New Electro-Biochemical Reactor for Treatment of Wastewaters

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ABSTRACT

A number of microorganisms, including undefined microbial consortia, are capable of directly accepting electrode provided electrons in a manner that enhances reduction of terminal electron acceptors; i.e., metals such as arsenic and selenium and various inorganics like nitrates and sulfates. Electrons are provided directly to a system via an electrode and through an electron distribution matrix, using low DC voltage. Providing electrons directly to microbes and reactor environments has a number of advantages over indirect electron provision: adding organic electron donors or hydrogen to bioreactors.

A patented electro-biochemical reactor (EBR) directly provides electrons and an electron receptor environment to microbes and reactors, enhances contaminant transformations, and allows for more precise control of system Eh/pH environments. In conventional treatment systems, nutrients and chemicals provide electrons and electron acceptor environments, in a rather inconsistent manner. Due to the inconsistent availability of electrons and hydrogen within these systems, a large excess of nutrients and chemicals are often required for contaminant transformations and to generate the desired Eh/pH conditions within a portion of the treatment environment.

The EBR provides electrons to microbes and reactor environments using low DC voltage (1–3 volts) in configurations that provide an electron density gradient or a controlled electron density. The provided electrons result in increased microbial contaminant transformation kinetics, reduced retention times, and reductions in the amount of nutrients and chemicals required for contaminant removal. Preliminary tests indicate that EBR technology will remove target contaminants to low ppb levels using ≤½ conventional bioreactor retention times and nutrient concentrations. The applied voltage reduces the use of nutrients, supplies some of the energy (electrons) required for bacterial growth, and supplies energy potential for contaminant transformation, reducing contaminant transformation energies that must be provided for through microbial metabolic processes.

INTRODUCTION

Treatment of various wastewaters is becoming ever more critical due to diminishing water resources, increasing wastewater disposal costs, and stricter discharge regulations that have lowered permissible contaminant levels. The treatment of wastewater for reuse and disposal is particularly important in arid regions of the US and the world. Metal pollutants can be introduced into waters through many activities including mining and mineral processing, abandoned petroleum processing, coal-fired power plants, and agricultural run-off. Over the past decades, appreciation for the role of microbes in the precipitation of minerals and the redox transformation of many elements has grown.

Microbes are now known to be involved in the environmental transformations of many elements. Understanding of the tremendous metabolic diversity of the microbial world has expanded in recent years with the discoveries that microbes can use a variety of elements and compounds, including Fe, Mn, Se, U, As, NO_3^-, CN^-, SO_4^{2-}, and others as terminal electron acceptors in anaerobic respiration. Metal and inorganic removal is highly dependent on the availability of electrons—redox, or Eh/pH, the available oxygen in the system, pH, etc. (Metcalf, 2002, Peoples and Adams 2010, Twidwell, et al. 2000). Microbes and chemicals alter or remove metal and inorganic contaminants by adding and/or removing electrons. It is based on this knowledge that an electrochemical bioreactor was developed to take further advantage of direct electron provision to aid in microbial and biochemical metal and inorganic transformation processes.

Research conducted at Utah Universities since 1995, drawing on related microbial fuel cell studies, has resulted in patented technology harnessing the ability of various microorganisms and undefined microbial consortia to directly utilize electrode provided electrons in a manner that enhances reduction of terminal electron acceptors (Adams, et al., 2009, Adams and Peoples, 2010, Peoples and Adams 2010, Newton, et al., 2008). Electrons are provided directly to a system via an electrode and through an electron distribution matrix, using low DC voltage. Direct electron transfer to cells and reactor systems has a number of advantages over indirect electron provision via addition of organic electron donors or hydrogen to bioreactors. While the mechanisms for direct electron transfer from electrodes to microorganisms are still being investigated, including methods to optimize various patented applications, their benefit is often immediately apparent.

Background

The ability of microorganisms to transfer electrons to electrodes without electron shuttling compounds has been known for about a century (Potter, 1911). Much more recently, acetate was shown to be oxidized to carbon dioxide with direct electron transfer to an electrode as the sole electron acceptor (Bond and Lovley, 2003, Bond et al., 2002). The history of direct electron flow from electrodes to microorganisms is rather short with the first report appearing in 2004 (Gregory et al., 2004). A recent review by Thrash and Coates (2008), indicates that there are a number of investigations on a wide diversity of redox-active molecules that can function as electron shuttles.

