

# **APPENDIX V**

## **Treatability and Bench-Scale Bioreactor Testing of Waters for Selenium Removal**

**January 31, 2008**



***TREATABILITY AND BENCH-  
SCALE BIOREACTOR TESTING  
OF WATERS FOR SELENIUM  
REMOVAL - FINAL REPORT***

January 31, 2008

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## EXECUTIVE SUMMARY

This report summarizes data presented in *Phase 1 and 2 reports* and presents new data gathered for Phases 3 and 4 that addresses bench-scale testing of three potential process waters and provides a cost breakdown for an on-site pilot-scale test. Good microbial growth and selenium reduction was obtained using a defined media and a molasses, yeast, phosphate media (MYP nutrient) at 3.75 gm/L molasses, 1.5 gm/L yeast extract, and 2.0 mg/L phosphate in waters containing ~4.3 mg/L selenium. DGGE profiling of the selected SF036, Z0, SF056, and Lorax-1 (L-1) microbial population and individual isolated microbial constituents was conducted and on-line database sequence comparisons yielded no matches with sequenced microorganisms. These microorganisms are unknowns at the genus and species level. Microbial stock cultures have been made and are available for pilot-scale testing.

Reactor testing was conducted in two new reactor types 1) a biochemical-enhanced material reactor (BEMR) and 2) an electro-biochemical reactor (EBR). Both had void volumes of ~700 mL and used mostly modified pumice as a microbial growth surface. *Phase 3* testing used water spiked with selenium to ~9 mg/L and nine buckets of visibly different waters, received as one process run. Two tests were made with these waters, one at ~4.3 mg/L selenium and one at ~15 mg/L selenium. The first tests used two BEMR in series with feed waters containing ~9 mg/L (spiked) selenium and nutrient addition to only the first bioreactor and produced effluents that averaged ~0.58 mg/L selenium using a total retention time of forty-four hours. In Test 2 the bioreactor configuration was changed to include a pretreatment step and used the feed waters containing ~9 mg/L selenium, with microbes and nutrient, to simulate a holding or equilibration pond, and removed an average of ~2.9 mg/L selenium. Test waters were again supplemented with 100 mL nutrient before entering reactor 1 as described for Test 1; no nutrients were added to the bottom of BEMR-2 during this test except what carried over from BEMR-1. A bioreactor retention time of forty-four hours produced a final effluent averaging ~0.029 mg/L selenium.

In Test 3 the bioreactor configuration was changed to eliminate the pretreatment step and waters containing ~4.3 mg/L selenium were introduced into BEMR-1 and BEMR-2 as in Test 1. In this test 100 mL of 3.75 gm/L MYP nutrient was added to the bottom of both BEMR-1 and BEMR-2 for a total of 200 mL nutrient once daily; the effluent was sampled at the top of BEMR-2. A total retention time of forty-four hours produced a final effluent averaging ~0.031 mg/L selenium. During Test 3, the EBR was started and operated in the same manner as the BEMRs. A retention time of twenty-two hours produced a final effluent averaging ~0.030 mg/L selenium. In Test 4 the bioreactor configuration was again changed to connect the BEMRs and EBR in series using new test waters containing ~15 mg/L selenium and a total retention time of sixty-six hours. Limited data gathered showed an effluent averaging ~0.47 mg/L selenium from the two stage BEMRs and a final effluent from the EBR averaging ~0.072 mg/L selenium. A number of additional metals identified as potential permitting criteria were also removed to a large extent by the bioreactors.

Testing and site conditions indicate that both pre and post treatment steps will be required and sizing is given for both a 1/10 and a 1/50 pilot-scale system. Treatment costing assumptions and items are provided for a 1/10 pilot-scale test running three months with a cost estimate just under \$163,000.00.

# ***TREATABILITY AND BENCH-SCALE BIOREACTOR TESTING OF WATERS FOR SELENIUM REMOVAL - FINAL REPORT***

## **INTRODUCTION**

This report summarizes data presented in earlier reports, *Phases 1 and 2*, addressing selenium treatability and initial testing of several different potential process and waste waters for a new mine at the Wolverine Project, YK. *Phases 3 and 4* address bench-scale testing of several different process water samples and provides a cost breakdown for an on-site pilot-scale test. Limiting conditions with respect to treatability at this site are temperature and high selenium concentrations; up to 14 mg/L selenium. It is planned to run the selenium biotreatment system seasonally; six months of the year - spring through fall. Water samples were obtained from tailings pilot plant runs that simulated actual full-scale tailings plant waters; samples were received from *Lorax Environmental Services Ltd.*, 2289 Burrard Street, Vancouver, BC V6J 3H9. The waters received were examined using different methods to assess potential selenium treatability, including two newly developed selenium removal technologies demonstrated to enhance biofilm growth and metal removal.

## ***Data Summary***

### **Phase 1 Goals**

- Evaluation of pilot plant process water chemistry for microbial toxicity and required nutrient supplementation
- Qualitative assessment of biotreatability effectiveness at temperatures of 20° C and 4° C using site and repository microbes
- Qualitative assessment of biotreatability effectiveness of several microbial mixtures

*Phase 1* testing produced the following conclusions:

- Microbial screening successfully demonstrated that site microbes and two microbial populations were capable of good selenium reduction in site waters at ~20° C and to a lesser degree at 4° C
- Overall, the tests were very positive and provided a strong demonstration that biological selenium reduction was achievable in the waters tested
- If effluents containing higher levels of selenium, e.g., <50 ppb can be considered, they would be possible with considerable less development and capital investment

*Phase 1* Recommendations:

- Continue testing the microbial populations and site isolates in *Phase 2*
- Continue investigation of microbial adaptation for lower temperature selenium reduction
- Perform preliminary assessment of selenium removal using selected nutrient combinations

### **Phase 2 Goals**

- Select an optimal microbial population for selenium removal in process test waters received
- Continue to qualitatively assess selenium removal at 20° C and 4° C
- Perform preliminary assessment of selenium removal using selected nutrient combinations
- Evaluate microbial selenium removal characteristics

*Phase 2* testing produced the following conclusions:

- Several microbial isolates and microbial populations were capable of selenium reduction in site waters at ~24° C using an economical nutrient and a 72 hour retention time
  - A microbial population – SF036, Z0, SF056, and Lorax-1 (L-1) will be used in bioreactor tests
  - Following mixed culture co-adaptation, site microbes volatilized selenium to a high degree
- pH was not an issue, but the waters provided for the *Phase 2* tests did affect selenium reduction

