BIOLOGICAL TREATMENT OF SURFACE AND GROUND WATER FOR SELENIUM AND NITRATE[1]

Tina Maniatis[2] and D. Jack Adams

Abstract. An ex situ biological treatment system was implemented at a gold mine site in South Dakota. The system removes selenium and nitrate from a mixture of groundwater and surface water runoff at ambient water temperatures. The Applied Biosciences' system replaced a denitrification system that did not perform consistently, did not treat for selenium and required the influent water to be heated. Installation was completed in phases, with the selenium circuit being brought into operation in February, 2002 and the nitrate circuit in March, 2002. System design can accommodate average flows of 380 L/min (100 gallons/minute) and a maximum flow of 1,136 L/min (300 gallons/min). Four cells were designed to remove nitrate from approximately 30 mg/L to below 10 mg/L and two cells were designed to remove selenium from 0.01 mg/L and higher to below 0.005 mg/L. No pretreatment is required to operate the system at ambient temperatures which can fall as low as -12° C in the winter and treat water at temperatures ranging between 8º C to 16º C. Nutrient requirements are met using a sugar based nutrient mixture that includes a balanced C:N:P:S ratio, trace elements and vitamins. Since start up, the system has consistently been in compliance for nitrate and selenium, treating water to below detection limits for both contaminants. This paper discusses laboratory biotreatability testing of a system designed to remove selenium, arsenic, and nitrate and full-scale implementation and optimization of a system to remove nitrate and selenium.

Additional Key Words: microbial, biological treatment, selenium reduction, denitrification, nitrate reduction, arsenic removal

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[2] Tina Maniatis is a Project Manager for Applied Biosciences, Salt Lake City, UT 84115. D. Jack Adams is a Vice President of Research and Development for Applied Biosciences, Salt Lake City, UT 84115.

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Introduction

Nitrate is a stable and highly soluble ion with a low potential for coprecipitation or adsorption. Therefore, nitrate removal is difficult using conventional water treatment technologies, such as lime softening and filtration (Hoek et al. 1992). More sophisticated technologies, such as ion exchange, reverse osmosis, electro dialysis, and biological denitrification, can be used to remove nitrates from water (Hoek et al. 1992, Kook 1989, Richard 1989, Murphy 1991, Dahab 1987). Ion exchange, reverse osmosis, and electro dialysis may produce high quality effluents but require high capital costs. Additionally, reverse osmosis and ion exchange produce a waste stream than may require treatment. Biological denitrification has been demonstrated as a cost-effective means of removing nitrate from water without high capital and operating costs. Nitrate reduction to nitrogen gas occurs as a series of steps as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

Both heterotrophic and autotrophic bacteria can perform denitrification. Heterotrophic anaerobes or facultative anaerobes utilize a carbon source such as sugars, methanol, ethanol, or acetic acid for the conversion of nitrate to nitrogen gas. Autotrophic bacteria utilize hydrogen or reduced sulfide compounds as substrates, and carbon dioxide or bicarbonate serve as the carbon source for cell synthesis (Mat et al. 1992).

Selenium is a naturally occurring metal that is commonly found in mining waters, petroleum waters and agricultural runoff. Selenium occurs in various valence states from -2 to +6. In water, selenium exists mostly as the ions selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Kapoor et al. 1995). Treatment options for selenium removal include iron coprecipitation, reverse osmosis, the use of constructed wetlands, and biological selenium reduction. Iron coprecipitation may be successful in removing selenite but may not be successful for selenate removal. One pilot test resulted in lower effluent selenite, but an increase in effluent selenate. This may have been attributable to oxidation of selenite to selenate by ferric iron (Merrill et al. 1986). Additionally, iron coprecipitation results in waste sludge that may require treatment and disposal. Constructed wetlands can be successful in removing selenium from water; however, due to selenium bioaccumulation in wetland plants and animals, constructed wetlands may create a toxic hazard for wildlife. (Lemly and Ohlendorf 2002). Finally, reverse osmosis can remove selenium with high efficiencies. However, reverse osmosis technologies can be expensive to implement and operate, may require pretreatment, and yield a waste stream that may also require treatment.

Many heterotrophic and autotrophic bacteria can perform biological selenium reduction, but most bacteria perform this transformation slowly. In mining wastewaters, these transformations slow even further and many bacteria are selective in their ability to transform selenate and selenite. Heterotrophic anaerobes or facultative anaerobes utilize a carbon source such as sugars, methanol, ethanol, or acetic acid for the conversion of selenate and/or selenite to elemental selenium.