The current model for electron transfer to anodes is that of conductive pili (Lovley, 2008, Reguera et al., 2006, Strycharz et al., 2008) and is indicated to proceed through outer-surface *c*-type cytochromes (Holmes et al., 2004, Nevin et al., 2009) required to facilitate electron transfer between the biofilm and the anode surface (Inoue et al., 2010, Kennedy et al., 2007). Additionally, gene expression patterns in current-consuming cells appear to differ from those in current-producing cells (Strycharz et al., 2008). The ability of microorganisms to respire using electrons from an electrode or an electron distribution matrix as a sole or primary electron donor or acceptor makes it feasible for these populations to be more self-sustaining. This mechanism also allows for control of excess microbial growth, thus, reduction of microbial plugging in various treatment system environments resulting from conventional treatment practices using large excesses of nutrients/chemicals.

Electro-Biochemical Remediation

As a historical perspective, microbial reduction of soluble U(VI) to insoluble U(IV) was proposed, but not demonstrated, for immobilizing uranium in subsurface environments (Anderson et al., 2003, Finneran et al., 2002, Gregory and Lovley, 2005) or to concentrate extracted uranium (Phillips et al., 1995). *Geobacter sulfurreducens* reduced U(VI) to U(IV) with an electrode serving as the electron donor (Gregory and Lovley, 2004). Additionally, *Geobacter lovleyi* has been shown to reduce tetrachloroethene (PCE) and trichloroethene (TCE) to *cis*-dichloroethene (*cis*-DCE) with an electrode serving as a sole electron acceptor (Strycharz et al., 2008). Additionally, degradation of complex mixtures of organic compounds has been shown to be enhanced using EBR technology, (Adams–unpublished results, Potter, 1911, Thrash and Coates, 2008).

Electron transfer from electrodes is beginning to receive increased attention; e.g., removal of nitrate in wastewater treatment. Arsenic and nitrate reduction has been examined by Waterland et al., 2010, Oremland and Stolz, 2005, and Virdis et al., 2008. EBR tests show two to ~nine-fold increases in denitrification kinetics in the laboratory (Adams et al., 2009, Adams and Peoples, 2010, Peoples and Adams, 2010, and Newton et al., 2008) and arsenic removal and denitrification in pilot field studies (Adams and Peoples, 2010, Peoples and Adams, 2010). Several studies have reported that mixed microbial cultures were able to denitrify nitrate with an electrode serving as the sole electron donor (Clauwaert et al., 2007, Jia et al., 2008, Park et al., 2005, Virdis et al. 2008). However, in all studies, the microorganisms responsible for this process, the mechanisms of interaction, and denitrification processes warrant further study.

Research conducted at the Utah Universities has shown that directly supplying electrons require less maintenance, monitoring and energy than conventional methods using only organic electron donors. Electrons can be supplied from solar panels, making the use of EBR technology for treatment of waters and contaminated soils an attractive sustainable practice, and pilot-scale tests have been completed and more are underway. Solar-powered and low voltage DC cathodes have been described and deployed at a pilot-scale field site for removal of arsenic and nitrate from contaminated mine waters and large-scale EBR applications are under development.

Though conventional bioreactors have proved successful in an operational sense, the issue of capital costs, treatment residence time, and nutrient costs has dictated their acceptance over other treatment approaches. In conventional systems, a large excess of nutrients/chemicals are required to provide the electrons and electron acceptor environments needed. Moreover, excess nutrients/chemicals are required in conventional systems to adjust reactor chemistry, for microbial growth and contaminant removal, and to compensate for system sensitivity and inefficient and fluctuating electron availability.

The electro-biochemical reactor (EBR) technology overcomes shortcomings in conventional systems by combining electrochemical and biological treatments. It supplies electrons to the reactor and microbes using low *DC* voltage; o*ne volt supplies about a trillion, trillion electrons.* These electrons cost-effectively replace the electrons normally supplied by excess nutrients/chemicals in a controlled manner, effecting a reduction in treatment residence times and nutrient costs; therefore, capital costs. The electrons needed for a full-scale facility can easily be supplied by a small solar grid. The EBR addresses improvements in conventional systems by application of a charge potential that provides electrons to:

1. Stabilize and control the conditions necessary to precipitate metals and reduce inorganics,
2. Reduce or eliminate the amount of nutrients and chemicals required for ORP adjustment,
3. Provide controllable ORP environments that occurs with distance from the electrode rather than the classical and variable ORP gradient produced with excess nutrients/chemicals, and
4. Provide easy microbial access to electrons needed for life functions and transformation of contaminants in aerobic and anaerobic environments.

The EBR technology makes conventional reactors more controllable, efficient, economical, and robust, reducing the reactor size and nutrient/chemical amounts required for effective contaminant transformations. In short, EBR technology starts with the best aspects of proven microbial and chemical systems and takes them to the next level of performance and cost-effectiveness. Both bench and pilot-scale tests indicate that the EBR technology will allow more effective and economical biotreatment of large volume low-contaminant metal and inorganic containing waters as well as provide economic benefits for treatment of high contaminant organic, metal and inorganic waters.