- Cyanide or another contaminant removed with hydrogen peroxide made a significant difference in the selenium removal obtained
  - Selenium concentration will require at least two treatment stages to remove selenium to <50 ppb
- Nutrient component levels formulated for *Phase 3* tests included sugars, protein (carbon), nitrogen, iron, magnesium, and phosphate
  - Nutrients were provided in test solutions containing molasses/soy (3.75 to 0.75 gm/L), yeast (1.5 to 0.75 gm/L), and phosphate (0.05 to 2 gm/L)
  - The best performing defined media contained 3.75 gm/L molasses/soy, 1.5 gm/L yeast, and .5 to 2.0 gm/L phosphate – MYP media
  - Growth rates in the MYP media were slower than in a rich commercially available trypticase soy microbial media
  - Microbial selenium reduction at ~4° C in the waters provided for the *Phase 2* tests was not acceptable

*Phase 2* Recommendations:

- Testing and evaluation should proceed to the next level of bench-scale reactor testing at 24° C
- Testing can be conducted in two new, but tested, biotreatment system designs
  - Electro-biochemical reactor (EBR)
  - Biochemical-enhanced materials reactor (BEMR)
- Profile microbial population using Denaturing Gradient Gel Electrophoresis (DGGE)
- Continue to track selected microbial adaptation and selenium volatilization

## BACKGROUND - BIOREACTOR TESTING

### *Phases 3 and 4*

*Phases 3 and 4* have not been covered in a formal report and are reported here. This document reports on the bench-scale process tests using three different waters, evaluates bioreactor testing for an on-site pilot-scale test, and provides a rough cost estimate for this test. The objectives include:

- Evaluate the new process waters received for microbial toxicity and required nutrient supplements
- Re-evaluate the selected microbial population in the process waters for relative selenium reduction
- Fingerprint the microbes identified as important for selenium reduction in site waters using DGGE
- Evaluate selenium reduction in two new bioreactor types – a bio-electrochemical (BEC) reactor and a biochemical-enhanced microbial support materials (BEMS) reactor
- Use the developed nutrient containing 3.75 gm/L molasses/soy, 1.5 gm/L yeast, and 1.0 gm/L phosphate – (continued *Phase 2* testing indicated a lower phosphate concentration could be used)
- Target a bioreactor effluent with selenium concentrations below 20 µg/L (ppb)
- Assess technologies for pilot-scale and provide a rough cost estimate for on-site tests

### *Water Samples*

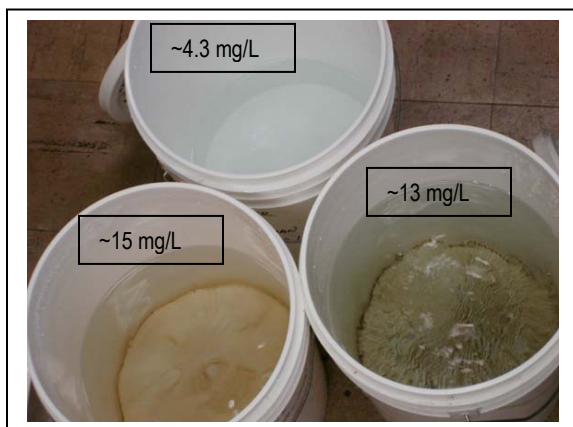
#### *Water Chemistry Analysis*

Selenium and other metal concentrations in samples analyzed were obtained by direct ICP MS analysis of the sample or through a dilution of the sample by a factor of 10:1 or 100:1. Samples and sample dilutions were run against calibration curves constructed using response measurements on known calibration standards. For example, for the lower selenium concentrations, the following 0, 40, 80, 120, and 240 ng Se/L calibration standards were used. When required the selenium or other metal concentrations in the calibration standards were revised, for example to 0, 25, 250 and 2500 ng/L. In addition, dilution factors were calculated so that the selenium and other metal concentrations in the resulting dilutions approximated one-half the Se concentration in the new high calibration standard. The new dilutions were then prepared and run on the ICP MS against the calibration standards at the revised concentration levels; detection limits were 2 ppb for selenium. Results presented are corrected for dilution.

### Water Samples Received

*Phase 3* testing was initiated with five buckets of waters initially received for *Phase 3* that did not contain any significant selenium or other contaminants. These pH ~7 waters did not have any visible precipitates, were initially thought to contain selenium, and be from a process run. Water samples received were re-analyzed three times with the same result, no selenium present. However, analysis of another water sample from the same run, held by *Lorax Environmental Services Ltd.*, was shown to contain selenium, but not enough to conduct the tests required. The five buckets of pH ~7 water were spiked to ~9 mg/L selenium and used to start reactor conditioning and initial reactor tests.

A second water sample received for *Phase 3* tests consisted of nine buckets of water, provided as one process run with the water chemistry listed under the Bulk Tailings analysis in *Attachment 1*. This set of water samples was reported to have a pH of ~10 and a selenium concentration of ~2.5 mg/L. The pH in all buckets was ~10; however, the selenium concentration varied considerably, from ~4.3 mg/L to ~15 mg/L selenium, as did the appearance of the waters, *Figure 1*. The clear solution, five buckets total, containing ~4.3 mg/L selenium was used along with the spiked solution described above for the bulk of the bioreactor testing.



*Figure 1*. Three different buckets of the nine buckets of test solution received for *Phase 3* testing. These buckets have a selenium concentration of between ~4.3 to 15 mg/L. This photo shows the difference in the solutions received for testing. The clear solution, five buckets total, was assayed at average value of ~4.3 mg/L selenium. The colored solutions contain the higher selenium amounts – there were two buckets each of the red and brown colored solutions. The red colored solutions contained ~15 mg Se/L and the brown colored solutions contain ~13 mg/L selenium; this analysis represents a 0.22  $\mu\text{m}$  filtered sample analysis. Observation: The amount of precipitate in the buckets increased two- to three-fold with time, over approximately two months, and was not a function of settling. Buckets were held at pH ~10, as received, until used in bioreactor or other testing.

## METHODS AND RESULTS

Standard and modified methods were used for all microbial analyses conducted. Standard and modified methods were used in all procedural protocols and modified or 'special' analysis methods were used for qualitative microbial selenium evaluations. Data presented represents summary, combined, and average data from individual tests and the screening conducted.

