlandite is mainly produced by the reaction of the calcium silicate phases, alite and belite (impure forms of Ca$_3$SiO$_5$ and Ca$_2$SiO$_4$, respectively), the principal phases in Portland cement, which also result in the formation of the C–S–H phase, which is the main phase responsible for the strength development. Alite reacts within hours to days, whereas belite hydration takes place over roughly 1–4 weeks, contributing to the strength development at later ages. After a few days, the formed C–S–H phase resulted in a dense material, slowing down the hydration of the remains of alite and belite, as the dissolution and precipitation reactions become diffusion controlled. Fly ash reacts after the hydration of alite and over longer time and thus the amount of portlandite in the cement matrix will decrease with time and increasing degree of fly ash reaction. Thus, the pH in the pore solution will decrease, in particular after the depletion of portlandite. The consumption of portlandite can explain the decrease in the pH when comparing cement specimens prehydrated for 1 month (pH > 11) to those prehydrated for 2 months (pH ~ 10) (Figure 3). For the specimen with 50 wt % fly ash substitution, the pH decreased from 10.5 to 9.75 for the specimens hydrated for 1 and 2 months. The pH inside the crack decreased even more from 10 to 9.25 in the part of the crack furthest away from the penetrating ASW. Thus, the addition of a significant amount of pozolanic material such as fly ash to the cement results in an accelerated decrease in pH, thereby potentially further facilitating bacterial activity.

Mapping Bacterial Activity in Well Cement. As discussed above, the measured pH regime within submillimeter-sized cracks of pure and fly ash substituted well cement suggest that only the fly ash substituted cement specimens will sustain bacterial activity. To test this prediction, freeze-dried superabsorbent polymers (SAPs) with B. alkalinitrilicus endospores were added to cracks in cement specimens and hydrated with a suitable growth medium. The aerobic respiratory activity of the bacteria inside the cracks was subsequently monitored with oxygen optodes, which enables the reversible visualization of oxygen concentrations in 2D (similarly to the

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pH optode images) to quantify bacterial oxygen consumption inside the cracks.

In all cement specimens, the oxygen air saturation within cracks containing hydrated SAPs decreased immediately upon exposure to the oxygen optode (Figure 4), demonstrating that

![Figure 4. Oxygen imaging of the bacterial activity of SAPs encapsulated endospores induced to germinated inside cracks of well cement. The air saturation decreases due to aerobic bacterial activity. Arrows indicate microbially active SAP particles inside the cracks of the cement specimens. The microbial oxygen consumption is detected in all images, even inside the cracks of pure well cement; however, the activity increases with increasing amounts of fly ash substitution. The oxygen consumption seen in the bottom of the images in the specimens is due to the bacterial activity originating from the crack transported downward by a thin flow of water between the cement specimen and optode.](image)

the embedded endospores of the bacteria were able to germinate and grow even in the pure well cement. However, the inferred respiratory activity was very low and spotty in the cracks of pure well cement but increased substantially with the increasing degree of fly ash substitution and thus decreasing pH (Figure 2). The pH decreased from above 10 to 9.25 inside the cracks in these samples with increasing fly ash substitutions. B. alkalinitrilicus has a pH range of 7–10.2 with an optimum at 9.33 which leads to more activity and O2 consumption in the samples with the most fly ash substitution, as the pH inside the cracks in these specimens resembles the pH optimum of the bacteria. Given that all other parameters, except fly ash substitution, were kept constant, this also highlights pH as a key factor limiting the bacterial activity in these experiments. In pure well cement, leaching of portlandite due to longer hydration times alone may therefore likely allow bacteria with a pH tolerance similar to that of B. alkalinitrilicus to metabolize and precipitate CaCO3.