MATERIALS AND METHODS

Microbes

Microbes employed to reduce selenium, arsenic, and nitrate in these tests were site indigenous microbes, isolated, screened for their respective contaminant reductive abilities, grown to high densities, and established within the EBR systems. Nucleic acid based individual microbe and microbial population identification used denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment (TRF) analysis.

Site Test Waters

Bench scale EBR tests evaluated process waters with a two stage conventional bioreactor system and a single stage EBR. Selenium and nitrate were present in the site water at concentrations of ~4.5 mg/L, nitrate concentrations were adjusted to ~100 mg/L nitrate-N, and the pH was adjusted to ~6.5. Retention times of 12 hours in the EBR and 24 hours in the conventional 2-stage CBR reactor were used. Nutrients were also required at ~1.5× amounts in the CBR system. All reactors were operated under up-flow conditions that approximated plug flow. The EBR system only, was operated at 3 volts DC potential, current was only measurable in the microamp levels. All other conditions in the CBR and EBR stages were identical. All analysis used ICP-MS.

Additional selenium removal tests were conducted at bench-scale using site contaminated waters and a two-stage, up-flow, EBR system; waters contained ~260 mg/L selenium. Because the waters received did not have significant nitrate present, site personnel advised that test waters be spiked with 100 mg/L nitrate-N. The retention time for each EBR stage was between 15 and 24 hours. All analysis used ICP-MS.

Bench scale selenium removal tests were performed with single stage EBR under two different electrode configurations. Tested waters contained 550–1000 ppb selenium. Retention times varied between 17 and 24 hours. All analysis used ICP-MS.

Pilot-scale tests. A two-stage EBR system was operated under conditions that approximated plug flow (up flow); total retention times varied from 10 to 18 hours or flow rates from

~0.5 gpm to ~0.8 gpm. Influent arsenic concentrations were ~750 mg/L and nitrate concentrations averaged about 24 mg/L nitrate-N. Pilot-scale tests were initiated in summer and finished in late fall with water temperatures ranging from ~23°C to ~9.5°C. Analysis was completed by a University certified laboratory and a third party lab.

Nutrients

In both the selenium and arsenic tests, a laboratory culture media, Trypticase Soy Broth at 15 to 30 g/L or a balanced molasses, urea (>0.25g/L to 2.5g/L), yeast extract (>0.25 g/L to 1.0 g/L), and phosphate (0.025g/L to 1.0 g/L) mixture was used. Nutrient concentrations were varied to examine nutrient components of C:N:P:S, etc., for growth of new microbial cells and were balanced to microbial cellular levels. Higher nutrient levels were used to establish the biofims to their desired density. Lower nutrient levels were used once the EBR and microbial populations reached more stabilized operating conditions. Nutrients were added on a daily basis by mixing the nutrient into an appropriate amount of pH adjusted test water and pumping them into each EBR separately over a five minute time period, then returning to normal flow rates.

RESULTS AND DISCUSSION

Microbes

Many species of microorganisms can affect the reactivity and mobility of metals and inorganics, and can be used to remove these contaminants from waters. The microorganisms used are naturally-occurring INOTEC/University repository microbes and site microbial consortia; not genetically engineered or modified microbes. The resulting products of the processes are elemental selenium, elemental arsenic and arseinc sulfides; nitrates and nitrites are converted to nitrogen gas.

Microbes present in the arsenic EBR included *Desulfobacterium sp.*, *Desulfotomaculum sp.*, *Shewanella sp.*, *Bacillus sp.*, and several uncultured (unidentified) microbes. Bacteria present in the selenium EBR included *Pseudomonas sp.*, *Desulfobacterium sp.*, *Shewanella sp.*, *Bacillus sp.*, and several uncultured (unidentified) microbes. The unidentified microbes were shown through site population analysis to be site indigenous microbes (data not presented). Microbial populations were examined after biofilm establishment using site waters containing indigenous microbes. Microbial density on the EBR support materials estimated through assays at $\sim 2 \times 10^{11}$/g.

Voltage, ORP, and Nutrients

The voltage required to increase contaminant transformation efficiencies varies with the bioreactor's microbial support materials, water chemistry, and microbes. Supplied voltage provides electrons at the bacterial surface and a readily available supply of elections to the bacterial-contaminant-surface environment that lowers the microbial contaminant interaction and transformation energy requirements. Providing electrons at the microbial support surface interface allows a more controlled oxidation-reduction potential (ORP) environment or gradients for more effective removal of single or multiple contaminants in a single bioreactor.

