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Project Title: Design of Mixed Batch Reactor and Column Studies at Oak Ridge National Laboratory

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Abstract

We (the Stanford research team) were invited as external collaborators to contribute expertise in environmental engineering and field research at the ORNL IFRC, Oak Ridge, TN, for projects carried out at the Argonne National Laboratory and funded by US DOE. Specifically, we assisted in the design of batch and column reactors using ORNL IFRC materials to ensure the experiments were relevant to field conditions. During the funded research period, we characterized ORNL IFRC groundwater and sediments in batch microcosm and column experiments conducted at ANL, and we communicated with ANL team members through email and conference calls and face-to-face meetings at the annual ERSP PI meeting and national meetings. Microcosm test results demonstrated that U(VI) in sediments was reduced to U(IV) when amended with ethanol. The reduced products were not uraninite but unknown U(IV) complexes associated with Fe. Fe(III) in solid phase was only partially reduced. Due to budget reductions at ANL, Stanford contributions ended in 2011.

Keyword: uranium, iron, sediments, bioreduction, reoxidation, microcosm, column, synchronal analysis

During the subcontract period (2008-2011), research subcontracted to the Stanford team has mainly focused on static microcosm (SM) testing including monitoring and investigation of biogeochemical changes in relation to the stability and reduction-oxidation of uranium at molecular scale. In 2010, MBR and column studies of relative dynamics of UVI reduction and speciation of UIV under Fe- and sulfate-reducing conditions. This research represented the combined efforts of Drs. Shelly Kelly (ANL), M.I. Boyanov (ANL), Edward O'Loughlin (ANL), Ken Kemner (ANL) and other ANL team members, Drs. Craig Criddle and Wei-Min Wu at Stanford University and Dr. Terry Marsh at Michigan State University.

Efforts at ANL focused on monitoring the U speciation and aqueous chemical composition (e.g., pH, [U(VI)], [Fe(II)], sulfate, etc.) with time in static microcosms. Six microcosms were initiated in July 2008 and were monitored continuously to study the long-term effect of geochemical changes on uranium speciation in relation to its immobilization and stability. Microcosm sediments and groundwater recovered using the surge block collection method from Area 3, US DOE IFRC Oak Ridge site. Sediments (220 ml) and groundwater were transferred under anoxic conditions to 500 ml polycyclic bottles. Two different level of sulfate (1.0 vs 5.0 mM) were added to test the effect of sulfate on bioreduction. The initial pH was 7.0 with alkalinity ~ 4.0 mM. The microcosms were stored in an anoxic Coy chamber, except for the 1 to 4 hours required to make X-ray measurements at the MRCAT beamline at the APS. The aqueous phase Fe(II), [U]tot and pH were recorded. The microcosms showed changes consistent with the development of reducing conditions: pH of the groundwater increased, aqueous [Fe(II)] decreased, and aqueous U(VI) levels fell below detection limits. In the microcosms with initial high sulfate, the pH gradually rose to above 8.0 and aqueous U(VI) concentrations rebounded.. Over the long-term, all sulfate was removed by microbial reduction and the pH stabilized.

The x-ray probe of 0.5 by 0.5 mm of each microcosm was scanned from the groundwater-sediment interface down vertically for the X-ray Absorption Near Edge Structure (XANES) of uranium valence and Extended X-ray absorption fine structure (EXAFS) for the the U chemical speciation. Detailed analysis of these spectra from the first series of microcosms show that U(VI)-C and U(VI)-P species are transformed to non-uraninite U(IV).

Dr. Criddle has lent his guidance in the SMs tests and interpretation of results. Dr. Wu contributed to the monitoring and maintaining the SMs, sample analysis arrangement and data reduction. Drs. Criddle and Wu communicated with ANL SFA team through email and conference call. Dr. Wu meet ANL SFA team at ANL in September, 2010 and 2011, the annual DOE BRE meeting in 2009, 2010 and 2011, and at the ASM annual meetings in 2009, 2010 and 2011. In these meetings, members of the Stanford team discussed project results.