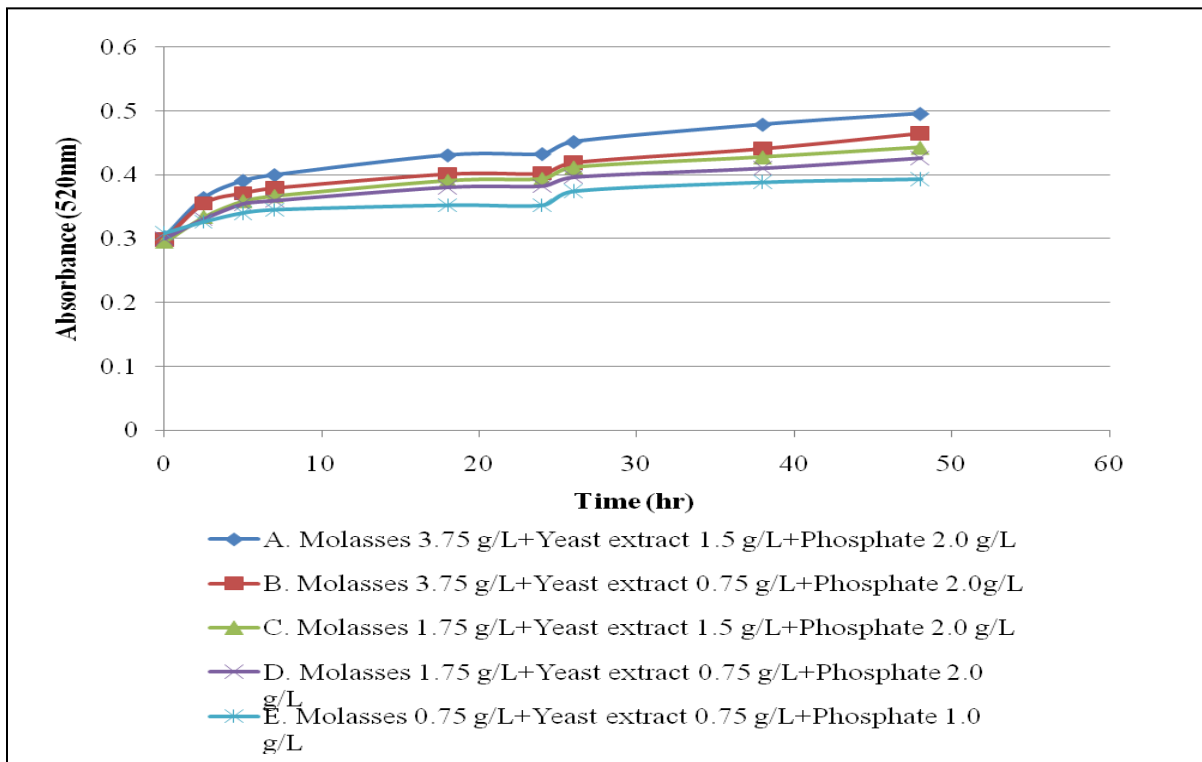
### *Re-evaluation of test microbes in new waters*

In this step, the new process waters received were evaluated for microbial toxicity and nutrient supplements required for good microbial growth and selenium reduction. Evaluation media was made with new test waters containing 4.3 mg/L selenium and various concentrations of nutrient components in a test matrix. Individual microbes were screened in this test procedure for relative growth and selenium reduction. *Figures 2, 3, and 4* depict good microbial growth and selenium reduction using a defined media and a molasses/soy, yeast, phosphate media (MYP nutrient) in waters

containing ~4.3 mg/L selenium. Growth tests were conducted in static test flasks held at ~24° C (Laboratory temperature); absorbance shown in *Figures 3 and 4* is correlated in a direct manner with microbial growth.



*Figure 2a and 2b.* 2a shows SF036, Z0, SF056, and Lorax-1 (L-1), the individual bioreactor test microbes, with good microbial growth and selenium reduction using a defined media. 2b shows a screen for selenium reduction using three different media containing different amounts of selected media components. The best growth and selenium reduction is on the plate on the right side of *Figure 2b*.



*Figure 3.* Sample growth curves of the selected microbial population in various media component concentrations.

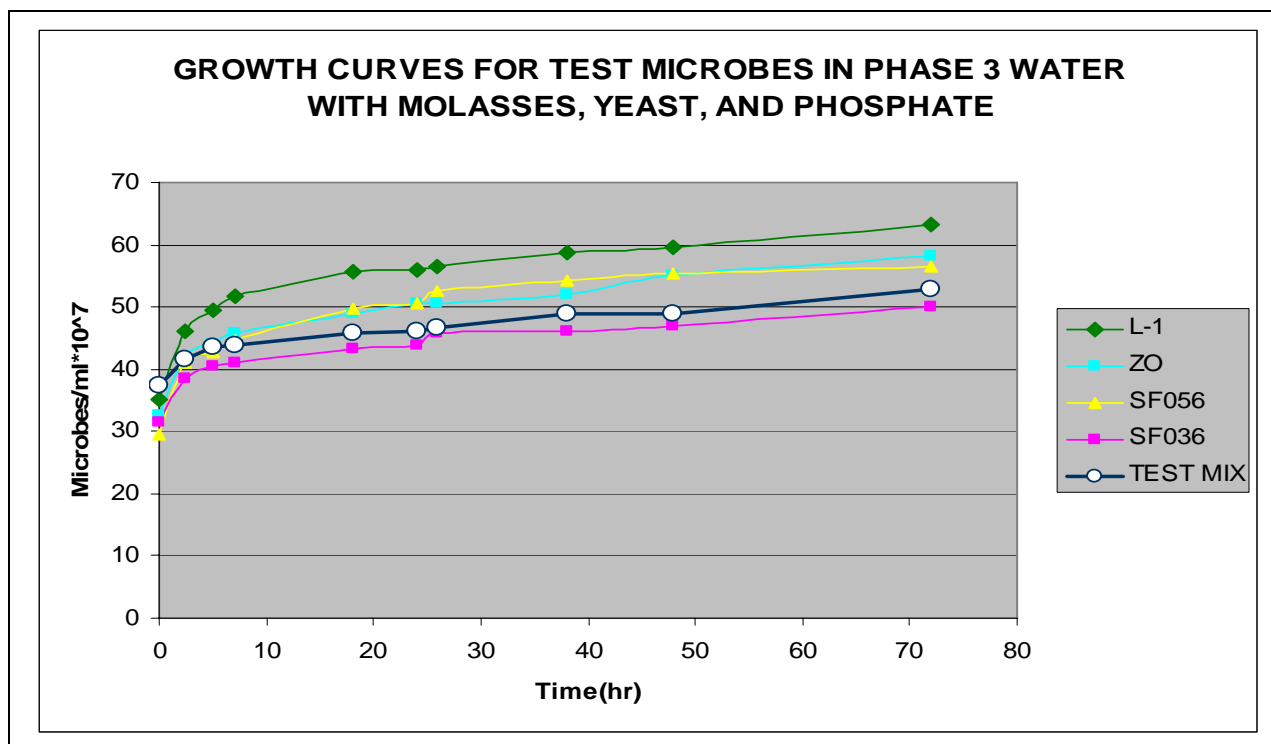


Figure 4. Growth curves of individual test microbes and the test population mix in MYP nutrient over a 72 hour period at 24° C (Laboratory temperature).

