Status of Biostimulation Experiments

May 1, 2004

This update summarizes results-to-date from work currently in progress at the NABIR FRC. An update is needed because we were obliged to submit our renewal proposal (entitled “Field-scale evaluation of biological uranium reduction and reoxidation in the near-source zone at the NABIR Field Research Center in Oak Ridge”) after only a single biostimulation run. We now have data from 10 runs. Accordingly, the purpose of this document is to provide a more complete and accurate understanding of project progress-to-date.

The dates of all biostimulation runs completed are summarized in Table 1. The first four runs focused on stimulation of microbial growth and removal of nitrate, while the subsequent six runs have focused on the establishment of U(VI) reduction activity.

Table 1: Summary of biostimulation runs as of May 1, 2004.

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<th>Biostimulation Run</th>
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<tr>
<td>10</td>
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Summary of Well System Operation and Establishment of Biostimulation

In this update, we assume access to the Figures in the Color Images section of our submitted proposal in which the layout of the field site is presented. As a brief reminder of operations, we offer this summary:

The well system is designated as follows:
- Well A (FW103): outer loop extraction well
- Well B (FW026): inner loop extraction well
- Well C (FW104): inner loop injection well
- Well D (FW024): outer loop injection well
- MLS (Multi-Level Sampling) wells (FW100, FW101, and FW102): ports 1-4 indicate the four deepest sampling ports (1 is the deepest, 4 is the shallowest).

In normal operation, we extract water from Well A (flow rate 0.44 LPM), augment it with treated or tap water adjusted to pH 5.8-6.0 using HCl and inject it into Well D at a combined flowrate of 1.25 LPM. The water extracted from Well B (flow rate 0.44 LPM) is adjusted to a desired final pH with K2CO3, degassed in an oxygen-free headspace vacuum stripper to remove dissolved nitrogen and carbon dioxide gasses, stored in a tank.
sealed with a He headspace, then reinjected into Well C. During biostimulation, the stored water is mixed with ethanol and K₂CO₃ to achieve a desired target substrate concentration and pH.

During runs 1 to 4, we adjusted the pH of water injected into Well C to ~6.5. At and below this pH, U(VI) sorbs strongly at and below this pH. The ethanol concentration of the injected water, measured as COD, was ~80 mg/L.

During run 5, we adjusted pH to 6.5 for the first 48 hours, then increased it to 7.5-7.6. Our aim was to release U(VI) from soil into the groundwater to enhance its bioavailability. During the initial 48 hours, we added ethanol at ~80 mg/L as COD. Thereafter we decreased ethanol to 40 mg/L as COD for 24 hours then increased it to 100 mg/L. For subsequent runs, except run 9, we adjusted the pH of the injected water to 7.2-7.5 and injected ethanol at 80 mg/L as COD during biostimulation. At the conclusion of each run, we stopped ethanol and K₂CO₃ addition to assess the rebound of nitrate and U(VI).

**Summary of bioremediation results (Day 160–245).**

Figures 1-3 summarize chemical changes during runs 2 to 10. Figure 1 illustrates a time series for the inner loop extraction and injection wells. Changes at the MLS wells in Figures 2 and 3 provide a picture focused on the target inner cell region between Wells B and C. Water level data summarized in Figure 4 illustrate well clogging patterns at the injection and extraction wells and recovery of these wells after we implemented clean-up measures.

Results for MLS well FW101-2 are typical. This well is located directly between the injection well screen and the extraction well screen, about 40 ft below ground surface. When we added ethanol, COD levels increased at FW102-2 in about 6-8 hours. After we stopped ethanol addition, residual COD at FW102-2 was consumed within 1-2 days.

**The results support the following conclusions:**

***A denitrifying community is well established***

To limit biofouling, we added ethanol in pulses. Ethanol and its intermediates were monitored as COD (Figure 1c). Most of the added COD was consumed in the subsurface. When we stopped injection, nearly all of the residual COD was rapidly consumed (within 24 hours). Nitrate concentrations fell during ethanol injection and rebounded when we stopped ethanol injection (Figure 1d). After repeated injections, peak nitrate concentrations dropped to ~0.2 mM. In the future, shorter pulses of ethanol addition will help further limit biofouling.