Microbes in the EBR system interact with the electrode through direct contact, mediating the transfer of electrons via conductive pilli and microbial surface interactions. Interaction can also occur through energy shuttle compounds that move energy to both electrode and

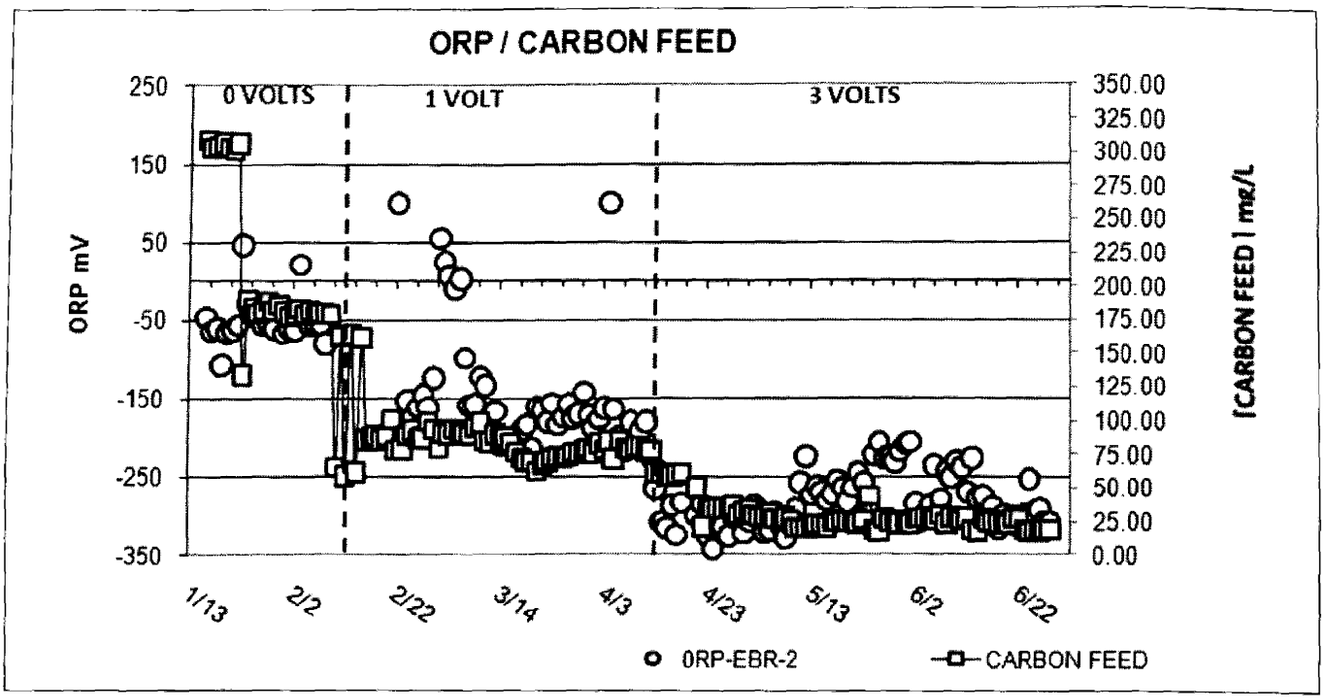

Figure 1. Relationships between voltage, ORP, and carbon input measurements made in a two-stage EBR at the end of the second stage

non-electrode and conductive matrix bound microbes throughout the EBR system. The applied voltage potential assists in controlled system ORP in a manner that eliminates some of the need for excess nutrients to lower ORP; thus reducing nutrient costs. Readily available electrons supply some of the energy (electrons) required for bacterial growth and contaminant transformation, again reducing nutrient costs. Figure 1 shows typical ORP measurements made in a two-stage EBR and a relationship with voltage and nutrient (carbon) amount. Note the stability of ORP obtainable within the EBR system at 3 volts and the lower amounts of nutrient required to achieve the desired ORP.

Bench-Scale Selenium Removal Tests

Figure 2 shows the results of bench-scale tests using mining process water containing ~4.5 mg/L selenium and spiked nitrate levels of ~100 mg/L. The figure shows comparisons with a two stage conventional bioreactor (CBR) system and a single stage EBR. Retention times were 12 hours in the EBR and 24 hours in the conventional 2-stage CBR reactor. Nutrient amounts were also ~1.5× in the CBR system. All reactors were operated under up-flow conditions that approximated plug flow. The EBR only, was operated at 3 volts DC. All other conditions in the CBR and EBR stages were identical. The EBR effluent was below 0.030 mg/L while the two state CBR produced selenium effluents averaging >0.060 mg/L. Monitoring points within the EBR indicated that nitrates were also completely removed in the first few inches of the system.