Applied Biosciences has developed a new biological treatment system that is capable of removing nitrates or a mixture of nitrates, metals, and other inorganic contaminants, such as selenium and arsenic from wastewaters (Adams and Pickett 1998, Adams et al. 2000, Adams et al. 1998, Adams et al. 1996). In a recent application, the ABMet™ technology was configured to remove nitrates, selenium and arsenic from a mixture of groundwater and surface water runoff at a gold mine site in the Black Hills of South Dakota. Mine operations deposited spent ore from blasting in a valley in accordance with their operating permit. Surface water runoff from the spent ore is collected in a lined pond. This near neutral water contains arsenic, selenium, and nitrate. Groundwater in the valley contains elevated levels of nitrate and is pumped out of the aquifer, combined with the surface water runoff and fed to the biological treatment system on the mine site. The combined waters result in a wastewater flow ranging from 380 L/min to 1,136 L/min and contain selenium at approximately 0.01 mg/L, nitrate at approximately 30 mg/L, and arsenic at approximately 0.06 mg/L. Treatment targets for nitrate and selenium are 10 mg/L or below and <0.005, respectively. Arsenic removal was addressed in laboratory studies but arsenic removal was not included in the full-scale implementation at this time.

The Applied Biosciences' biological treatment system replaced an existing system that was only partially effective for biological denitrification. The old system consisted of four in-ground concrete cells that partially removed nitrate from the water using methanol as a nutrient and biorings and gravel as a microbial support. The system did not remove arsenic or selenium and required heating of the influent water for nitrate removal. Even with heating, denitrification rates were not adequately meeting site requirements. A system was needed to bring mine waters into compliance for selenium and nitrate and reduce operational costs associated with heating the water for treatment. The Applied Biosciences' biotreatment system removes nitrate and selenium at ambient temperatures using a balanced sugar-based nutrient and a microbial support that maintains higher densities of microbes.

This paper discusses laboratory biotreatability studies that included bench scale studies for selenium, arsenic, and nitrate removal, screening of microbes, and nutrient formulations tailored for contaminant removal in site waters. Additionally, the paper will address the full-scale implementation of the biotreatment system including results from the on-site microbial scale-up, system inoculation under winter temperatures, and full-scale treatment.

Methods

Laboratory Studies

Laboratory studies consisted of microbial characterization of site water, bench-scale biosystem tests for selenium, arsenic, and nitrate removal, and nutrient formulation testing. All samples were stored at 4 ° C to inhibit microbial growth until analysis was performed. Baseline microbial characterization included microbial isolations and plate counts. Site waters were characterized for microbial content by identifying isolates by colony morphology and gram stain, and slanting isolates on appropriate media for future testing. Samples were plated on modified Trypticase Soy Agar (TSA) plates, and resulting colonies were counted using Standard and Modified Plate Counting Methods. Additionally, isolates and selected combined cultures were characterized through arsenic and selenium reduction, and denitrification assays. Microbial analysis and characterization data is used by Applied Biosciences to insure that the microbial inocula established in site bioreactors meet predetermined specifications.

A bench-scale treatment system was inoculated with selenium-reducing, arsenic-reducing, and denitrifying microbes and tested with site water. The system was sampled daily after a 24 hour equilibration period. Effluent samples were filtered and preserved with hydrochloric or nitric acid and analyzed for selenium, nitrate, and arsenic.

Nutrient screening tests were conducted to choose a site specific nutrient blend by examining the growth and contaminant removal characteristics of selected cultures in various nutrient formulations. The nutrient used was a site-specific balanced C:N:P:S sugar based mixture that included trace elements, and vitamins.

On-site Scale-up and Inoculation

Scale-up required culturing microbes to sufficient volume to inoculate the entire biotreatment system for selenium reduction or denitrification. Following inoculation, the microbes in each segment of the biotreatment system were allowed to grow to predetermined levels before bringing the system on-line.

Startup and Operation

The biotreatment system was operated to remove nitrate in four denitrification tanks and two selenium reduction cells. Nutrient requirements were met using a sugar-based nutrient containing a mixture of a balanced C:N:P:S ratio, trace elements and vitamins. The system startup was monitored for pH, temperature, oxidation/reduction potential, and contaminant removal.

Results and Discussion

Laboratory Studies

The site water contained a moderate and diverse microbial population with a low population of selenium reducing microbes. The surface water contained 1.5 E6 colony forming units/milliliter (cfu/ml) with 0.18% of the colonies capable of reducing selenium. The groundwater contained microbial counts of 1.1 E4 cfu/ml with 0.29% of the colonies capable of selenium reduction.

Growth of selected microbes was tested using different concentrations of a nutrient formulated for this specific site. Nutrient composition varies with bioreactor type, aerobic and/or anaerobic bioreactor conditions, microbes used, and site water chemistry. There are components that are usually present in a nutrient mix; these are included in the components listed in Table 1. The amount of any individual component changes depending on the variables listed above, for example, if the site water is high in sulfate and iron, these components will not be provided in the nutrient mix. The optimized nutrient mixture is balanced to a pH of ~ 7 with site water for testing.

Table 1. Nutrient Components.