### Nucleic Acid Profiling

The following discussion is presented because initially these bacteria were sub-cultured together to enhance selenium reduction in the site microbes and in a bioreactor test population that later became defined as containing SF036, ZO, SF056, and Lorax-1 (L-1). It is now accepted that the vast majority of microbes have not yet been isolated, identified or characterized. This is largely due to a lack of knowledge of how these organisms survive and grow in natural habitats. When one also considers that a bacterium is often part of a larger more complex community or ecosystem with possible co-dependence on other members, it is understandable why traditional culturing techniques fail to accurately reflect the large microbial diversity in an environmental sample. Identification and classification of microbes is further confounded by a general absence of morphologically distinct features, thousands of bacterial species are classically categorized by a few different (~17) morphologies.

The advent of culture-independent techniques has transformed the field of microbiology. PCR-based techniques allow the classification of microorganisms based on particular genetic markers and the profiling of complex microbial communities on the basis of nucleic acid sequence diversity (including the uncultured majority). For the past 10 –15 years, microbiologists have relied upon DNA sequence information for microbial identification, based primarily on the genes encoding the small subunit RNA molecule of the ribosome (16S rRNA or SSU rRNA).

Functional constraints on the cellular nucleic acid translational apparatus limit variability in the 16S rRNA molecule, resulting in a high degree of sequence conservation; therefore, a high degree of matching between the same microbes from different sources and different growth conditions. So, even if microbes exchange genes for selenium reduction, the conservation of the rRNA gene sequence permits bacterial characterization and identification based on sequence information obtained from pure cultures or cloned genes from mixed communities. This is possible because rRNA sequence data is used to design phylogenetically conserved probes that target both individual and closely related groups of microorganisms without cultivation.

A principle repository of 16S rRNA sequences, the Ribosomal Database Project (RDP), currently maintains over 17,000 aligned entries representing 850 of 940 formally recognized prokaryotic genera, which are placed into 1,149 phylogenetic groups. Comparative DNA sequencing analysis is widely considered to be the best genotypic method for microbial identification. The most common approach is PCR amplification and sequencing of all or a 500 Base Pair portion of the 16S ribosomal RNA gene. Sequence data is then compared against a sequence database, which ideally contains only validated microbial sequences.

One technique, based on PCR amplification and sequencing of 16S ribosomal RNA, now routinely used is denaturing gradient gel electrophoresis (DGGE). DGGE is a genetic fingerprinting technique that is used to separate individual sequences from a complex mixture. DNA sequences with differing base composition have different melting properties when passed through an acrylamide gel containing an increasing gradient of a chemical denaturant. The melting temperature of a double stranded DNA fragment is influenced by hydrogen bonds formed between complementary base pairs and also by the attraction between neighboring bases on the same strand (known as stacking interactions). The order of bases on a strand determines the degree of stacking. A DNA molecule may therefore have several melting domains with characteristic melting temperatures. DGGE profiles of the selected microbial population and individual isolated microbial constituents were conducted and results are shown in *Figure 5*. As can be seen, all test microbes are quite similar, but slightly different, and the bands found on the individual test microbes are present in the population profile 4M.

DGGE bands were extracted, amplified, and sent for sequence analysis. The DGGE and amplified sequence analysis shows that all microbes tested appear closely related, but their sequences matched no known microbial sequences in the on-line databases; they are unknown at both the genus and species level but closely related. Metabolic tests have shown that these microbes are quite different metabolically. A previous database check of repository microbe SF036 had shown no matching sequences and cultures of all test microbes exhibited the same DGGE profile before and after sub-culturing.

Microbial stock cultures have been made and are available for pilot-scale testing.



*Figure 5.* DGGE profiles of site microbial RNA from L-1 and ZO, two repository microbes, 4M - a mixture of all bioreactor test microbes, and a *A. ferrooxidans* control.

## BIOREACTOR TESTING

### *Bioreactor Configuration and Operation*

Both reactors contained modified pumice materials as the bulk of the microbial support surface. Each bioreactor has a void volume of approximately 700mls and test waters have a twenty-two hour retention time in each bioreactor. Each reactor has three sampling ports used to monitor conditions within the bioreactor at the bottom, middle, and top. Bioreactors were operated at Laboratory temperatures of ~24° C. Bioreactor feed water was added to a clean feed water container on a daily or every other day basis followed by adjustment of the pH between 6.8 and 7.2 using hydrochloric acid. For one test, nutrients, at 3.75 gm/L, were added every other day to a pretreatment container that contained two days of test waters spiked to 9 mg/L selenium. In all other tests shown, nutrients were added to one or each reactor separately at a rate of 3.75 gm/L on a daily basis by mixing the nutrient into a 100mls of pH adjusted test water and pumping it into the reactor over a five minute time period. The flow rate through the bioreactor was then re-adjusted for a twenty-two hour retention time and re-connected to the feed water container.

All reactors were tested with an economical nutrient solution containing 3.75 gm/L MYP nutrient added to test waters. Testing was initially conducted in two new biochemical-enhanced material reactors (BEMR) while a third electro-biochemical reactor (EBR) was being constructed and undergoing preliminary tests. The BEMRs were operated in series for a total retention time of forty-four hours. *Figure 6* depicts the two initial BEMRs tested; no photo of the EBR is provided because patent approval is still in progress. The EBR was constructed and operated in a manner similar to the BEMR; a twenty-two hour retention time was used in this reactor. In one test all three reactors were connected in series, two BEMRs followed by the EBR for a total retention time of sixty-six hours.

