Structural Iron Reduction and Illitization of Nontronite by Bacteria *S. oneidensis*

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Introduction

In situ biological interactions in geologic systems are of great interest for deep geological disposal of spent nuclear fuel (SNF). Specifically, the role that biological pathways may play in the transformation of smectite to illite (illitization) is important due to the potential use of smectite as a backfilling material in a repository. Illitization is defined by the following chemical reaction (Mulligan et al., 2009):

Smectite+ K^++ Al^{3+} (Fe^{3+}) → Illite+ Silica+ (Cation)

where the exchangeable interlayer cations in smectite (Na^+ or Ca^{2+}) is exchanged with K^+.

Microbial reduction of structural Fe^{3+} in the octahedral sheets of iron-rich smectites may promote the smectite-to-illite transformation (bio-illitization), where previous studies have observed smectite dissolving.

This study aims to:

1. Determine if smectite can be transformed or degraded by the existing microbial environment
2. Inform the performance assessment (PA) of a geological repository clay barrier
3. Help design effective strategies for environmental management.

This work is focused on understanding and quantifying the mechanism of microbial reduction of Fe^{3+} in the nanostructure of nontronite (an iron-rich, Ca^{2+}-smectite) to soluble Fe^{2+} and its effect on the alteration of clay crystallinity, specifically illitization.