NUTRIENT COMPONENTS			
Carbon	Potassium	Zinc	Vitamins
Nitrogen	Manganese	Magnesium	Protein
Phosphate	Calcium	Amino Acids	Trace Elements
Sulfur	Iron	Lactate	alcohol

Figure 1 shows the microbial growth obtained in site waters by varying the Trace Elements, Amino Acids, and Vitamin amounts once optimal levels of other components had been determined.

Bench-scale biotreatment system tests showed that nitrate, selenium and arsenic could be removed to desired levels from the mine water using appropriate microbes and nutrients. Bench-scale testing results are summarized in Figures 2 through 4.

<u>Site Preparation</u>

Biotreatability testing determined that selenium removal to below detection limits at ambient winter temperatures and the higher expected flow rates of 1,136 L/min (300 gallons/min) would require the use of all four existing in-ground cells. A biotreatment system was designed to utilize existing equipment and tankage. Two cells were cleaned out to accommodate the new selenium reducing biosystem. The two other denitrification cells were kept running until the conversion to the selenium/nitrate system was complete. Four new above ground tanks were constructed of steel to denitrify the water prior to selenium biotreatment. Distribution systems were installed in all of the cells and tanks to provide a modified plug flow biotreatment system. Once the distribution systems were in place, the tanks and cells were filled with microbial support materials.

Figure 1. A nutrient mixture with the amount of base nutrient, trace elements, amino acids, and vitamins optimized for site waters produced the best microbial growth.

<u>On-site Scale-up and Inoculation</u>

Laboratory studies screened indigenous and Applied Biosciences' microbes to produce an optimized, durable, and site specific inocula. Both microbes and nutrient formulations were tailored for nitrate and selenium removal in mine waters. Microbial inocula were prepared in the lab, trucked to the site, and scaled-up for inoculation of the biotreatment cells. The selenium reducing culture was delivered to the site on December 18, 2001. The scale up finished on December 26, 2001 and the two selenium cells were inoculated on December 27, 2001.

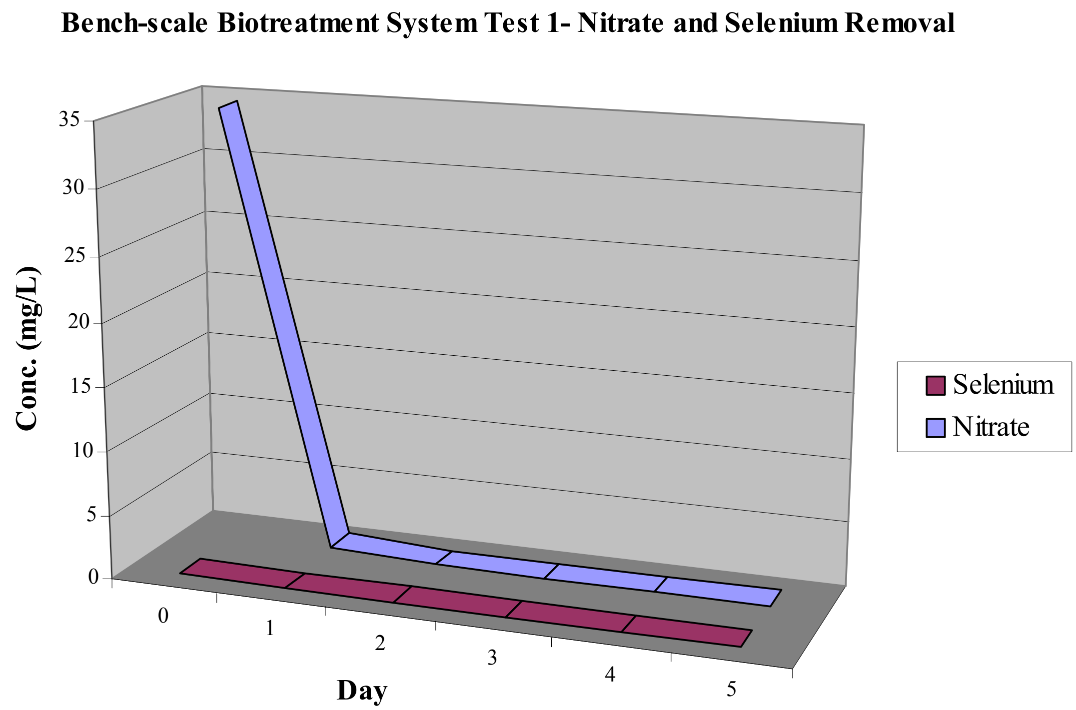

Figure 2. This graph displays selenium and nitrate removal using a site specific staged biotreatment system. Complete nitrate and selenium removal was obtained.