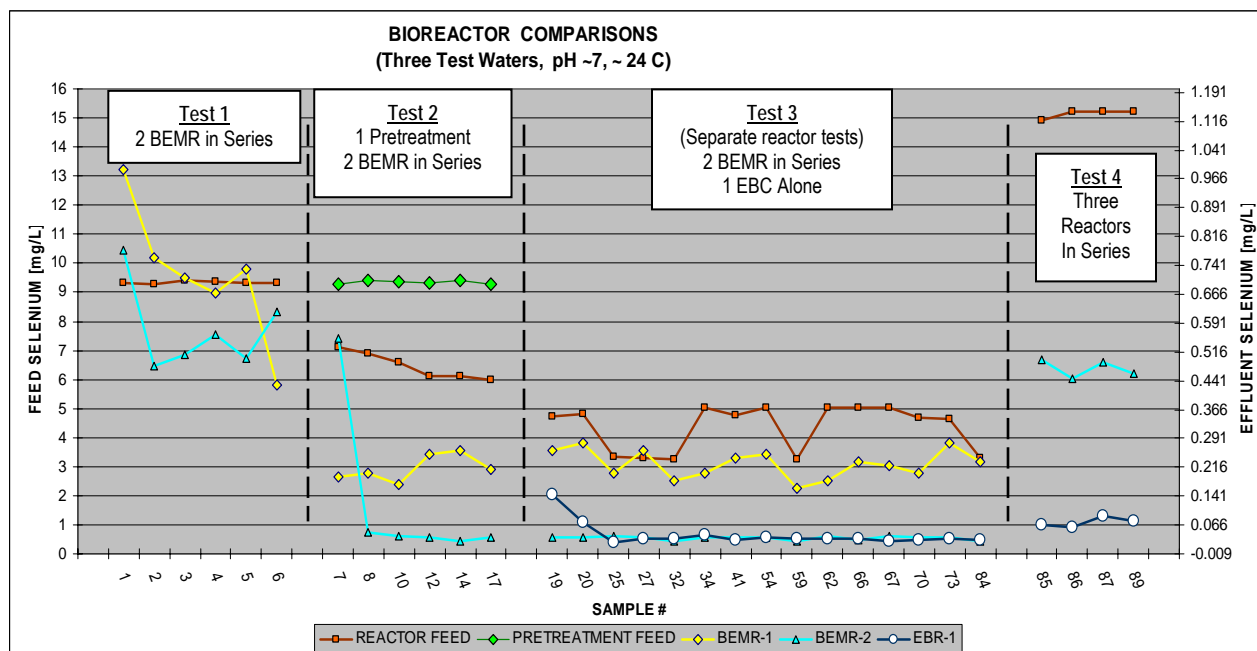
Bioreactor effluent samples were collected during nutrient addition into a sterile 50 ml tube. All samples, including paired feed samples were centrifuged for 30 minutes and filtered through a pre-filter followed by a 0.22 micron filter. Samples were preserved by adjusting the pH below 1 with nitric acid and stored at 4° C until analysis. pH and ORP measurements were made before nutrient addition using the three sampling ports; fifteen milliliter samples were collected, centrifuged for 10 minutes, and pH and ORP were measured.

### *Bioreactor Testing Results and Discussion*

BEMR inoculation and start-up was initiated with waters spiked to 9 mg/l selenium at pH ~7. This water was from the first five buckets of waters initially received for *Phase 3* that did not initially contain any significant selenium or other contaminants. Bioreactors were started and operated for one month using these waters. A second water sample was received for *Phase 3* tests that consisted of nine buckets of water; five buckets containing a clear test solution were used for the bulk of the bioreactor testing; these buckets were the ones that closely matched the water chemistry shown in attachment 1. The selenium content of these waters was ~4.3 mg/L. The EBR was started using test waters containing ~4.3 mg/L selenium. The final tests, conducted with three reactors in series, two BEMRs followed by the EBR, with a total retention time of 66 hours used water from a bucket containing a redish precipitate and ~15 mg/L selenium. Testing results are presented in *Figure 7*.



*Figure 6.* Two BEMR in series using a retention time of twenty-two hour each for a total retention time of forty-four hours.



**Figure 7.** A comparison of a two-stage BEMR and a separate single-stage EBR comparison using a twenty-two hour retention time per stage for a forty-four hour overall retention time in the BEMRs and a twenty-two hour retention time in the EBR. Dashed lines show breaks in reactor testing that included reactor start-up, down time, and re-equilibration of bioreactors for new test solutions or test configurations. The BEMRs and EBR comparisons were made with one test water at ~4.3 mg/L selenium; the BEMRs tests used three different test waters. The BEMRs were used to test three different test water selenium concentrations ~9 mg/L (spiked), ~4.3 mg/L, and ~15 mg/L. The reactor data shown represents operation at one nutrient concentration; 3.75 g/L. Reactors were operated at Laboratory temperature ~24° C.

In Test 1 the bioreactors appear to still be approaching a steady state. In this test, waters containing ~9 mg/L selenium were introduced into the bottom of BEMR-1 and flowed from the bottom to the top of BEMR-1 then into the bottom of BEMR-2; effluents were sampled at the top of BEMR-1 and BEMR-2. Nutrients were added to the feed solution entering the bottom of BEMR-1 using 100 mL of 3.75 gm/L nutrient once daily; no nutrients were added to BEMR-2 during this test except what carried over from BEMR-1. Retention time in each BEMR stage was twenty-two hours for a total retention time of forty-four hours and produced a final effluent averaging ~0.58 mg/L selenium.

In Test 2 the bioreactor configuration was changed to include a pretreatment step in which the feed waters containing ~9 mg/L selenium were held at ~24° C for forty eight hours with microbes and 100 mL of nutrient before entering the bioreactor. This simulated holding pond, with no microbial support materials, removed an average of ~2.9 mg/L selenium. At this point, the test water now containing ~6.5 mg/L selenium was again supplemented with 100 mL of nutrient and continued to flow into BEMR-1 and BEMR-2 as in Test 1; no nutrients were added to BEMR-2 during this test except what carried over from BEMR-1. Retention time in each BEMR stage was twenty-two hours for a total retention time of forty-four hours and produced a final effluent averaging ~0.029 mg/L selenium, if the first data point in this test is not included.

In Test 3 the bioreactor configuration was changed to eliminate the pretreatment step in Test 2. In this test, waters containing ~4.3 mg/L selenium were introduced into BEMR-1 and BEMR-2 as in Test 1, but 100 mL nutrient was added to the bottom of both BEMR-1 and BEMR-2 for a total of 200 mL nutrient once daily; the effluent was sampled at the top of BEMR-2. Retention time in each BEMR stage was twenty-two hours for a total retention time of forty-four hours producing an average final effluent of ~0.031 mg/L selenium.

During Test 3, the EBR was started after a period of preliminary tests and operated independent of the BEMRs; no pretreatment step was included. Separate test waters were introduced into the bottom of the EBR and flowed to the top where the effluent was sampled. One hundred milliliters of 3.75 gm/L nutrient was added to the bottom of the EBR once daily. Retention time in the EBR was twenty-two hours and produced a final effluent of ~0.030 mg/L selenium if the initial data point is discarded.

In Test 4 the bioreactor configuration was again changed to connect the BEMRs and EBR in series using new test waters containing ~15 mg/L selenium and a different water chemistry than in Tests 1, 2, and 3; no pretreatment step was included. In this test, waters were introduced into the bottom of BEMR-1 - to the bottom of BEMR-2 - to the bottom of the EBR; 100 mL of 3.75 gm/L nutrient was added to the bottom all reactors for a total of 300 mL nutrient once daily. Retention time in each reactor stage was twenty-two hours for a total retention time of sixty-six hours and produced an effluent averaging ~0.47 mg/L selenium from the two stage BEMRs and a final effluent from the EBR averaging ~0.072 mg/L selenium.