Figure 3 shows the results of a second bench-scale test using a two stage, up-flow, EBR system to treat waters containing ~260 mg/L selenium. Because the waters received did not have significant nitrate present, site personnel advised that test waters be spiked with 100 mg/L nitrate-N. The retention time for each EBR stage was ~15 hr for a total retention time of 30 hr. The EBR system produced final selenium effluents averaging ~13 µg/L. Effluents from EBR-2 ranged from a high of 55.4 µg/L to a low of 2.8 µg/L. If the high and low selenium effluent readings were discarded for the 3 volt test period, the average selenium effluent from

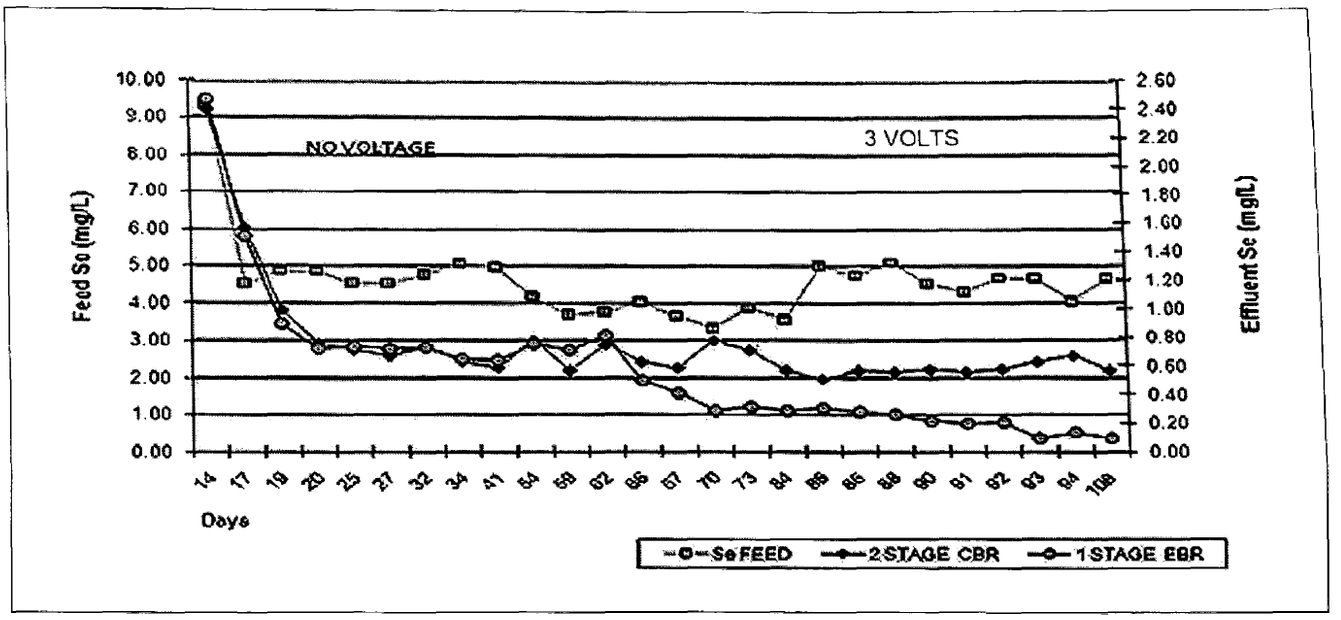

Figure 2. Comparison of treatment of mining process water containing selenium using two bioreactor types. A two-stage conventional bioreactor (CBR), configured identically to a single-stage EBR, but without applied potential, using twice the retention time, and ~1.5 times the nutrient amount as the single-stage EBR.