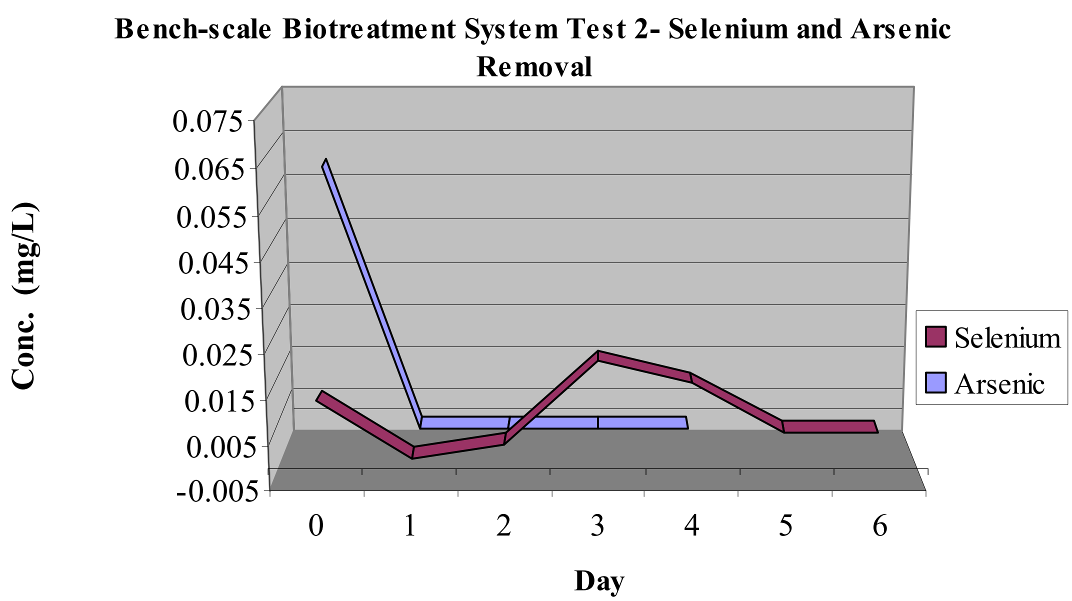

Figure 3. Selenium and arsenic removal was evaluated in a second test run. Arsenic was removed to below detection in a single pass. Effluent selenium levels peak at day three due to nutrient depletion. Feeding of the system reduced selenium levels.

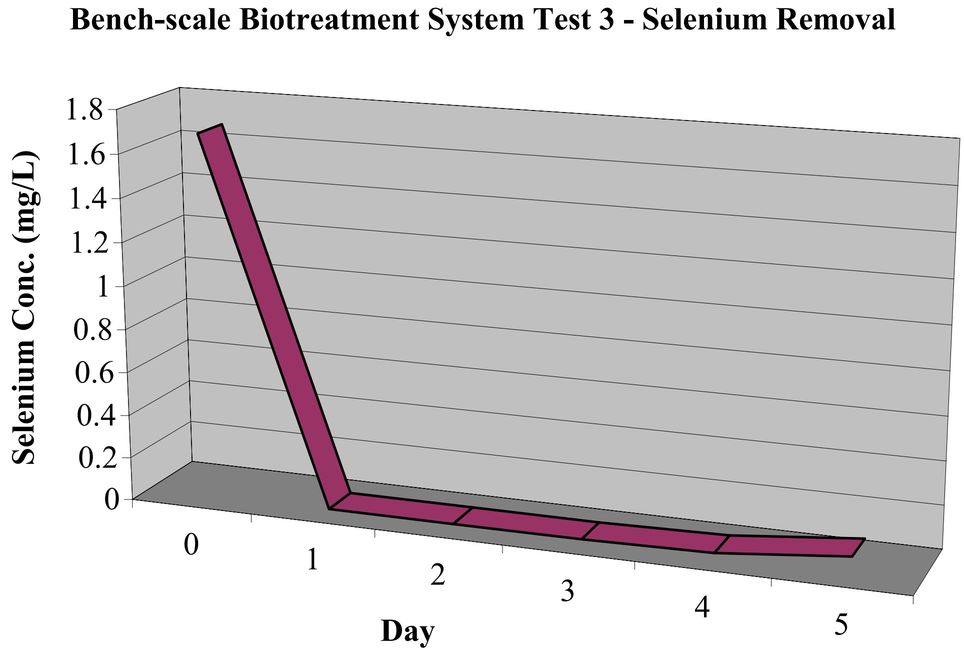

Figure 4. Selenium removal to method detection limit (0.002 mg/L) was achieved in test run 3. Values increased slightly on days 4 and 5 due to nutrient depletion in the system.

The first nitrate culture was brought on-site on January 30, 2002. The scale-up was completed on February 7, 2002. On February 8, 2002, two of the four nitrate cells were inoculated with the nitrate reducing culture. The final nitrate culture was brought on-site on March 7, 2002. The scale-up was completed on March 15, 2002 and the final nitrate tanks were inoculated on March 16, 2002.