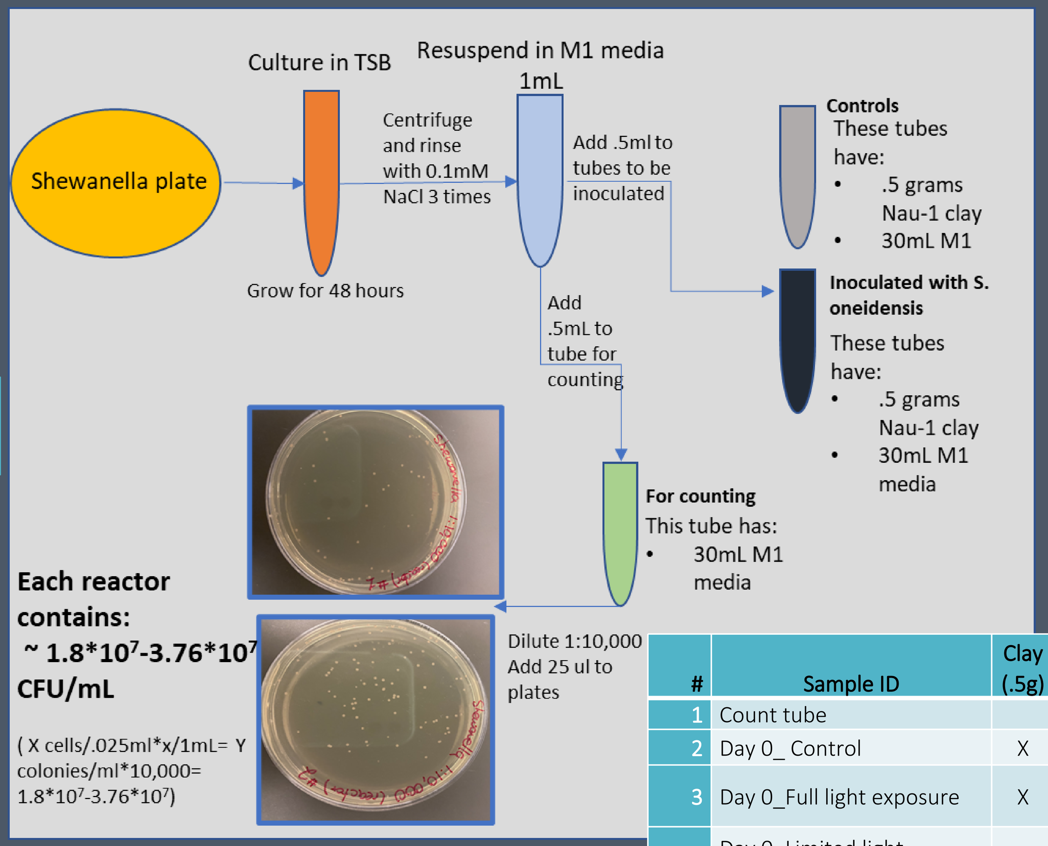
Experimental Design

Batch reactors were prepared as shown (right). The M1 media recipe* used is modified from Kostka et al. 1999 (Chapter 3: *Techniques in Microbial Ecology)*. Sample vials were put in anaerobic chambers with *GasPak* gas generating pouch with methylene blue O2 indicator. Chambers were placed in a 30°C shaking incubator.

*Modified M1 media by removal of EDTA and FeSO4

Reactors were centrifuged for 20 minutes, and the supernatant was collected for pH, Fe assay, and elemental analysis. Iron was measured using spectrophotometry at 590nm (*BioAssay Systems QuantiChrom* iron assay kit with VICTOR3 multi-label plate reader). Elemental concentrations were measured by ICP-OES (*Perkin Elmer Optima 8000*).

The nontronite clay pellet was agitated with 1 mL deoxygenated water, then the clay/water slurry was used to prepare an XRD oriented mount as shown. The XRD mount was allowed to dry at 50°C for 1 week in an anaerobic chamber. Samples were analyzed on a *Bruker D2 Phaser* XRD.



Each reactor contains:
~ $1.8*10^7$-$3.76*10^7$ CFU/mL

(X cells/.025ml*x/1mL= Y colonies/ml*10,000= $1.8*10^7$-$3.76*10^7$)

#	Sample ID	Clay (.5g)	Media (30mL)	Inoculated with S. oneidensis
1	Count tube		X	X
2	Day 0_ Control	X	X	
3	Day 0_Full light exposure	X	X	X
4	Day 0_Limited light exposure	X	X	X
5	Day 1_Full light exposure	X	X	X
6	Day 1_Limited light exposure	X	X	X
7	Day 14_Full light exposure	X	X	X
8	Day 14_Limited light exposure	X	X	X
9	Day 35_Full light exposure	X	X	X
10	Day 35_Limited light exposure	X	X	X
11	Day 35_Control	X	X	

Conclusions

After reactions, a visible color change in the clay was observed, indicating microbial induced reduction. At day 35, pH in the full light exposed sample was lower, which could have potential implications to microbial populations.

XRD:

Between day 0 and day 35, layer collapse is shown in the samples. The D-spacing for the ethylene-glycolated (001) peak decreases as time progresses. While XRD analysis showed a shift to the right on the 001 peak, there is no indication illite has formed (001 peak typically at 10Å).