Test procedures and results

Microcosm test with added sulfate and ethanol as electron donor source.

Six microcosms of the same size were set up to test the effect of amendment with ethanol and impact of sulfate concentrations on U(VI) reduction. Each microcosm contained 580 mL of sediment slurry taken from Area 3, IFC Oak Ridge site (about 215 g dry weight per microcosm). Two different initial sulfate concentrations (1.0 vs 4.0 mM) were used. Ethanol (10 mM) was added to the four microcosms (two with 1.0 mM sulfate and two with 4.0 mM sulfate). The control microcosms did not receive ethanol (Figure 1). The monitoring procedures for this set of microcosms was the same as that published by Shelly et al. (2010).

These microcosms were monitored by withdrawing aqueous samples for pH, sulfate, U(VI), ethanol, acetate, Fe and Ca periodically. The XANES and EXAFS were used to determine the valence and U chemical speciation as described about.

The results indicated that significant U(VI) removal in aqueous phase and sulfate reduction occurred when ethanol was added. Slow removal of U(VI) and sulfate was observed in the control. Slightly more U(IV) content was observed in the microcosms with added higher sulfate concentration. The sediments in the microcosms were reduced sequentially from the upper to lower portion over a long time period (Figure 2).

Figure 3 illustrates the change in aqueous geochemistry of each microcosm. Ethanol was consumed rapidly and acetate produced was then consumed. Sulfate was gradually reduced, resulting in increase in pH. U concentration declined after amendment except for that contained high sulfate. In that case, U was increased due to pH raising and finally decreased as pH become stable or sulfate was consumed.

Microbial analysis

Sediments have been archived for microbial analysis in progress. Samples were collected from the initial U(VI) microcosms inoculum and with depth from the microcosms.

Clone libraries of 16S sequences were used to study the shifts of microbial communities. The twelve samples were i.) the initial FRC sediment (ECFW026), ii.) an aliquot of the initial sediment stored in 4°C in which uranium reduction was observed (May0308_FW026Pulled12706), iii.) Microcosm (Microcosm 1 vs. Microcosm 2) samples taken eleven months apart (May 3rd, 2007 vs. April 21st, 2008) at two different positions for aqueous and sludge samples (top vs. bottom), and iv.) the inocula for Microcosm 10 (high SO_4^{2-} vs. low SO_4^{2-}). Clone libraries of at least 138 sequences per sample were constructed with the total of 1888 cloned 16S rRNA genes. The resulting 16S sequences were analyzed for ecological parameters using distance matrix with DOTUR (DOTUR 1.53), the Ribosomal Database Project (RDP) Classifier, and neighbor joining implemented in ARB.

Ecological parameters. Individual samples were analyzed with Dotur 1.53 to generate diversity indices and estimates of operational taxonomy units (OTU) . Shannon and Simpson diversity indices were consistent with each other (Table 1.), ranging from 2.73-3.75 and 0.03-0.14 respectively. We also report values for Chao-1 and ACE as estimates of the number of species in these samples. These indices suggested that the Microcosm 10 inocula (May2008_Micro10LowSO4 and May2008_Micro10HighSO4) and the FRC sediment aliquot stored in 4°C (May0307_FW026Pulled12706) were less diverse compared to the remaining samples. Analysis of the initial FRC sediment (ECFW026) revealed a complex community that was used as the inoculum for Microcosm 1 and 2. The communities of the ‘early’ microcosm enrichments (May0307_Micro1Top and Bottom, May0307_Micro2Top and Bottom) appeared less even and less diverse compared to the starting inoculum. Eleven months later, an increasingly diverse and complex community that approached the complexity of the original

FRC sediment community was detected (Apr2108_Micro1Top and Bottom, Apr2108_Micro2Top and Bottom). Thus, the detectable diversity of the U(VI) microcosm communities experienced an initial decrease in richness and diversity, presumably as a result of strong selection, followed by increases in richness and diversity as the original selection pressures diminished. As reported above by ANL, U(VI) reduction was observed in Microcosm 1 and 2 during the same eleven month period. Thus, the detectable diversity might be directly associated to the concentration of U(VI). Variations in richness and evenness were also observed amongst aqueous and sludge samples taken during the same period of time. However, no interpretable patterns in diversity indices could be detected between aqueous (Top) and sludge (Bottom).