Nitrate concentrations dropped more rapidly in the MLS wells than in the extraction wells (Figures 2d and 3d). This was expected since the inner cell extraction well pulls in water from outside the focused inner cell treatment area. In FW101-2, the nitrate concentration decreased when ethanol was present, then rebounded after ethanol addition was stopped. The peak rebound concentration decreased over time, approaching zero by day 245 (Figure 2d). The same trend was observed in FW102-2 (Figure 3d).
**The pH in the inner loop has increased to a level suitable for microbial activity and U(VI) reduction**

The pH of the subsurface increased due to the injection of K$_2$CO$_3$ and the production of alkalinity during ethanol consumption. The pH of the inner loop extraction well was the slowest to increase. We attribute the persisting low pH of this well to the presence of Al-rich soil nearby. Chemical analyses of the water from this well revealed significant levels of dissolved Al, and samples of the foulants in this well and its tubing confirm the formation of aluminum precipitate. Despite this complication, we have now succeeded in raising the pH to 6.2-6.4 (**Figure 1b, 2b, and 3b**) in all wells.

**Sulfate reduction activity is underway**

Several indicators of sulfate reduction have been observed. Sulfate decreased in Well B (FW026), illustrated in **Figure 1e**. In addition, sulfate has declined in MLS wells FW101-2 and FW102-2 (**Figures 2c** and **3c**). After Day 219, we noted a strong H$_2$S odor during sampling. We are currently confirming the presence of both sulfide and sulfate-reducing bacteria at these wells.

**Biological reduction U(VI) is underway**

Uranium (VI) concentrations in extraction Well B (FW026) have continuously decreased during successive runs of biostimulation (**Figure 1a**). At the same time, pH has increased from 5.4 to above 6 (**Figure 1b**). In future reoxidation and drilling experiments, we plan to further confirm that the mechanism of the U(VI) removal is microbial reduction.

As expected, the pH of the MLS wells has tracked addition of K$_2$CO$_3$ at the injection wells (**Figure 2b** and **3b**) and U(VI) levels increase and decrease as pH increases and decreases. We are using carbonate addition to manipulate the bioavailability of sorbed U(VI). A decrease in U(VI) at elevated pH is indicative of a microbial removal mechanism (**Figures 2** and **3**) when it correlates with ethanol addition. We note that:

1) When the pH > 5.8-5.9, aqueous U(VI) concentration increases. U(VI) concentrations generally track changes in pH.

2) During successive biostimulation runs, however, we have observed an overall decrease in peak aqueous U(VI) concentration.

3) During run 5 (day 185-194) and after in FW101-2 the U(VI) concentration increased in concert with pH (**Figure 2a**), but also rapidly decreased while pH remained relatively high in the presence of COD in the well. **This is evidence of a microbial removal mechanism.** The same phenomenon was observed during run 6 (day 201-209) and in another MLS well FW102-2 during the same time periods (run 5 and run 6 in **Figure 3**).

4) Suspended sediment samples recovered from Well C (FW104) and Well B (FW026) indicated that the soil near the injection well is black in color, indicating a reduced condition (**Plate 1**). The black color is likely the result of Fe, Mn or U(VI) reduction. We will monitor the composition of further suspended sediment samples to watch for U(IV).
Our strategy for management of well screen clogging has succeeded

One of the most important factors affecting the success of bioremediation generally, and of this site in particular, is clogging of the well system by chemical and/or biological foulants. We have developed a workable strategy for managing this problem.

As indicators of well clogging, we monitor well water levels. Figure 4a shows the recent water level history in inner loop injection well (Well C/FW104) and inner loop extraction well (Well B/FW026).

We first observed clogging in the injection well (Well C/FW104). Water levels rose rapidly during the 2nd biostimulation run (day 163-166). After we cleaned up the well, the water level returned to the baseline level. Suspended solids collected during cleanup were chiefly composed of Al and O, with U, Si, S, and P also present (Plate 2). This composition is consistent with the occurrence of Al(OH)₃ precipitate. We conclude that low pH flowstreams containing Al converged and mixed with higher pH flowstreams, resulting in the formation of Al(OH)₃ precipitate, which accumulated at the well screen. This type of Al precipitate was also observed in the extraction well’s pump head (Plate 3). We cleaned up Well C (FW104) a second time on day 212. Again the clogging was mainly due to precipitation of aluminum.

Over time, as more and more ethanol has been injected into the subsurface, bio-clogging has become more of a concern. We have monitored a gradual decrease in the water level of the extraction well (Well B/FW026), and an increase in water level at the injection well (Well C/FW104). Both wells were cleaned up on day 247 and the water levels return to normal levels. The pump head from Well B (FW026) was covered with thick biofilm (Plate 4).