The oxygen consumption originates from B. alkalinitrilicus loaded SAP particles placed inside the cement cracks in all the cement specimens (Figure 4, arrows). The aerobic B. alkalinitrilicus cells use oxygen to oxidize the supplemented electron donor (Na-lactate), and the decrease in oxygen concentration around cracks is due to their activity. In the 2D images of the oxygen concentration inside cracks (Figure 4), downward streaks of lower oxygen concentrations from the crack opening are evident. These are caused by a flow of a thin watery film between the cement specimen and optode (Figure 4). Unfortunately, surface roughness makes it impossible to avoid such flow-induced movements. Contrary to the pH optode that is composed of a swelling hydrogel, the oxygen optode used in this study is more sensitive to surface roughness. This explains the observed flow-induced water movement. For structurally complex surfaces, optical sensor particles have been shown to be suitable alternatives, enabling oxygen imaging even on complex biological surfaces. Nevertheless, for the current study, the oxygen optode system used here was sufficient and enabled the estimations of bacterial activity in 2D.

pH has not previously been measured in cementitious materials for the application of microbial self-healing compatibility. The evidence of bacterial precipitation of CaCO3 in concrete specimens inoculated with B. alkalinitrilicus is strong. Thus, the pH in ordinary Portland cement based concrete must be within the biocompatible range. Concrete surfaces exposed to accelerated carbonation or biogenic acid attack show the pH values, measured optically, in the range of above 12 to below 10 similar to the results presented here.23–25 It is therefore not surprising that the alkaliphile B. alkalinitrilicus would metabolize in both concrete and hardened well cement specimens.

### CONCLUSIONS

Oil and gas well cements are very alkaline with the pH above 11 for pure hydrated cements. Here, we found that the buffering capacity can be reduced to an extent where bacterial activity occurs and thrives by the addition of supplementary pozzolanic materials. Although significant amounts of fly ash may hardly be introduced in well cements for other reasons, the present work shows that the partial replacement of cement by pozzolanic materials may reduce the pH of the cement pore solution to a level at which the bacterial activity can occur. Thus, this study is a step toward the design of suitable bioactive self-healing well cement formulations that may contribute to a more sustainable construction of future oil wells with longer service life times and an improved material for well abandonment.

### EXPERIMENTAL SECTION

#### Cement Specimen Preparation

The main component used for the specimen preparation in this study was a class G cement, commonly used for wells in the oil and gas industry.26,35 The mix was prepared with 0.25% by volume of polypropylene fibers to keep the specimens attached after the introduction of cracks, and with varying additions of fly ash (Table 1). The ingredients were mixed by a blender-type homogenizer for 1 min and then poured into 40 mm × 40 mm

| Table 1. Composition of Cement (C), Fly Ash (FA), and Water (W) Fraction by Weight in the Analyzed Specimens |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| sample ID | 0%             | 10%            | 20%            | 30%            | 40%            | 50%            |
| C:FA:W     | 1:0:0.45       | 0.9:0.1:0.45   | 0.8:0.2:0.45   | 0.7:0.3:0.45   | 0.6:0.4:0.45   | 0.5:0.5:0.45   |
× 160 mm prismatic molds. The specimens were demolded after 48 h and then postcured in demineralized water at 20 °C for 20 days.

After 20 days of curing, cracks were induced in a 3-point bending setup (Figure 5). The cracks were induced by deformation controlled tests by applying a loading rate of 0.1 mm/min of cross-head displacement. When a crack mouth opening displacement (CMOD) of 0.8 mm was reached, a plastic wedge was installed at the widest part of the crack to keep this size. After cracking, specimens were submerged in demineralized water at 20 °C for an additional 30 or 60 days to induce portlandite leaching and reduction in pH before conducting the pH measurements.

**Preparation of pH and Oxygen Planar Optodes.** Luminescence-based pH and oxygen imaging was performed using planar optodes. For the oxygen-sensitive foil, 1.5 mg of the indicator dye platinum(II) meso-tetra(pentafluorophenyl)-porphyrin (PtTFPP; Frontier Scientific, Logan, Utah), 1.5 mg of the reference dye Macrolex fluorescein yellow (Lanxess, Köln, Germany), 100 mg of diamond powder (1–2 μm, Microdiamant.com), and 100 mg of polystyrene (MW ~192.000, Sigma-Aldrich, Taufkirchen, Germany) were dissolved/dispersed in 1 g of chloroform and knife-coated (~12 μm thickness) on a dust-free poly(ethylene terephthalate) (PET) foil (Puetz Folien, Taunusstein, Germany). The indicator emits in the red part of the spectrum (red channel) while the reference dye emits a green light (green channel).