Table 1. Diversity indices of microcosm communities.

Samples	**Indices**				**OTUs**	**No. of Clones**
	Ace	**Chao I**	**Shannon**	**Simpson**		
May2008_Micro10LowSO4	109.07	74.14	2.73	0.11	38	163
May2008_Micro10HighSO4	76.76	55.83	2.83	0.12	40	149
May0307_FW026Pulled12706	79.95	76.50	2.75	0.14	38	139
ECFW026	166.55	159.66	3.74	0.03	64	138
May0307_Micro2Bottom	126.22	134.67	3.12	0.09	52	175
May0307_Micro1Top	86.63	74.60	3.26	0.06	47	156
May0307_Micro1Bottom	118.75	105.38	3.28	0.06	51	170
May0307_Micro2Top	118.00	106.38	3.31	0.06	52	165
Apr2108_Micro2Top	94.94	107.50	3.35	0.05	49	151
Apr2108_Micro1Top	177.26	161.50	3.38	0.05	59	163
Apr2108_Micro1Bottom	197.87	240.50	3.71	0.03	68	171
Apr2108_Micro2Bottom	180.68	136.00	3.81	0.03	70	148

*Values were calculated at 97% similarity via DOTUR-1.53

Classification. The sample sequences were submitted to the Ribosomal Database Project (RDP) for classification at phylum and genus level (80% confidence level). All sequences were classified as bacteria (Table 2.). Approximately 4-12% of clones per sample were identified as unclassified bacteria at phylum level. Three phyla (*Proteobacteria, Acidobacteria* and *Chloroflexi*) were detected in all samples at variable abundance. *Proteobacteria* was the predominant group in all twelve samples (30-60%), especially in the initial FRC sediment. The early U(VI) microcosm samples (May0307_Micro1&2 samples) revealed a substantial decrease in detectable *Proteobacteria* population. However, *Proteobacteria* populations increased in the two microcosms eleven months later (Apr2108_Micro1&2 samples). Although *Acidobacteria* was present in all of the samples, the *Acidobacteria* population detected in Microcosm 10 inocula (May2008_Micro10LowSO4 and May2008_Micro10HighSO4) and FRC sediment aliquot stored at 4°C (May0307_FW026Pulled12706) was significantly lower than the remaining nine samples. The *Acidobacteria* group behaved exactly opposite to the Proteobacteria in that relatively low abundance of *Acidobacteria* was detected in the initial FRC sediment but appeared to increase in the early sampling of the U(VI) microcosms (May0307_Micro1&2 samples). The abundance of *Acidobacteria* in the same microcosms eleven months later was found to decrease and became comparable to the initial FRC sediment (Apr2108_Micro1&2 samples). The rise and fall of *Proteobacteria* and *Acidobacteria* population suggested that these populations were responding to the U(VI). As remediation continued and U(VI) was coverted to U(IV), the community responded and rebounded to a structure that resembled the original inoculum as measured by diversity indices. Besides *Proteobacteria and Acidobacteria,* phylum *Chloroflexi* was also reported in all of the samples. The abundance of *Chloroflexi* was significantly higher in Microcosm 10 inocula than the rest of the samples. The Classifier results suggested that both Micro10 inocula samples has substantial numbers of *Firmicutes* as well. Interestingly, although *Firmicutes* were present in low abundance in Microcosm 1 and 2 sludge samples, none were detected in the aqueous portions of microcosms. At genus level, approximately 30-70% of the sequences could not be identified by the RDP Classifier. Amongst all classifiable microorganisms, genus *GP8* (phylum *Acidobacteria*) and *Geobacter* were present in all samples. The Classifier data at the phylum level indicated that the detectable abundance of *GP8* was significantly lower in May2008_Micro10LowSO4 and May2008_Micro10HighSO4 but higher in the microcosms that were abundant with U(VI). Cluster analysis with the Bray-Curtis similarity index using genus level Classifier results showed that twelve samples could be grouped into three clades, i.) the FRC sediment aliquot stored at 4°C, ii.) the two Microcosm 10 samples, iii.) the initial FRC sediment and eight Microcosm 1 and 2 samples. The dendrogram revealed that the FRC sediment aliquot stored at 4°C was different from the rest of the samples. Hence, temperature was also an important parameter that determined the community structure of these uranium reducing samples.