Unlike the inner loop, the water levels in both injection well (Well D/FW024) and extraction well (Well A/FW103) of the outer loop have been stable during last three months and there was no sign of clogging (Figure 4b).

A quick response to both chemical and biological clogging, as detected by continuous water level monitoring, is critical to the success of this project. We will continue to address clogging as it occurs to limit the impact to a short time and the (relatively) easy task of cleaning the well screens and pump heads. If the clogging were allowed to extend farther into the aquifer, it could become more difficult to properly clean and maintain the desired flow rates.

Next steps

Over the past few months, bioactivity has flourished as indicated by denitrification, metal reduction and sulfate reduction. In the next 2 to 3 months, we will continue to focus on U(VI) reduction. Our operational strategy will is designed to limit clogging. The following experiments and operational methods are planned:

a) Continue pulse injections of ethanol and K₂CO₃ to build up bioactivity.

b) When a relatively stable pH around 6.8-7 is established, we will test the concentration change of U(VI) in the presence and absence of ethanol to estimate U(VI) reduction kinetics versus desorption/adsorption of U(VI).
c) Continue to monitor the impact of nitrate, Fe and sulfate reduction on U (VI) reduction.
d) Continue to monitor the water levels and clean up the wells when necessary;
e) Continue to sample microbial communities during the remediation process.
Figures

Figure 1: The operational results of inner loop extraction well FW026 (Well B) and injection well FW104 (Well C).

a) Uranium (U(VI)) in FW026 (Well B) and FW104 (Well C).

b) pH in FW026 (Well B) and FW104 (Well C).
Figure 1 (continued):
c) COD in FW026 (Well B) and FW104 (Well C).

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Figure 1 (continued):
d) Nitrate in FW026 (Well B) and FW104 (Well C).

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Figure 1 (continued):
e) Sulfate in FW026 (Well B) and FW104 (Well C).

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Figure 2: The Operational results of inner loop MLS well FW101-2.

a) Uranium (U(VI)) concentrations in FW101-2.

b) pH in FW101-2.
Figure 2 (continued):

c) COD and Sulfate in FW101-2.

d) Nitrate in FW101-2.
Figure 3: The Operational results of inner loop MLS well FW102-2.

a) Uranium (U(VI)) concentrations in FW102-2.

b) pH in FW102-2.
Figure 3 (continued):

c) COD and Sulfate in FW102-2.

![COD and Sulfate in FW102-2](image)

- Time, days: 160 to 250
- COD or Sulfate, mg/L
- 2nd Run: 163-166
- 3rd Run: 170-174
- 4th Run: 177-180
- 5th Run: 185-194
- 6th Run: 201-209
- 7th Run: 219-224
- 8th Run: 227-230
- 9th Run: 233-238
- 10th Run: 240-245

- FW102-2(COD)
- FW102-2(Sulfate)


d) Nitrate in FW102-2.

![Nitrate in FW102-2](image)

- Time, days: 160 to 250
- Nitrate, mM
- 2nd Run: 163-166
- 3rd Run: 170-174
- 4th Run: 177-180
- 5th Run: 185-194
- 6th Run: 201-209
- 7th Run: 219-224
- 8th Run: 227-230
- 9th Run: 233-238
- 10th Run: 240-245

- FW102-2(Nitrate)
Figure 4: Water level changes in inner loop (a) and outer loop (b) extraction/injection well.

a) Inner loop injection and extraction well water levels.

b) Outer loop injection and extraction well water levels.
Plate 1: Particulate matter taken from the inner loop extraction well (Well B/FW026) in the left two tubes, and from the injection wells (Well C/FW104) in the right two tubes, during well clean-up on day 247 have different colors. The solids in Well C (FW104) were black, indicating a reducing environment. The solids from Well B(FW026) were reddish-brown, indicating a less reduced environment. The black color could be due to microbial reduction of Fe, Mn and U(VI) near the injection well. This hypothesis is now being confirmed.
Plate 2: SEM image showing the structure of the chemical precipitates that clogged the inner loop extraction well during the second clogging event (day 212). These precipitates were mainly composed of Al precipitates with small amounts of Si and uranium also present. **Source:** D. Watson, March 2004.
Plate 3: This photo was taken after the second run of ethanol injection on Day 166. The pump head from the inner loop extraction well (Well B/FW026). It was clogged by aluminum precipitate.

Plate 4: On Day 245, after 10 biostimulation runs, the pump head from the inner loop extraction well (Well B/FW 026) became clogged by biofilm. To minimize this type of clogging, we are using pulse injections of ethanol, but periodic well cleaning is still necessary.