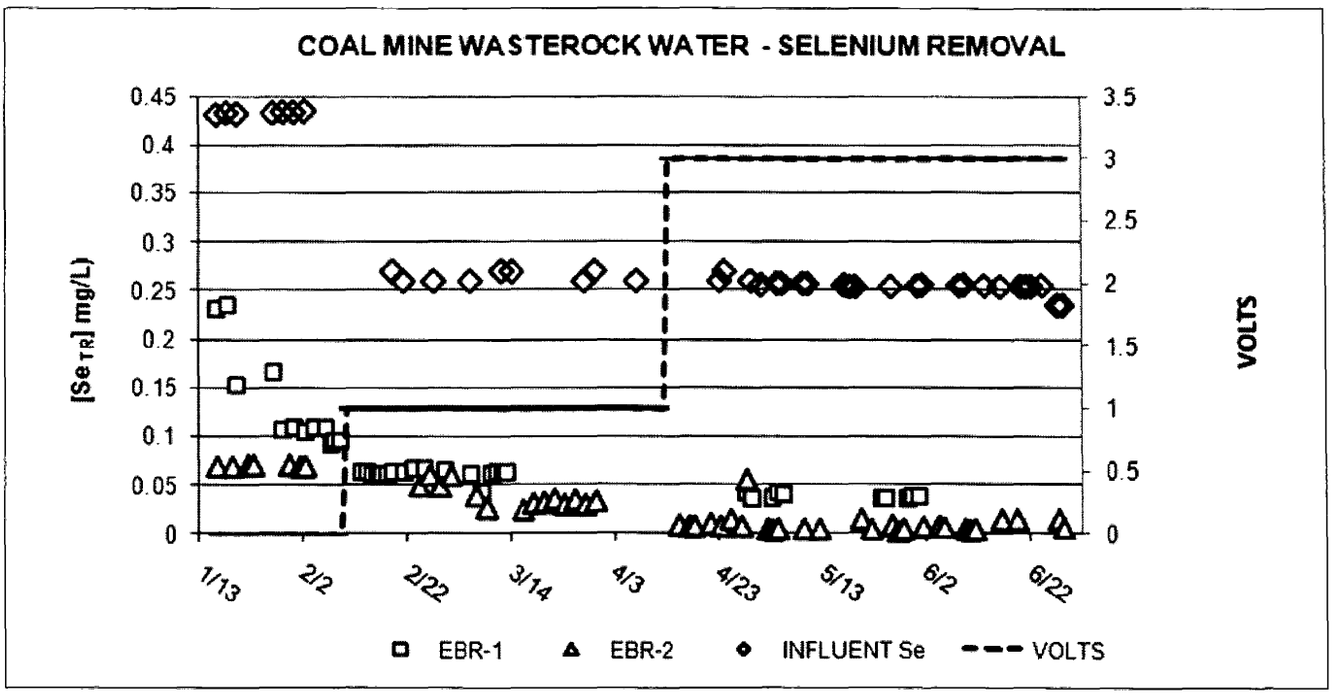

Figure 3. Two-stage EBR treating mining influenced wastewaters containing selenium. EBR stages were compared with applied voltage and selenium removal.

EBR system drops to ~11µg/L. Nitrate in the EBR system was removed in the first few inches of the EBR 1st stage.

In mining solutions, for example those shown in Figures 2 and 3, spiked with 100 mg/L nitrate-N nitrate was at zero at the first monitoring point in the EBR. Once equilibrated, EBR denitrification rates reached >95 mg nitrate-N per hour.

Additionally, a mine process water containing 550–1,000 ppb selenium was tested using EBR with different electrode configurations. Multi, where eight electrodes evenly spaced

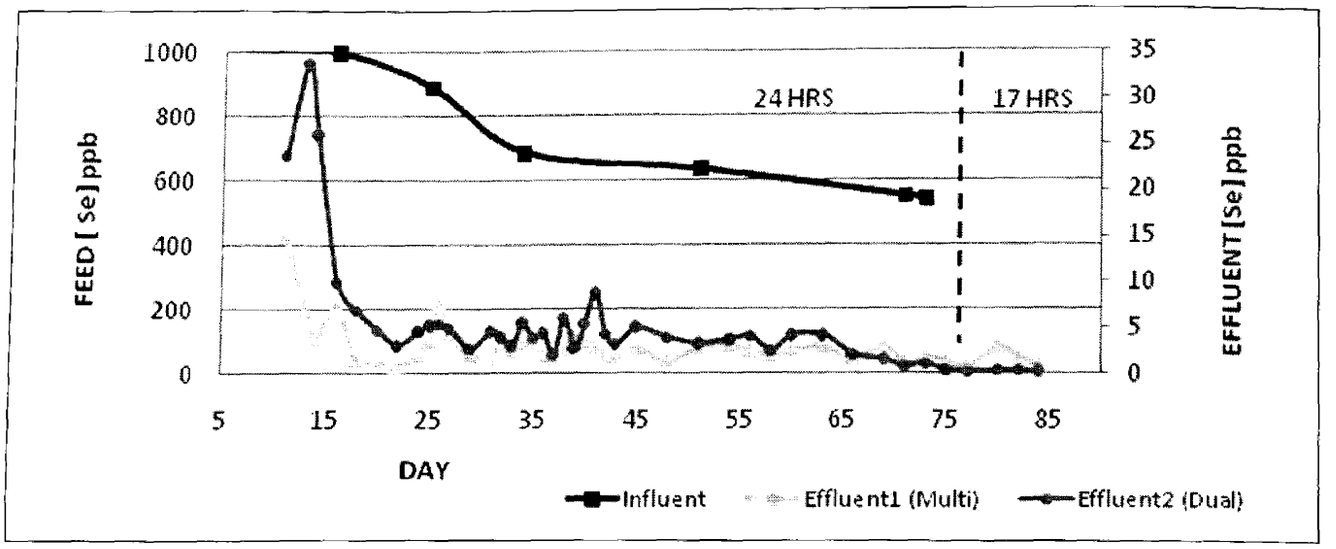

Figure 4. Process mine water treated in two different EBR configurations and with two retention times