Phylogenetic Analyses. The sequences were also aligned to the Arb Silva database for more rigorous phylogenetic analyses. To date, all sequences have been aligned and the results are consistent with the classifier outputs. The neighbor joining trees revealed several large groups of previously unidentified bacteria (e.g., within the *Chloroflexi* group). Further grouping and identification is in progress. In the coming 6-12 months, we will i.) complete the phylogenetic analyses of the 16S sequences, ii.) compare the communities via UniFrac and Libshuff, iii.) determine microorganisms that are essential for uranium reduction within the communities, iv.) target the dominated uncultured bacteria for isolation and investigate their ability in heavy metal reduction. As our ultimate goal, we will synthesize a community that is capable for rapid uranium reduction with the least complexity.

Publications and Conference presentations contributed by Stanford Team

The results of this project have been partially published in peer-reviewed journals and presented as posters at several conferences as below.

1. Kelly, S.D., K.M. Kemner, J. Carley, C. Criddle, D. Phillips, P.M. Jardine, T. L. Marsh, D. Watson, W.-M. Wu. 2008. Speciation of uranium in sediments before and after *in situ* bioreduction. *Environmental Science & Technology*. 42: 1558–1564.
2. Kelly, S.D., W.-M. Wu, F. Yang|, C. Criddle, T. L. Marsh, E. J. O'Loughlin, B. Rave, D. Watson, P.M. Jardine, K.M. Kemner 2010. Monitoring uranium transformations in static microcosms. *Environmental Science & Technology*. 44(1): 236-242.
3. Kelly, S.D., K. M. Kemner, E. J. O'Loughlin, W.-M. Wu, C. Criddle, T. L. Marsh. 2008. Monitoring uranium transformations. Poster presented at Goldschmidt *Conference 2008. Vancouver, Canada*. July 13-18, 2008.
4. Boyanov, M., E. O'Loughlin1, K. Skinner-Nemec, M. J. Kwon, S. Kelly, F. Yang, T. Marsh, W.-M. Wu, C. Criddle, K.Kemner. 2009. Combined x-ray, chemical, and biological characterization of biostimulated and sulfate-amended sediments from the Oak Ridge Field Research Center. Poster presented at 2009 AGU Fall Meeting, San Francisco, California, USA. December 14-18.
5. Boyanov, M, E. O'Loughlin, S. Kelly, K. Skinner-Nemec, M.-J. Kwon, W.-M Wu, C. Criddle, T. Marsh, F. Loefler, R. Sanford, C.Giometti, M.Scherrer, K. Kemner. 2009. The interplay between sulfate- and iron-reducing conditions: effect on uranium speciation studied in static and flow-through columns. Poster presented at 4th DOE-ERSP PI Meeting, Lansdowne, VA, April 20-23, 2009.
6. O'Loughlin, E., M. Boyanov, E. Carpenter, C.Criddle, J. Fredrickson, C. Giometti, P. Jardine, M. Kwon, L. Liang, T. Marsh, M. Mccormick, R. Sanford, M. Scherer, D. Sholto-Douglas, K. Skinner-Nemec, W. Wu, K. Kemner. 2009. The Argonne subsurface science program scientific focus area. Poster presented at 4th DOE-ERSP PI Meeting, Lansdowne, VA, April 20-23, 2009.
7. Boyanov, M , E.J. O'Loughlin, M.J.Kwon, K. Skinner, B. Mishra, C. Criddle, W.-M. Wu, F. Yang, T. Marsh, K.E. Fletcher, F.E. Löeffler, K.M. Kemner. 2010. The Influence of ligands on the formation of non-uuaninite U(IV) phases during biotic and abiotic U(VI) Reduction. Poster presented at 5th DOE-ERSP PI Meeting, Washington, D.C., March 29-31, 2010.
8. Kemner, K., E. O'Loughlin, M. Boyanov, D. Antonopoulos, S. Brooks, E. Carpenter, C. Criddle, J.Fredrickson, T.Henne, M.-J. Kwon, B.Lai, D. Latta, F. Loeffler, T. Marsh, M. McCormick, B.Mishra, R.Sanford, C. Segre, M. Scherer, D. Sholto-Douglas, K. Skinner, W.-M. Wu, C. Giometti. 2011. The Argonne Subsurface Science Program scientific focus area. Poster presented at The Department of Energy's SBR 6th Annual PI Meeting, Washington, D.C., April 26 – 28, 2011.
9. Boyanov, M.I., E. J. O'Loughlin, K. Skinner-Nemec, S.D. Kelly, W.-M. Wu, C.Criddle, F. Yang, T. March, Mueller, T. Melhorn, K. Lowe, D. Watson, S.Brooks, K. M. Kemner.