Startup and Operations

Startup of the completed system began in April, 2002. The system began running water through the system at a slower rate than normal to aid in microbial population development and stabilization. The plant operated the system in two treatment trains: Nitrate tank 1 (N1), nitrate tank 4 (N4), and selenium cell 1 (S1) ran parallel to nitrate tank 2 (N2), nitrate tank 5 (N5) and selenium tank 2 (S2) (Figure 5). By mid-April, the system was treating water at normal flow rates, approximately 100 gpm. By the last nitrate tank in each train, the concentration of nitrate was below the target limit of 10 mg/L. Selenium concentrations at the end of the selenium circuit were below detection. The first month of operations are displayed in Figures 6 and 7. Summertime operations are displayed in Figures 8 and 9.

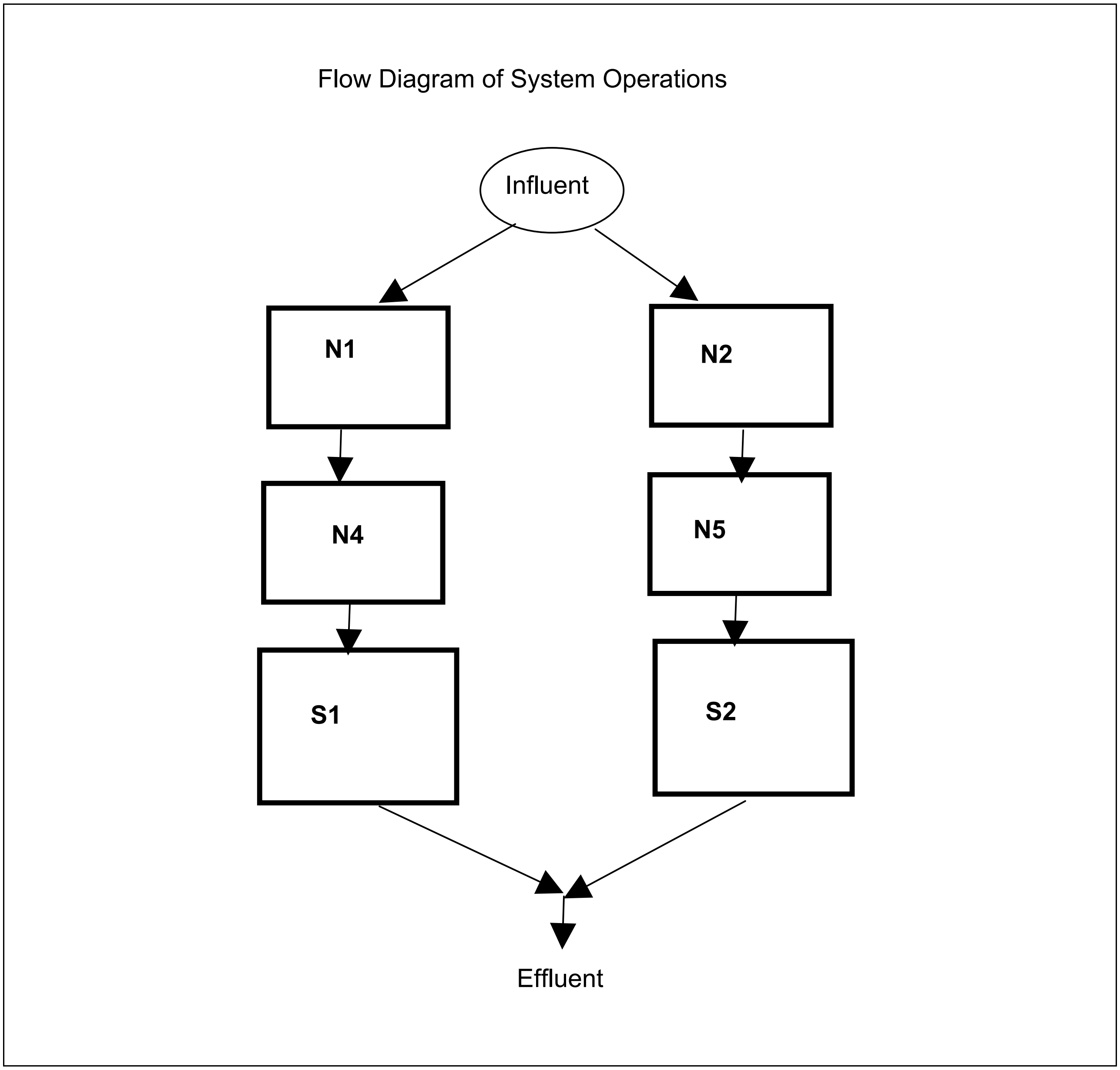

Figure 5. The figure displays a diagram of system operations. N1 through N5 represent the denitrification tanks and S1 through S2 represent the selenium reduction cells.