over the length of the EBR and connected to a power supply providing a 3 V potential, and dual, where two electrodes, one at the top and one at the bottom of the EBR also providing 3 V potential. Each EBR was operated as single-stage reactor with 24 hours retention time, Figure 4. Upon reaching steady state, >99.8% of selenium was being removed using both configurations; effluent selenium concentrations were <1 ppb. Initial results with a 17-hour HRT show similar results. Optimal EBR electrode configurations are dependent on selenium concentration and water chemistry.

Pilot-Scale Tests—Arsenic Removal

Pilot-scale tests were conducted on site using EBR components configured within a shipping container. The two stage EBR pilot system was operated under conditions that approximated plug flow (up flow) using total retention times varying between 10 to 18 hours; flow rates from ~0.5 gpm to ~0.8 gpm. The light colored circles in Figure 5 indicate replicate analysis splits, with the submitted samples shown in light diamonds being diluted ½. This analysis control check was completed because University laboratory analysis indicated between 10 and 20 ppb arsenic concentrations; significantly lower than those reported by a third party lab. However, the third party lab analysis met site arsenic treatment goals. Influent NO_3 was about 24 mg/L and was removed in the first EBR stage.

Note that after about three days, the system arsenic effluent in EBR1 increases during flow rates of ~0.8 gpm. However, during the same test periods, the efficiency of EBR2 increased, maintaining the final average arsenic effluent at about 15 ppb (University analysis) or ~40 ppb (third party analysis). Third party analysis met target discharge goals.

Pilot-scale tests were conducted through summer and late fall covering a >15°C water temperature drop. EBR system maintained a consistent metal and nitrate removal throughout the testing period, Figure 6.

Thus far, nine fully successful bench scale tests on various waters have been completed for mining companies to treat both process and wastewaters from hard rock mines and coal mines containing selenium and nitrate, arsenic and nitrate, and mercury. A pilot-scale testing for selenium removal from mining waters has also been completed successfully.