2011. Non-uraninite U(IV) phases in biostimulated sediments from the Oak Ridge IFRC. Poster presented at The Department of Energy's SBR 6th Annual PI Meeting, Washington, D.C., April 26-28, 2011.

10. Wu, W.-M., C.S. Criddle, D.Watson, S. Brooks, C. Schadt, T. Gihring, G. Zhang, T.Mehlhorn, K. Lowe, J. Phillips, J. Earles, C. Brandt, P. Jardine, K.Kemner, M. Boyanov, J. E. Kostka, W. Overholt, S. J. Green, P. Zhang, J. Von Nostrand, J. Zhou. 2011. Biological reduction of uranium in the contaminated subsurface by slow-release electron donor. Poster presented at The Department of Energy's SBR 6th Annual PI Meeting, Washington, D.C., April 26 – April 28, 2011.
11. Wu, W.-M., D. Watson, G. Zhang, T. Gihring, C. Schadt, T. Mehlhorn, F. Zhang, S. D. Kelly, M. Boyanov, K. M. Kemner, J. D. Van Nostrand, P. Zhang, J. Zhou, W. A. Overholt, S.J. Green, J. E. Kostka, C. S. Criddle, P. M. Jardine, S. C. Brooks. 2011. U(VI) reduction in contaminated sediments with oleate, emulsified vegetable oil and ethanol as electron donor. Poster presented at American Society for Microbiology 111th General Meeting, New Orleans, LS, May 21-24, 2011