### *Evaluation for pilot-scale tests*

Several different water chemistries were tested in *Phase 3*, one was close to the provided target process water chemistry with ~4.3 mg/L selenium, and all three were samples were initially thought to be samples of potential water chemistry. A two stage BEMR system of a single stage EBR came close to meeting target discharge criteria of 20 ppb on two of the potential target water chemistries. Addition of another reactor stage or increased retention times should meet these goals, but selenium removal is dependent on the water chemistry, and initial selenium concentration. In assessment of reactor performance, using the five buckets containing ~4.3 gm/L selenium, good selenium reduction and removal was achieved in the BEMRs with a forty-four hour retention time and in the EBR with a twenty-two hour retention time. Oxidation-reduction potential and pH were measured at three points within each reactor and provided for the BEMRs as Attachment 2.

Testing results indicate that a holding pond can be used for slower but significant selenium reduction if treated with nutrients and microbes. The effectiveness of the holding pond could be increased by addition of a microbial support growth surface; however, this would reduce the effective size of the pond. There was no attempt to determine the trade-off between pond size and addition of microbial growth surface which would increase the reaction kinetics.

The single stage EBR, with a twenty-two hour retention time, had the same performance as the two-stage BEMRs with a forty-four hour retention time. The level of selenium removals should be improved in both systems with the addition of a holding pond with microbes and nutrients. *It is likely that target selenium goals of 20 ug/L would have been obtained if a holding pond, with microbial support materials, had been used ahead of the bioreactors in Test 3, Figure 7.*

Selenium removal was good within the BEMRs even as the oxidation-reduction potential dropped into the -200 to -250 mV range between samples 40 and 45. This range is lower than is often considered optimal for the best selenium removal and is due to the amount of nutrients added to the bioreactors. This profile decrease in ORP has been noted in bench- pilot- and full-scale bioreactors and indicates that even though lower nutrient levels exhibited lower growth rates, *Figure 3*, in the long term these lower nutrient concentrations will still develop a substantial biofilm that should function well for selenium removal with significantly lower nutrient concentrations. *In all full-scale biotreatment systems implemented to date, significantly less nutrients were required once biofilm establishment was complete.*

Therefore, it is appropriate to start out a pilot- or full-scale bioreactor with higher nutrient levels to establish a biofilm and then reduce the nutrients significantly once the biofilm is developed. In colder water temperatures the formation of microbial biofilms is slowed considerably and unless the water can be heated, the development of a robust biofilm can take many months. This means the drop in ORP, observed in the bench-scale reactors, may not be observed for a much longer time, but is a good indicator of a mature biofilm and excess nutrients.

The pH increased within the BEMR environment as expected because the concentrated nutrient solution was slightly acidic even when made up with pH adjusted test water; it initially degrades into less acidic byproducts. The bench-scale reactors exhibited pH profiles similar to those observed in full-scale bioreactors. A similar pH profile would be expected during pilot-scale tests.

When nutrient levels in biotreatment systems are not well balanced and in some instances where they are well balanced, post treatment to reduce biological oxygen demand (BOD) is required. In colder climates and instances where the biotreatment systems are shut down for any period a post treatment system is required to reduce BOD.

### Additional Water Quality Criteria

As Phase 3 was being completed, a number of additional metals were identified as potential permitting criteria. As indicated in Table 1, the pumice materials used as a microbial growth support were a significant source of several metals of interest. This source of metals could be removed by using gravel or activated carbon, but the gravel would provide significantly less surface area for a microbial biofilm, therefore lower selenium reduction kinetics, and virgin activated carbon would be considerably more expensive. The two different bioreactor types removed different amounts of different metals; however a significant reduction in all metals of interest except copper and nickel was achieved. Copper, at these levels is a component in many nutrients; but the origin of the nickel increase is currently unknown in the BEMRs. Table 1 data is average data from 14 points throughout the experiment.

Table 1. Additional Water Quality Criteria - Bench-Scale Bioreactors

ITEM		ELEMENT	Al	S	Fe	Ni	Cu	Zn	As	Mo	Ag	Cd	Sb	Pb	Hg
			µg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
	AVERAGE FEED WATERS		998.95	460.67	32.00	6.23	3.00	19.48	2.61	632.52	2.04	1.77	14.93	5.02	2.15
TWO BR IN SERIES	AVERAGE BEMR-1 EFFLUENT	22 HR RETENTION	162.63	421.73	177.37	8.31	3.00	21.77	2.67	57.64	0.30	0.16	6.21	0.77	3.41
	AVERAGE BEMR-2 EFFLUENT	44 HR RETENTION	58.17	339.88	255.68	11.49	4.05	32.51	1.93	12.25	0.21	0.06	3.05	2.61	1.46
	AVERAGE EBR EFFLUENT	22 HR RETENTION	23.21	176.09	339.41	10.41	3.04	31.65	1.40	55.65	0.00	0.18	10.69	5.31	2.74
BR FILL	AVERAGE ELUTED FROM PUMICE*	(gm)	200.07	0.00	175.19	1.22	1.07	7.73	0.00	0.00	0.00	0.00	0.00	0.00	0.08

(BEMR) – Bio-enhanced Materials Reactor

(BER) – Bio-electrochemical reactor

\* - Average of two pumice elutions in amount per gram of pumice. Second elution amounts generally decreased by 50% to 75% with the exception of mercury which did not decrease with the 2<sup>nd</sup> elution. Elutions carried out in a manner to approximate release over the course of the experiments. No new elements showed up in 2<sup>nd</sup> elution; might expect some elution of these elements to continue over the course of the experiments conducted.

## BACKGROUND FOR PILOT-SCALE TESTING

### *Pre- and Post-Treatments*

Considering the range of selenium values and water chemistry differences in the water samples tested in *Phases 1-3*, a pre-treatment stage is strongly recommended. This system should be sized to handle any larger than normal flow events such as those that normally occur in the spring and fall. A recommended system would consist of a holding or equilibration pond either unfilled or partially filled with a microbial growth support. Based on water chemistry and temperature, this pond should have a minimum retention time of seven days to two weeks; or based on a flow rate of 9 L/s, a void volume of ~13,000 m<sup>3</sup> for two weeks retention time. A better estimate of pond size, optimal retention time, and an economical trade-off in microbial support surface fill depth can be determined during pilot-scale testing.

Post-treatment is required to reduce treatment system BOD, and will be required at this site due to temperatures and operational requirements; a post-treatment system is also usually required to deal process water chemistry changes. Post-treatment consists of an aerobic stage where the selenium treatment effluents would be aerated in contact with a dense microbial population. Usually this microbial population will be available in site waters and will ultimately consist of a portion of the selenium reducing population; some of the facultative anaerobes. The type of post-treatment system required is dependent on nutrient load and can vary from a conventional trickling filter to an actively aerated system.