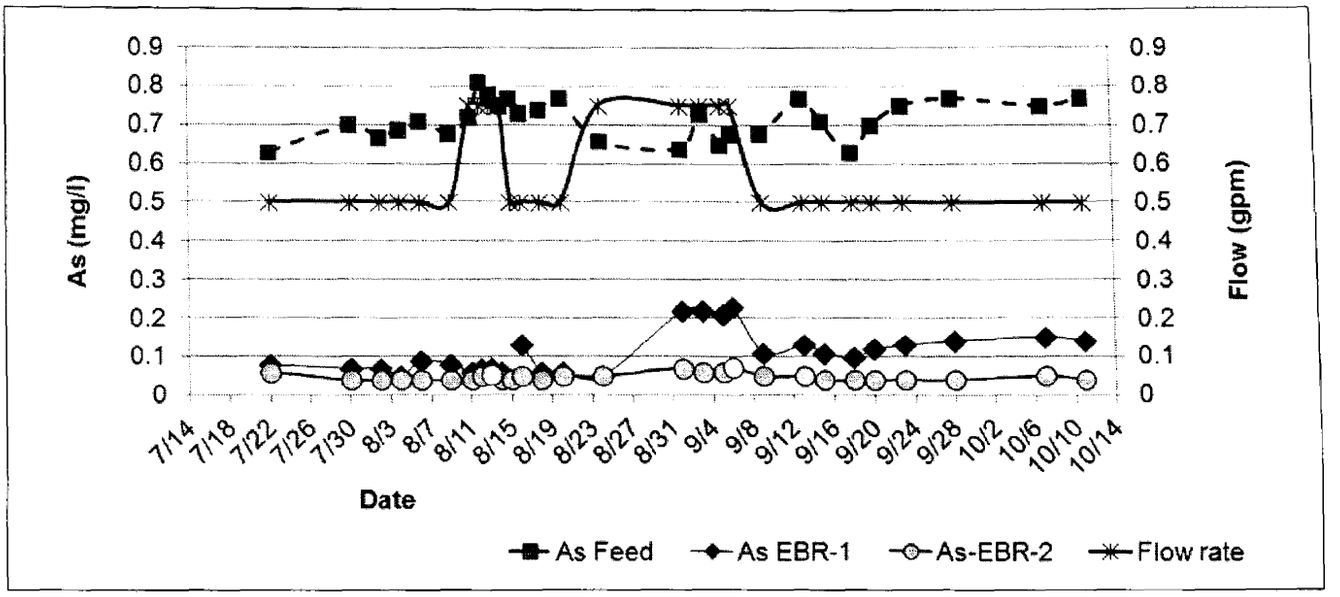

Figure 5. Arsenic removal in a pilot-scale EBR system operated on-site using 3 volts and a total retention time of 10 to 18 hours

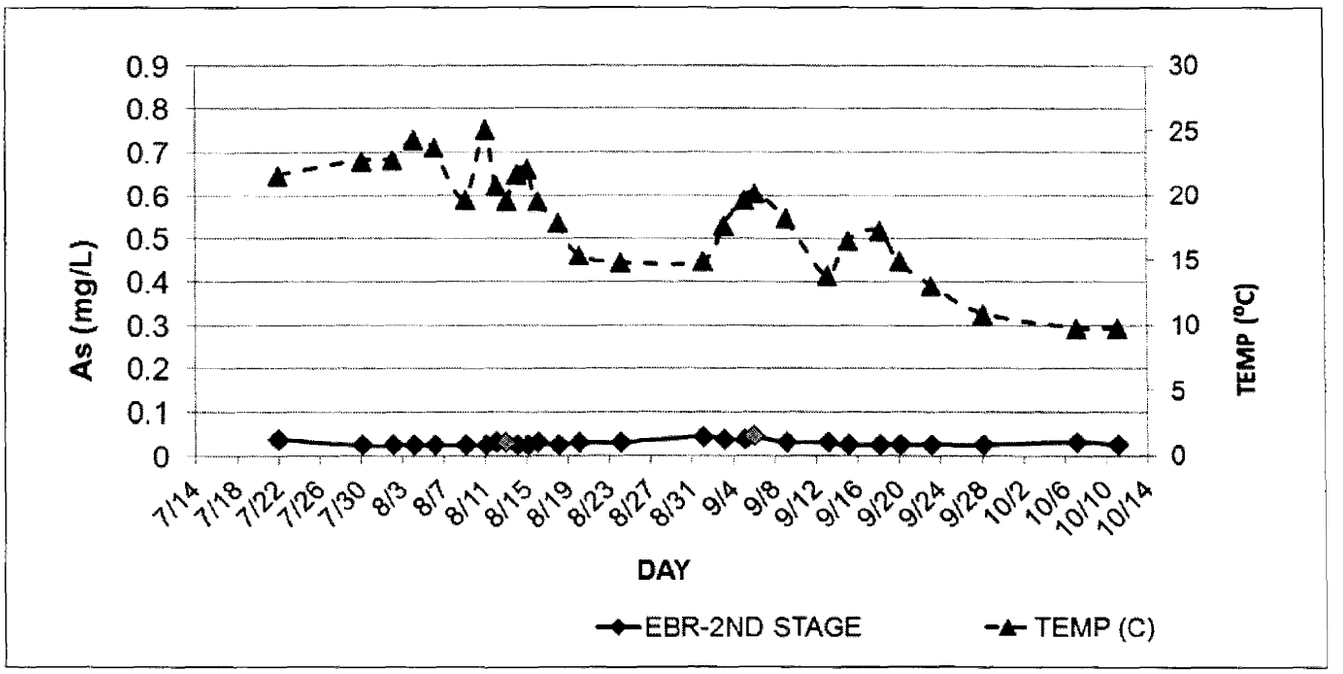

Figure 6. Relationship between temperature (°C) and arsenic effluent levels during on-site pilot-scale testing

CONCLUSIONS

The EBR has been shown to be effective in removal of selenium, arsenic, nitrate as well as other metals and inorganics.

- The EBR system provides a more precise system control; better controlled ORP, produces a more robust biofilm, and increases contaminant transformation/removal kinetics and efficiency.
- EBRs are capable of removing target contaminants to low ppb levels.
- From the results presented and for all tests conducted to date, EBRs show ≥40% lower capital costs and ≥40% less operational nutrient/reagent costs than conventional bioreactors.

- The EBR system produces more controllable and stable bioreactor environments.
- Because of the low DC voltage potential used, power requirements for a full-scale facility can be supplied by a small solar grid.
- EBR technology starts with the best aspects of proven microbial and chemical systems and takes them to the next level of performance and cost-effectiveness.

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