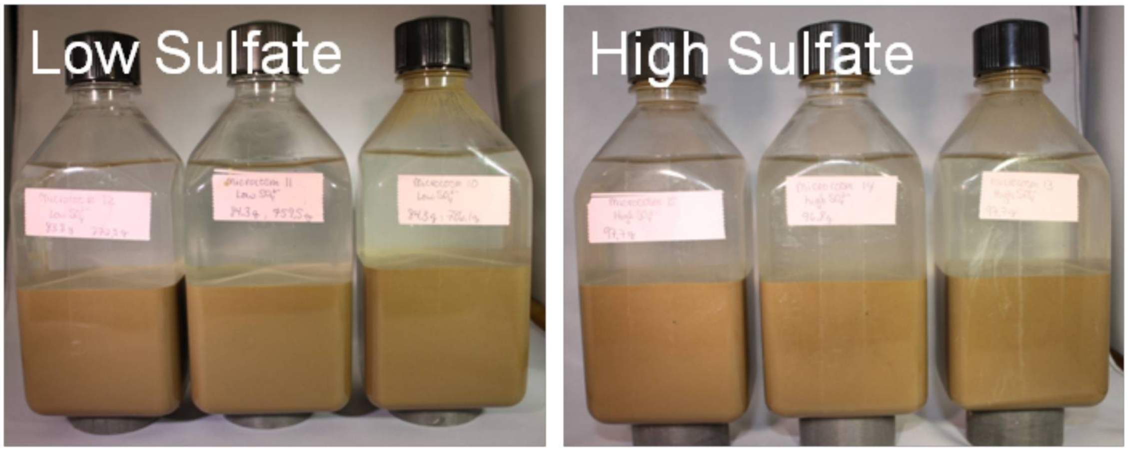

Figure 1. Microcosms prior to adding ethanol for bioreduction. Low sulfate microcosms contained 1 mM and high sulfate was 4 mM.

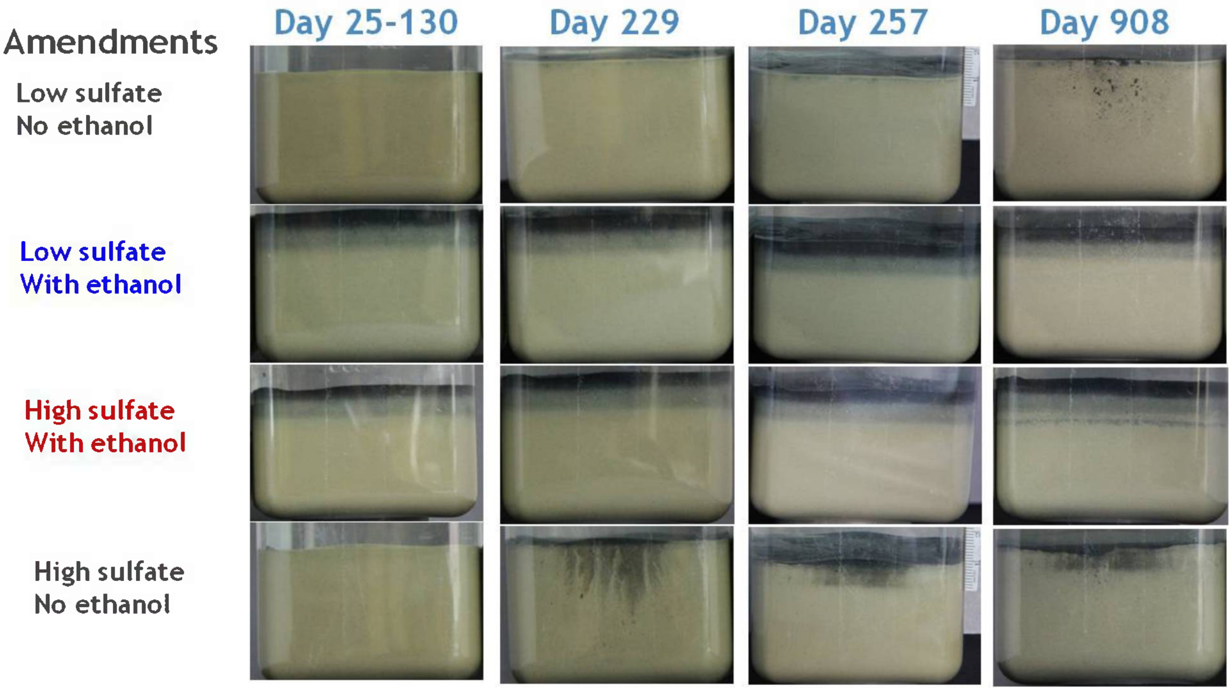

Figure 2. Progress of sediment bioreduction in microcosms after ethanol amendment.