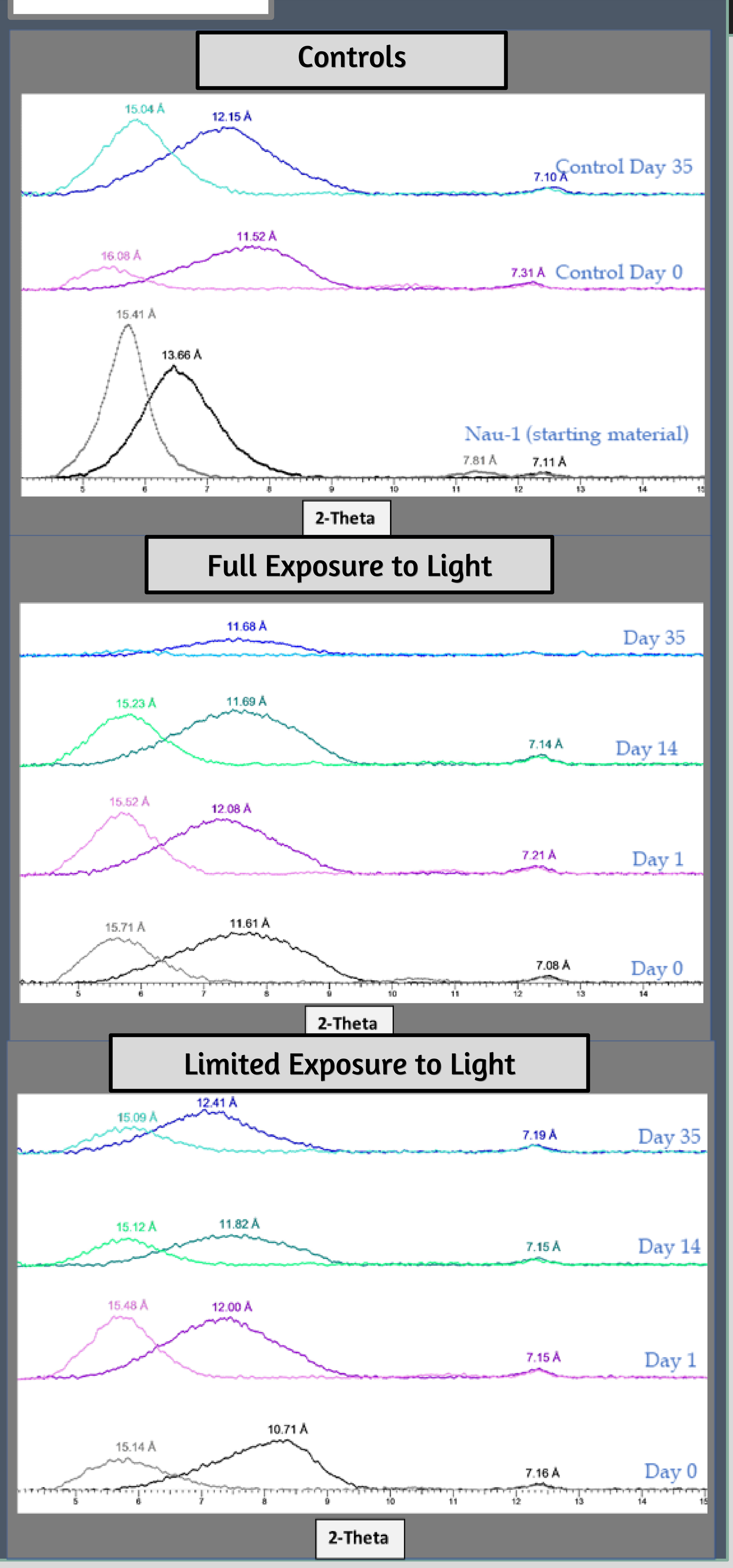
Iron in solution:

Increased Fe^{2+} in solution showed microbial iron reduction occurred in inoculated samples. Iron concentration (total Fe and Fe^{2+}) increases in inoculated samples between day 0 and day 14. After day 14, samples exposed to light showed a slight decrease in iron. However, iron concentration in samples not exposed to light decreased significantly. Controls showed minimal iron in solution.

Elemental analysis:

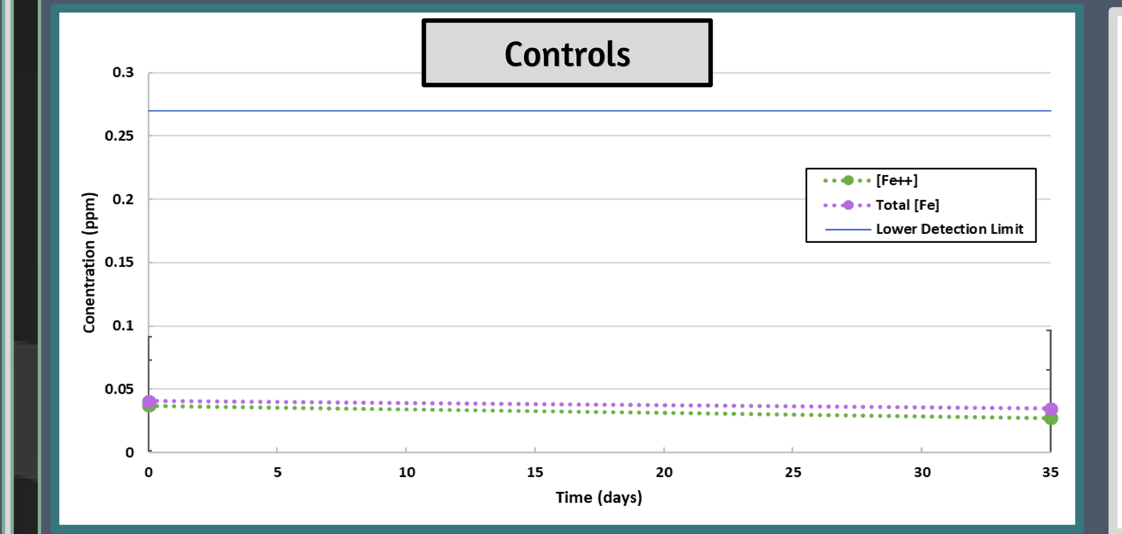
ICP-OES analysis indicates potassium and calcium uptake by the clay in inoculated samples. Elemental concentrations in solution differ between the samples that were exposed to light versus those that had limited light exposure. More potassium uptake was seen in full light exposure samples, while more calcium uptake is observed in samples with limited light exposure. Controls show only calcium uptake. Silica in solution shows an increasing trend over time, indicating dissolution of nontronite. By day 35, the limited light exposed sample shows decrease in Si, which could indicate pre-liminary secondary mineral precipitation.