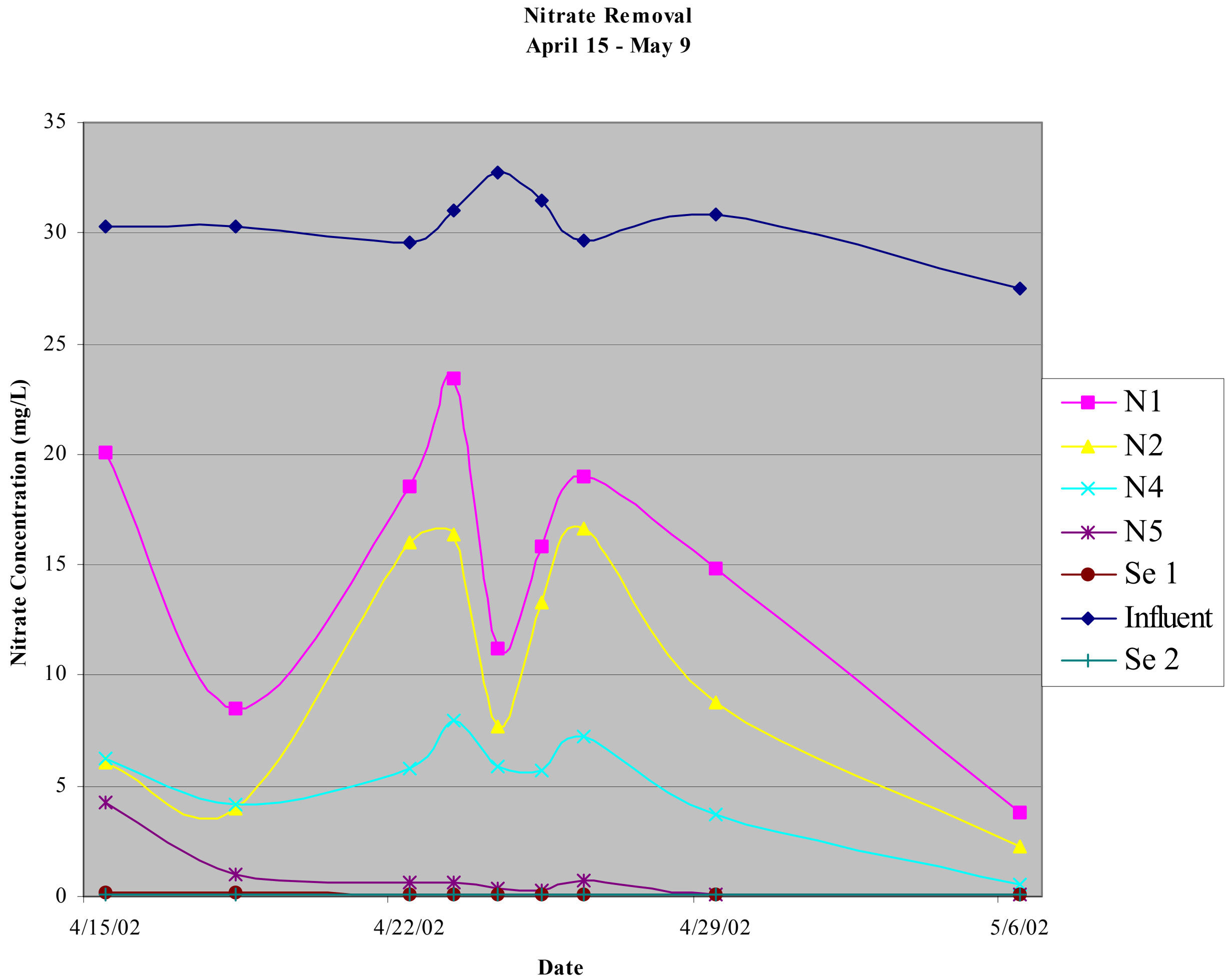

Figure 6. Graph displays the first month of operations. The final nitrate cells, N4, N5 are consistently below target limit of 10 mg/L.