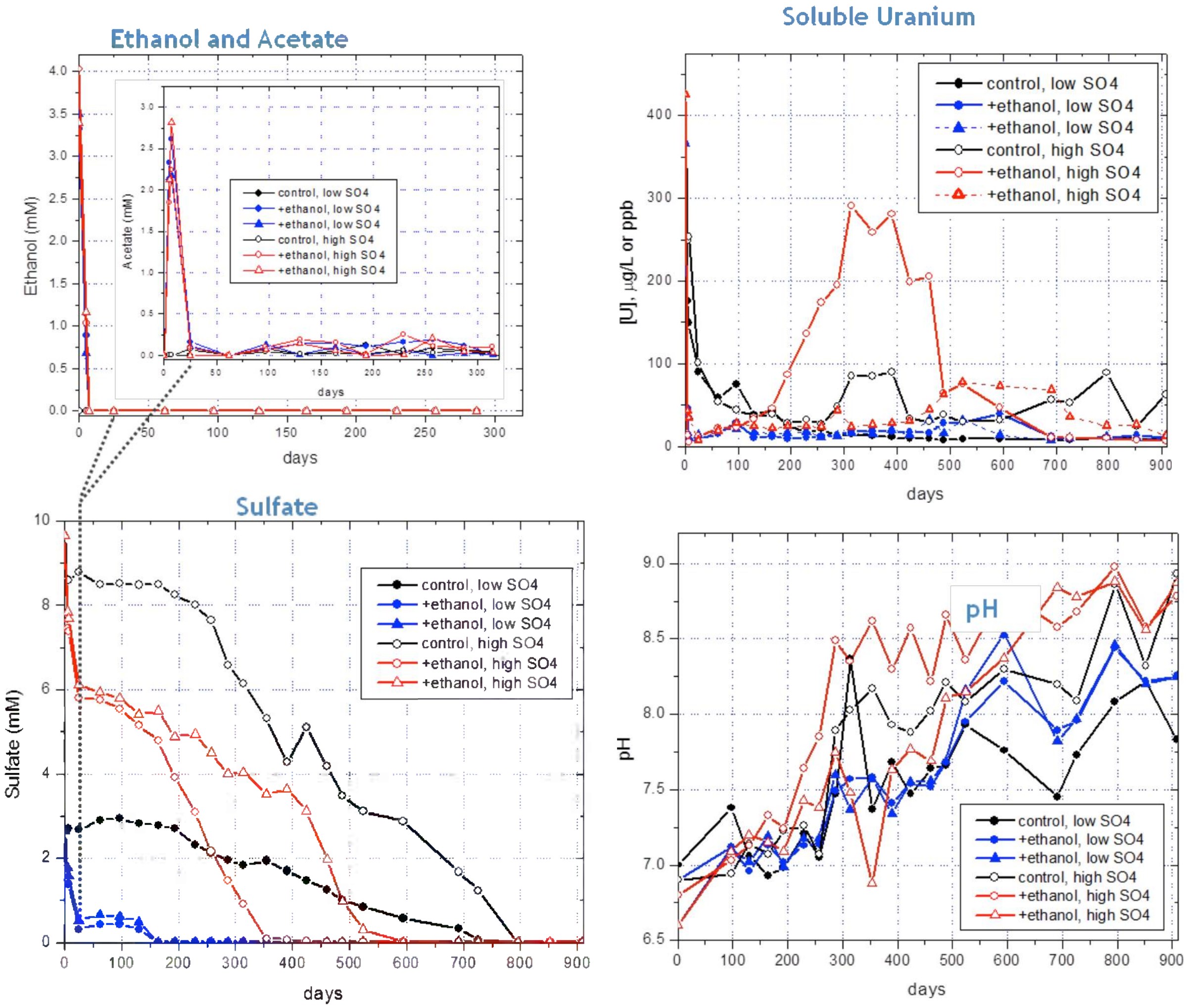

Figure 3. Change in geochemistry during the test (ethanol + acetate, sulfate, uranium and pH).