### *Pilot-Scale Treatment Size*

The project flow rate for full scale treatment is approximately 9 L/s; therefore, recommended flow rates for a large pilot-scale test would be 0.7, 0.9, and 1.1 L/s or flow rates about one-tenth of the full-scale flow. Smaller pilot-scale tests can be conducted and are often designed around flow rates of 0.02% of the target flow rate or 0.14, 0.18, and 0.22 L/s. Test flow rates are not fixed and should be adjusted to meet site requirements. Based on 50% void volumes and retention times needed, estimated system sizes for the pilot-scale tests are presented in *Table 2* below.

*Table 2.* Treatment System Component Size – Void Volume 50%.

Flow Rate (L/s)	Total Flow/Tmt Cycle (L/Day)	Pre-treatment Size (m <sup>3</sup> )	Treatment System Size (m <sup>3</sup> )	Post-treatment Size (m <sup>3</sup> )
0.14	12,096.0	406.4	79.8	14.5
0.18	15,552.0	522.5	102.6	18.7
0.22	19,008.0	638.7	125.5	22.8
0.7	60,480.0	2032.1	399.2	72.6
0.9	77,760.0	2612.7	513.2	93.3
1.1	95,040.0	3193.3	627.3	114.0

Total Flow – Flow Rate L/sec\*60 sec/min\*60 min/hr\*24 hrs/day

Pre-treatment Size – (Flow Rate L/sec\*60 sec/min\*60 min/hr\*24 hrs/day\*14 days)/1,000 L/m<sup>3</sup>\*2 vv\*1.2 sizing factor

Treatment System – (Flow Rate L/sec\*60 sec/min\*60 min/hr\*66 hrs)/1,000 L/m<sup>3</sup>\*2 vv\*1.2 sizing factor

Post-Treatment Size – (Flow Rate L/sec\*60 sec/min\*60 min/hr\*12 hrs)/1,000 L/m<sup>3</sup>\*2 vv\*1.2 sizing factor

### *Treatment Costing Assumptions*

The following assumptions are made for the included pilot-scale cost estimate.

- Site lodging and meals will be provided during time at site
- Testing time is June through August – three months
- Labor assistance by on-site personnel
- Weekends or cycle time in Whitehorse, YK (~24 days) - Transportation to Whitehorse
- Power, pumps, tanks/ponds, piping, and water heater at site
- Near-by gravel or pumice supply – provided on site
- Inoculum scale-up tanks – can use treatment system tanks
- Nutrient tank, costs, and transportation to site – ~106 tons OR ~20,000 gal – covered in cost estimate
- 1/10 flow pilot-scale test




ATTACHMENT 1. BULK TAILINGS ANALYSIS

**ALS Laboratory Group**  
ANALYTICAL CHEMISTRY & TESTING SERVICES



Environmental Division

ANALYTICAL REPORT	
LORAX ENVIRONMENTAL SERVICES ATTN: JONATHAN MACKIN 2289 BURRARD STREET VANCOUVER BC V6J 3H9	Reported On: 27-SEP-07 11:13 AM
Lab Work Order #: L550876	Date Received: 06-SEP-07
Project P.O. #: Job Reference: 474-1 YUKON ZINC Legal Site Desc: CofC Numbers: Other Information:	
Comments:	
 Timothy Guy Crowther General Manager, Vancouver	
For any questions about this report please contact your Account Manager: <b>Andre Langlais</b>	

THIS REPORT SHALL NOT BE REPRODUCED EXCEPT IN FULL WITHOUT THE WRITTEN AUTHORITY OF THE LABORATORY.  
ALL SAMPLES WILL BE DISPOSED OF AFTER 30 DAYS FOLLOWING ANALYSIS. PLEASE CONTACT THE LAB IF YOU  
REQUIRE ADDITIONAL SAMPLE STORAGE TIME.

**ALS Canada Ltd.**  
7471th, **ALS Laboratory Group**  
1988 Triumph Street, Vancouver, BC V5L 1K5  
Phone: +1 804 263 4188 Fax: +1 804 263 8700 [www.alsglobal.com](http://www.alsglobal.com)  
A Campbell Brothers Limited Company

## ALS LABORATORY GROUP ANALYTICAL REPORT

Sample ID Description Sampled Date Sampled Time Client ID		L550876-1	L550876-2	L550876-3	L550876-4	L550876-5
		05-SEP-07	05-SEP-07	05-SEP-07	05-SEP-07	05-SEP-07
		BULK TAILINGS 1 (9/10/11)	BULK TAILINGS 2 (12/13/17)	BULK TAILINGS 3 (14/15/16)	PB RO CONC	CU RO CONC
Grouping	Analyte					
<b>WATER</b>						
<b>Physical Tests</b>	Hardness (as CaCO3) (mg/L)	802	834	697	411	463
	Conductivity (uS/cm)	1730	1830	1530	1110	1190
	pH (pH)	10.3	10.7	10.2	7.62	7.91
	Total Dissolved Solids (mg/L)	1550	1560	1330	875	952
	Total Suspended Solids (mg/L)	9.3	<3.0	5.3	<3.0	<3.0
	Turbidity (NTU)	7.36	0.57	3.48	13.9	19.9
<b>Anions and Nutrients</b>	Ammonia as N (mg/L)	0.265	0.389	0.164	0.235	0.197
	Alkalinity, Total (as CaCO3) (mg/L)	106	151	101	7.3	81.3
	Bromide (Br) (mg/L)	<0.050	<0.050	<0.050	<0.050	<0.050
	Chloride (Cl) (mg/L)	4.44	4.91	5.27	6.07	5.89
	Fluoride (F) (mg/L)	0.167	0.161	0.134	0.114	0.093
	Sulfate (SO4) (mg/L)	597	632	534	393	460
	Nitrate (as N) (mg/L)	0.556	0.550	0.459	0.434	0.473
	Nitrite (as N) (mg/L)	0.0167	0.0085	0.0115	0.0067	0.0059
	Ortho Phosphate as P (mg/L)	0.0042	0.0048	0.0029	0.0035	<0.0010
Total Phosphate as P (mg/L)	0.213	0.142	0.130	0.242	0.0038	
<b>Cyanides</b>	Cyanide, Weak Acid Diss (mg/L)	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050
	Cyanide, Total (mg/L)	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050
	Thiocyanate (SCN) (mg/L)	<0.50	0.58	0.55	<0.50	0.69
<b>Total Metals</b>	Aluminum (Al)-Total (mg/L)	1.19	1.26	1.11	0.0219	0.0117
	Antimony (Sb)-Total (mg/L)	0.0287	0.0294	0.0301	0.0253	0.0598
	Arsenic (As)-Total (mg/L)	0.00789	0.00532	0.00611	0.00379	0.00458
	Barium (Ba)-Total (mg/L)	0.190	0.188	0.174	0.120	0.160
	Beryllium (Be)-Total (mg/L)	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025
	Bismuth (Bi)-Total (mg/L)	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025
	Boron (B)-Total (mg/L)	<0.050	<0.050	<0.050	<0.050	<0.050
	Cadmium (Cd)-Total (mg/L)	<0.0020	<0.0020	<0.0020	0.00229	0.00601
	Calcium (Ca)-Total (mg/L)	321	334	279	155	175
	Chromium (Cr)-Total (mg/L)	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025
	Cobalt (Co)-Total (mg/L)	<0.00050	<0.00050	<0.00050	<0.00050	0.00195
	Copper (Cu)-Total (mg/L)	0.00335	0.00095	0.00125	0.0118	0.0652
	Iron (Fe)-Total (mg/L)	0.308	0.039	0.158	0.048	1.02
	Lead (Pb)-Total (mg/L)	0.469	0.748	0.239	0.294	0.622
	Lithium (Li)-Total (mg/L)	<0.025	<0.025	<0.025	<0.025	<0.025
	Magnesium (Mg)-Total (mg/L)	<0.10	<0.10	<0.10	5.81	6.14
	Manganese (Mn)-Total (mg/L)	0.00548	0.00048	0.00208	0.146	2.98
	Molybdenum (Mo)-Total (mg/L)	0.661	0.696	0.562	0.0183	0.00529
	Nickel (Ni)-Total (mg/L)	<0.0025	<0.0025	<0.0025	<0.0025	0.0546
	Phosphorus (P)-Total (mg/L)	<0.30	<0.30	<0.30	<0.30	<0.30