The pH-sensitive optode consisted of a NIR pH indicator with a high pK_a, namely, DiF-OH-aza-BODIPY (compound 4 in ref 31), and the green emitting coumarin based reference dye Bu3Coum. A 0.5 mg of the indicator, 0.4 mg of the reference dye, 100 mg of diamond powder, and 100 mg of Hydromed D4 (purchased from AdvanSource Biomaterials) were dissolved/dispersed in 1 g of tetrahydrofuran (THF) and again knife coated on a poly(ethylene terephthalate) (PET) foil to give a ~12 μm thickness sensor layer.

**Optical Sensing of pH and O_2 in Well Cement.** The pH and O_2 planar optodes were mounted at the sidewall of a 6 L aquarium with an electrical tape (carefully avoiding the formation of air bubbles between the glass and the optode). Cement specimens were pressed against the optode with a specially designed distance spacer made of rubber to ensure that the specimen was pressed against the optode as tight as possible (Figure 1). The aquarium was filled with sterile artificial seawater (ASW, void of sulfates to eliminate sulfate attack, salinity of 35%, pH 8) and kept at 30 °C using a temperature-controlled Lauda or RAS water bath (LAUDA–Brinkmann, LP, Delran, New Jersey). Both optodes were excited using the same blue (470 nm) LED (i-led, ILH-GD01-DEBL-SC201; r-s components, Copenhagen, Denmark) powered by a USB-controlled LED driver unit. Images were taken after 60 min of contact with an SLR camera (EOS 1300D; Canon, Tokyo, Japan), modified by removing the near-infrared filter (NIR filter) to obtain sufficient signal from the pH indicator. The camera was equipped with a macro objective (100 mm f/2.8 AT-X M100 AF Pro D; Tokina, Tokyo, Japan) and a 530 nm long-pass filter (OG530 SCHOTT, 52 mm × 2 mm) to block out the excitation light. LED excitation and image acquisition were controlled and synchronized with the software look@RGB.37 The optodes were calibrated in the aquarium in the same ASW prior to the experiment. Oxygen calibrations were obtained by flushing the water in the aquarium with a known air/N_2 mixture (using red-y mass flow controllers from Vögtlin, Switzerland), while the oxygen saturation in the aquarium was checked using a calibrated commercial oxygen sensor (OXROB3 oxygen sensor connected to a FireStingGO2; both from Pyroscience, Aachen, Germany) (Figure S1). In a similar fashion, the pH optode was calibrated by adjusting the pH within the aquarium adding appropriate amounts of 5 M NaOH and performing simultaneous reference measurements using a calibrated glass pH electrode (Radiometer Analytical—Hach Company, Loveland, Colorado) (Figure 1).

Image processing was performed with Image J® using the plugin Ratio Plus as described in detail elsewhere.38,40

**Microbial Activity in Cement Cracks.** To assess the potential for sustaining bacterial activity inside cracks of well cement, endospores of *B. alkalinitrilicus* were encapsulated in a superabsorbent polymer (SAP) and manually introduced into cracks. *B. alkalinitrilicus* endospores were produced on modified Schaeffer’s medium and encapsulated in an acrylamide polymer cross-linked with bis-acrylamide to give superabsorbent properties. The endospore amended SAPs were freeze-dried until all water had sublimated and ground to <0.5 mm diameter particles in an agate mortar. The SAP...
particles were placed inside the entire length of the cracks of the well cement specimens with sterile tweezers. The cement specimens with 0–50 wt % fly ash substitution were prepared identically to the specimens above and prehydrated for 60 days at 20 °C before the endospore-amended SAPs were added to the cracks. The SAPs were hydrated inside the cracks with a concentrated sodium lactate based medium, Na-lactate 70 mM, NaCl 0.1 M, yeast extract (Sigma-Aldrich, St. Louis, Missouri) 1 g/L, which caused the SAP particles to swell and fill out the entire void of the crack. The specimens were then incubated in a closed humidified plastic container (0.5 m × 0.3 m × 0.3 m) for 5 days. Every 24 h, 2 mL of sterile medium was added to the cement cracks to support the germination and growth of endospores to a substantial cell density before oxygen measurements by planar optodes in the aquarium setup described above (Figures 1, S1, and S2).

## ASSOCIATED CONTENT

| Supporting Information |
|------------------------|
| The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02541. |

Calibration curve of the oxygen-sensitive planar optode; Microscopy images of the germination and growth of endospore-forming bacteria (Figures S1 and S2) (PDF)

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### Notes

The authors declare no competing financial interest.

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