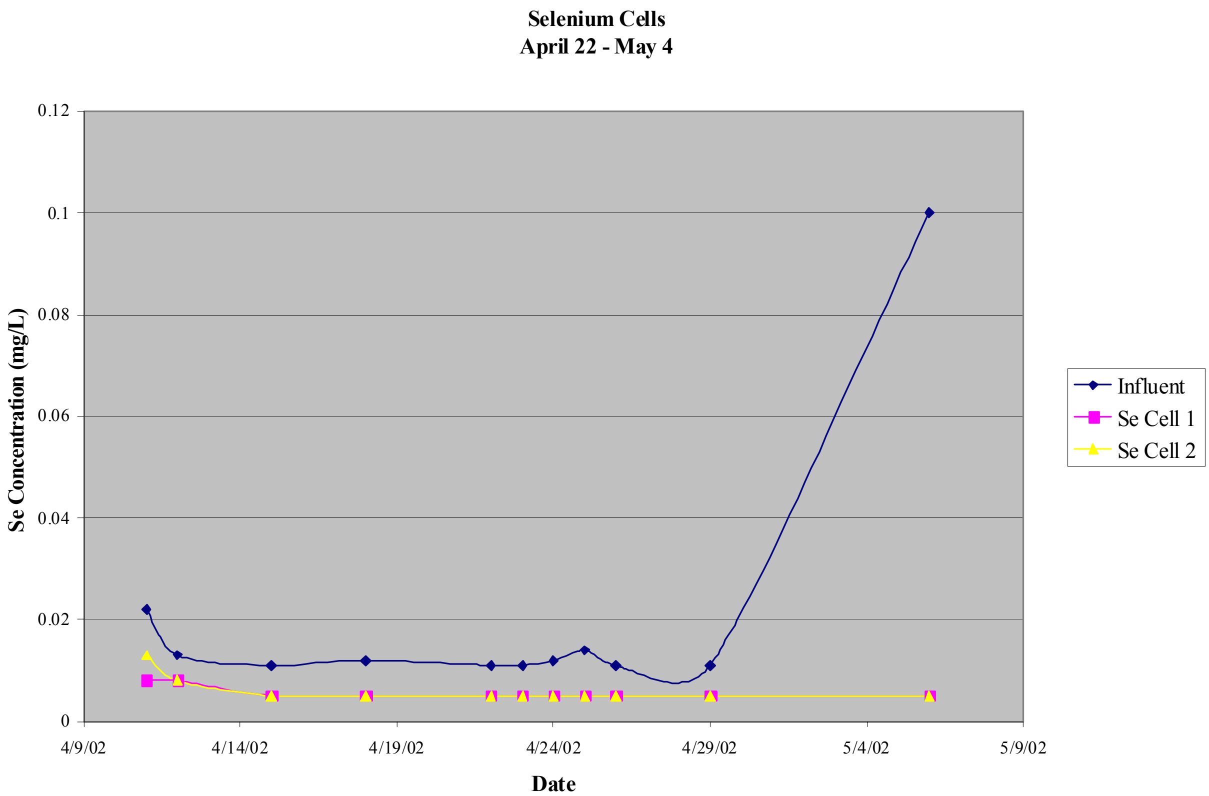

Figure 7. Effluent selenium values are consistently at detection limit of 0.005 mg/L.