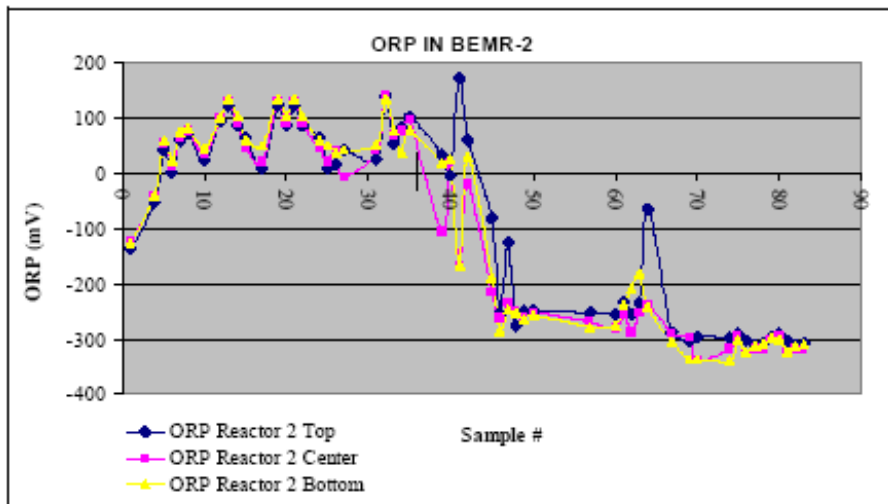
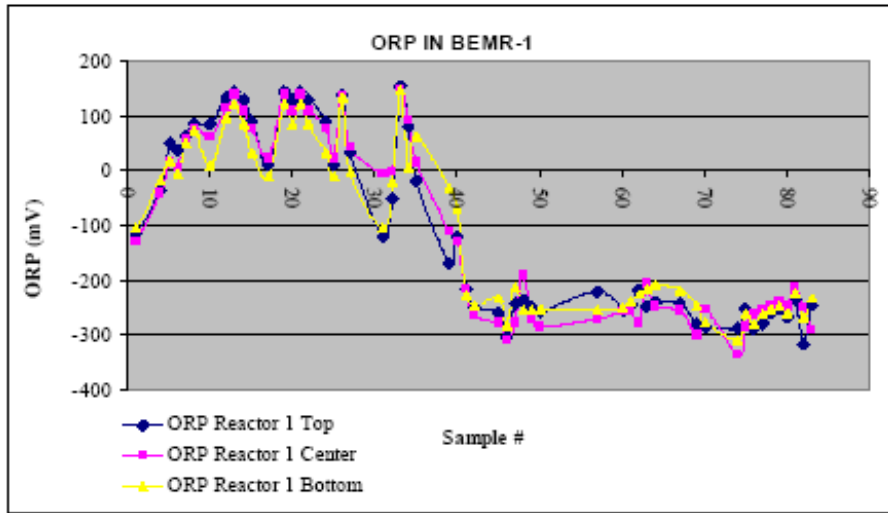
ALS LABORATORY GROUP ANALYTICAL REPORT

Sample ID Description Sampled Date Sampled Time Client ID		L550876-1	L550876-2	L550876-3	L550876-4	L550876-5
Grouping		BULK TAILINGS 1 (9/10/11)	BULK TAILINGS 2 (12/13/17)	BULK TAILINGS 3 (14/15/16)	PE RO CONC	CU RO CONC
Analyte						
<b>WATER</b>						
<b>Total Metals</b>	Potassium (K)-Total (mg/L)	8.2	7.4	6.4	5.7	5.4
	Selenium (Se)-Total (mg/L)	3.05	3.21	2.54	0.907	0.676
	Silicon (Si)-Total (mg/L)	0.149	0.110	0.148	0.145	1.05
	Silver (Ag)-Total (mg/L)	0.00262	0.00217	0.00122	0.00317	0.00849
	Sodium (Na)-Total (mg/L)	80.8	87.6	70.5	65.8	66.3
	Strontium (Sr)-Total (mg/L)	0.859	0.876	0.747	0.396	0.433
	Thallium (Tl)-Total (mg/L)	0.00152	0.00154	0.00187	0.00721	0.0103
	Tin (Sn)-Total (mg/L)	0.00825	0.00897	0.0165	0.00126	0.00194
	Titanium (Ti)-Total (mg/L)	<0.010	<0.010	<0.010	<0.010	<0.010
	Uranium (U)-Total (mg/L)	<0.000050	<0.000050	<0.000050	<0.000050	0.000520
	Vanadium (V)-Total (mg/L)	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050
	Zinc (Zn)-Total (mg/L)	0.314	0.486	0.293	0.190	2.44

ATTACHMENT 2. BEMR ORP and pH PROFILES

ATTACHMENT 2. OXIDATION REDUCTION POTENTIAL AND pH IN BEMRs

*Oxidation Reduction Potential*



pH in Bemrs