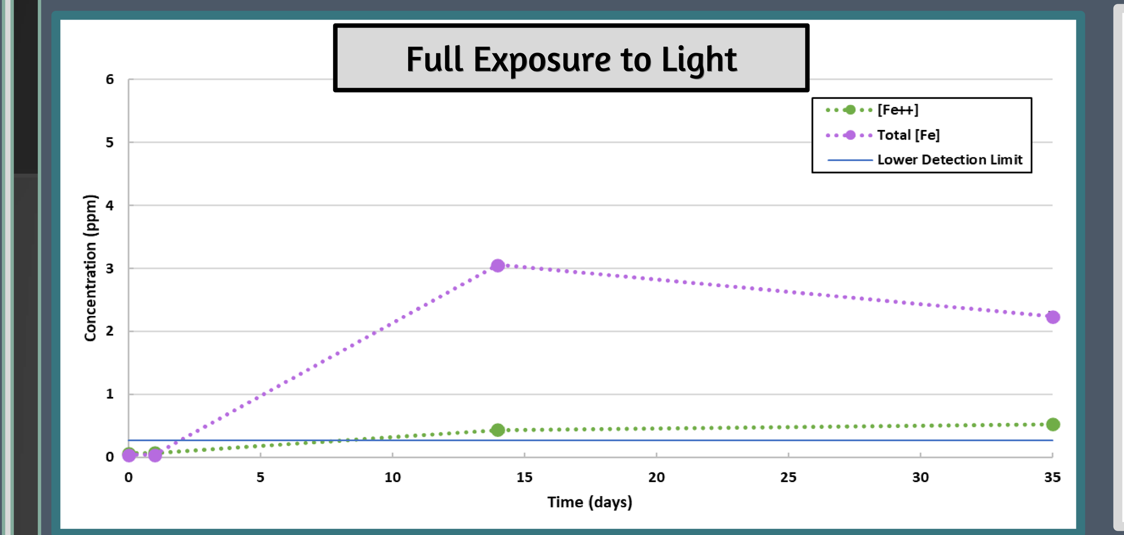
XRD

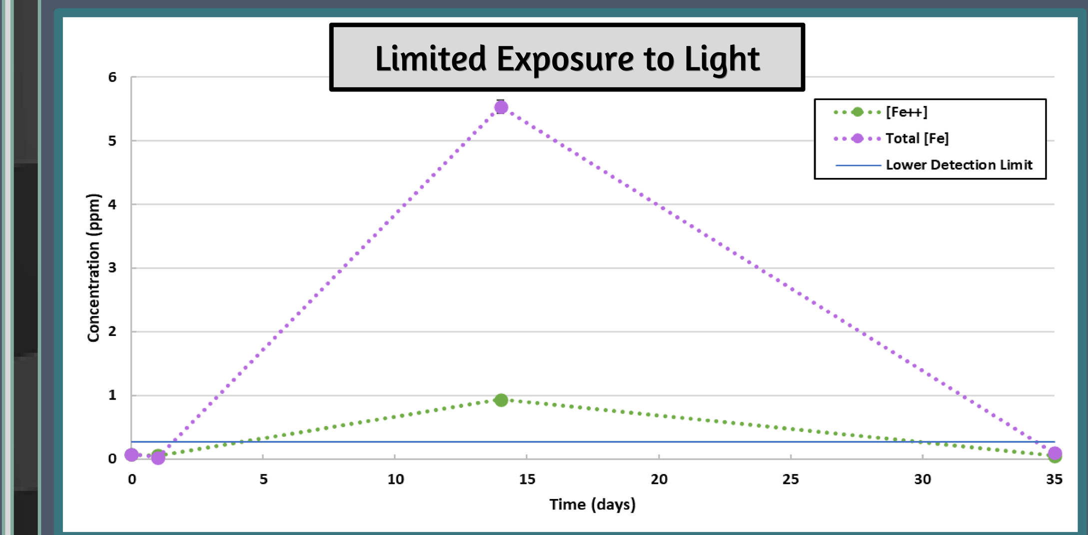


Solution Chemistry

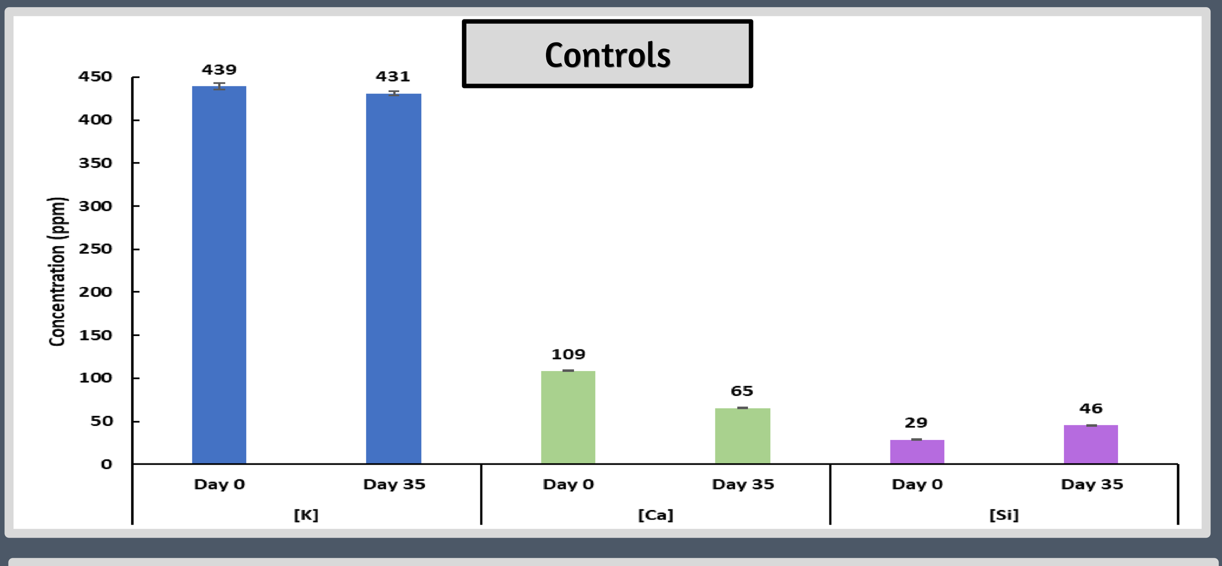
Iron Assay

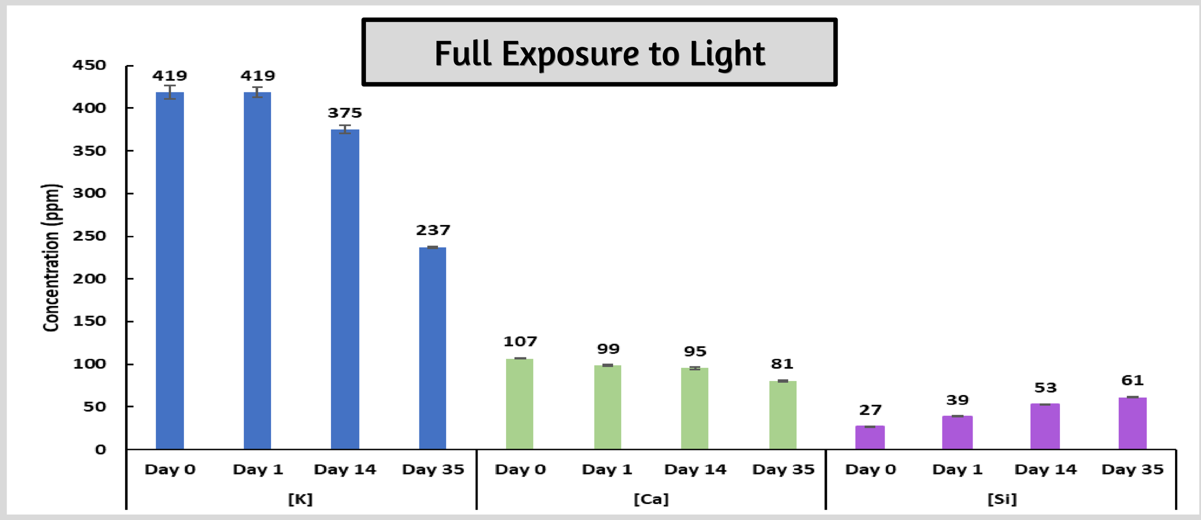


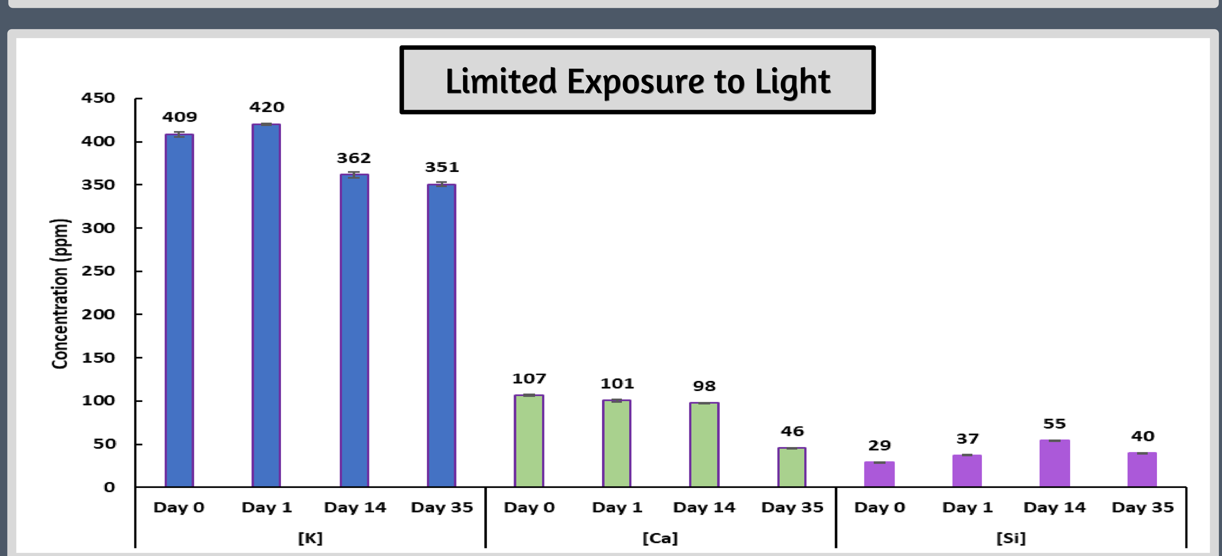




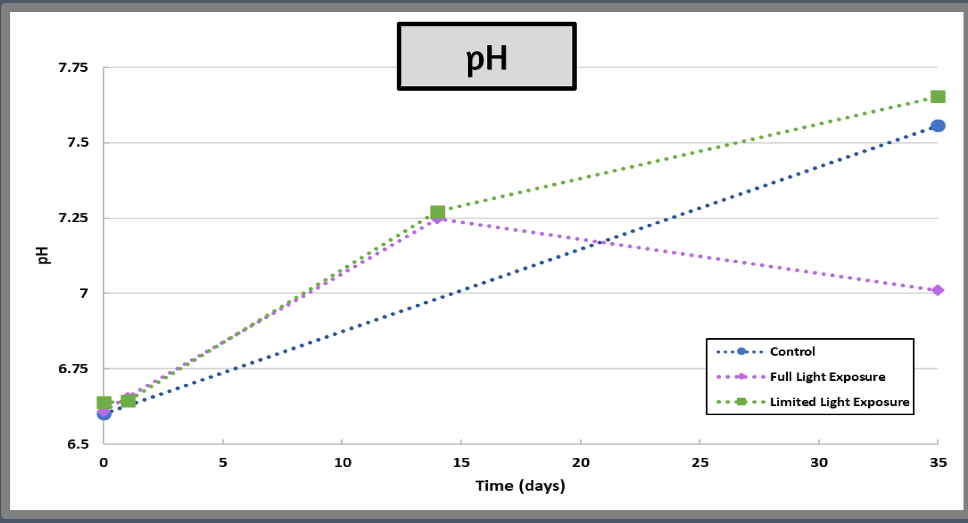
ICP-OES







Reacted clay suspensions after 35 days.
Left: Control
Center: Full exposure to light
Right: Limited exposure to light



Future Work

This proof-of-concept study developed a method for further assessment of bioillitization.

Future analysis will include:

- Longer incubation periods
- More sample replicates to verify repeatability
- Microbial population examinations through plating and sequencing during experiments
- Simulated subsurface conditions
- Analysis of secondary mineral precipitation

References

Kim, J., Dong, H., Seabaugh, J., Newell, S.W., Eberl, D.D. (2004). *Role of microbes in the smectite-to-illite reaction.* Science 303, pp. 830-832.

Koo, T., Lee, G., Kim, J., (2016). *Biogeochemical dissolution of nontronite by Shewanella oneidensis MR-1: Evidence of biotic formation.* Applied Clay Science 134. pp.13-18

Kostka, J., Wu, J., Nealson, K. and Stucki, J., 1999. The impact of structural Fe(III) reduction by bacteria on the surface chemistry of smectite clay minerals. *Geochimica et Cosmochimica Acta*, 63(22), pp.3705-3713.

Mulligan, C., Yong, R., Fukue, M., (2009). *Some effects of microbial activity on the evolution of clay-based buffer properties in underground repositories.* Applied Clay Science 42. pp 331-335.

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