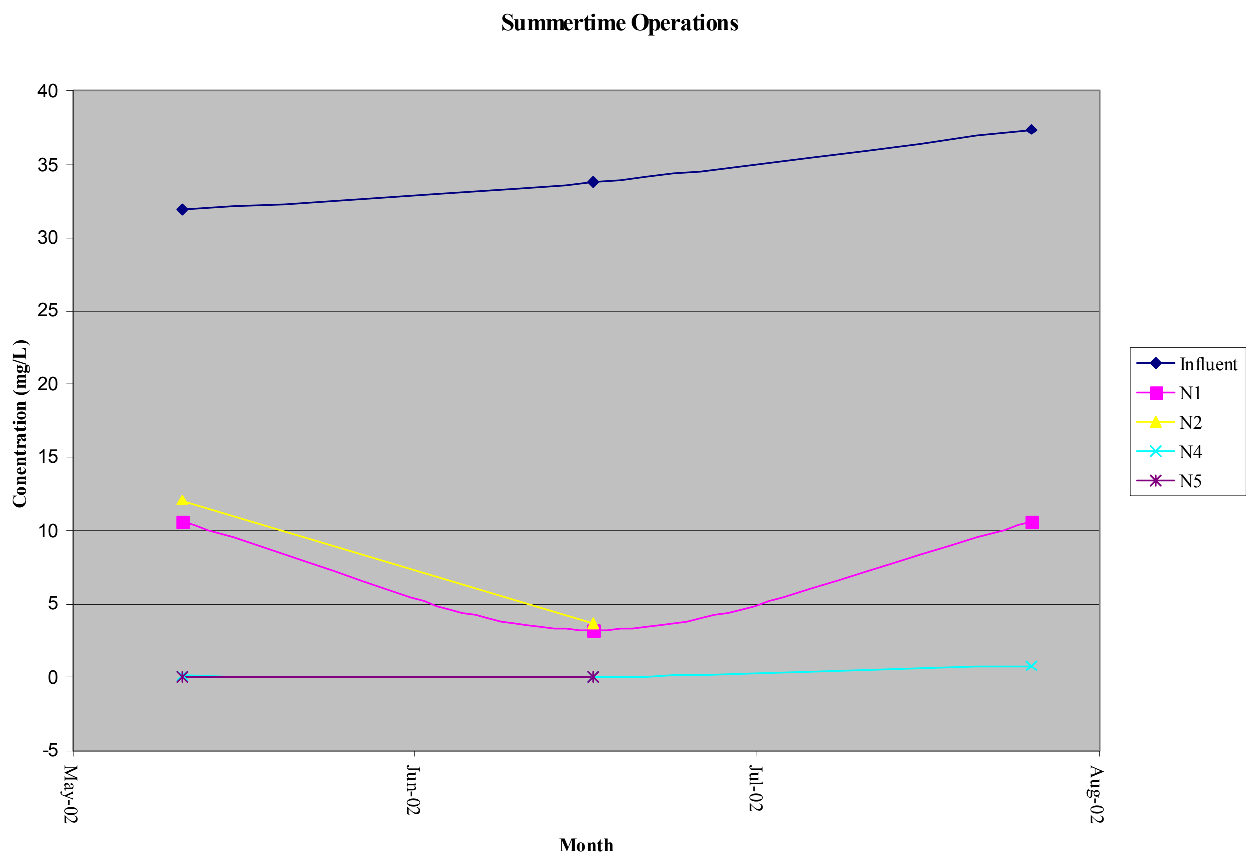

Figure 8. Graph reflects nitrate removal during summertime operations. In August, train 2 was shut down and all flow went through train 1. Flowrates in the summer decreased to approximately 70 gpm due to low precipitation.

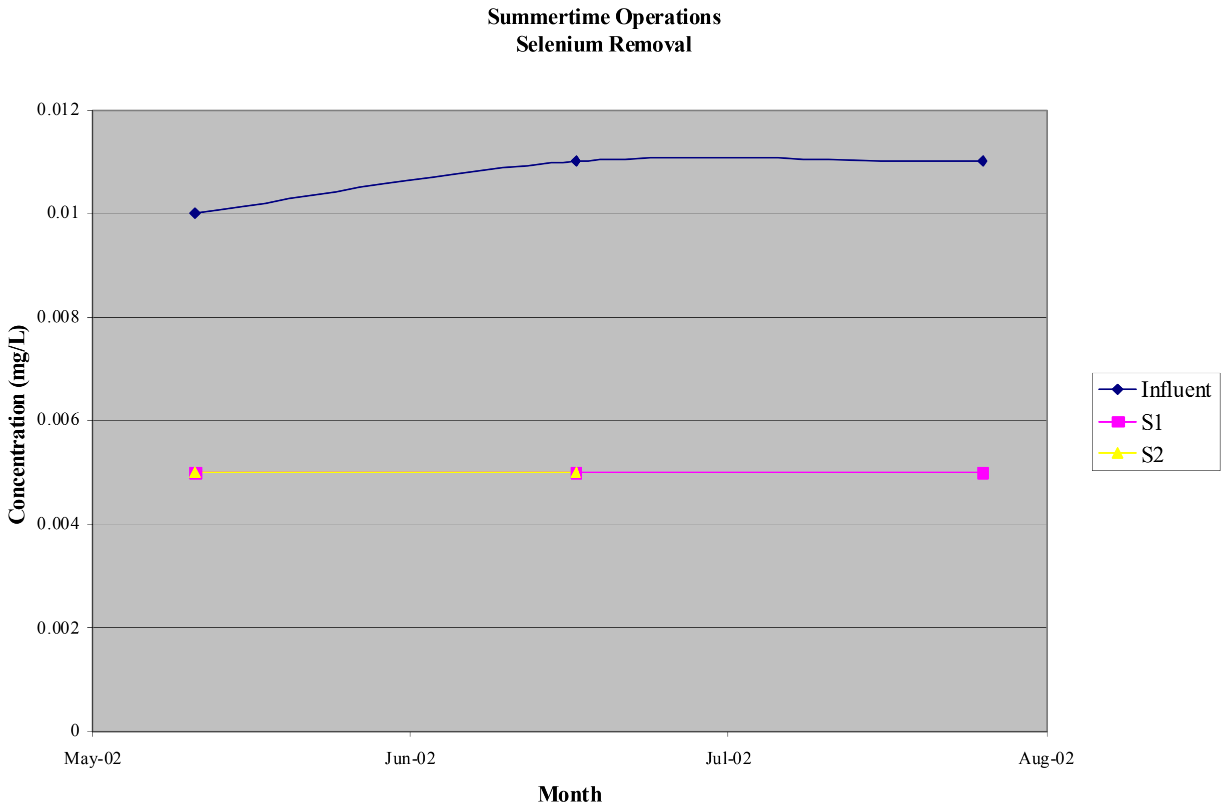

Figure 9. Graph reflects selenium removal during summertime operations. In August, train 2 was shut down and all flow was diverted to train 1. Flowrates in the summer decreased to approximately 70 gpm due to low precipitation.

Conclusions

As of December 2002, full-scale implementation of the Applied Biosciences biotreatment system at the South Dakota site is fully successful. Consistent removal of both selenium and nitrate at ambient temperatures to target limits of 10 mg/L for nitrate and <0.005 mg/L for selenium have been obtained since start-up in April 2002. The site-specific balanced nutrient formulation produced significant cost savings over the methanol-phosphate nutrients used in the replaced system. The microbial populations screened in site waters have proven to be highly stable and durable and have been maintained at stable population densities since startup.

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