

The Effects of Increased Salinity on the Algal-Bacterial Reduction of Selenium from Excess Wastewater in the Panoche Drainage District of the San Joaquin Valley

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Abstract Selenium (Se) is a semi-metallic element that has been proven to be toxic when present in aquatic environments. The two forms of concern are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). In 1986, selenium-related birth defects were observed in wild fowl living along the outreaches of Kesterson Reservoir in California's San Joaquin Valley. This led to the development of a pilot study known as the Algal-Bacterial Selenium Removal System. Under this system contaminated agricultural drainage water is treated in a series of reduction ponds with a combination of algae and bacteria to reduce the water's selenium content. The incoming water's mineral content can be affected by changing weather conditions and increased erosion rates. This study, therefore, looks at the effectiveness of the ABSR system under varying concentrations of salinity. Four different salinity concentrations were prepared from a collected water sample, using standard procedures prepared by Lawrence Berkeley National Laboratories. The concentrations consisted of an influent standard (4765mg NaCl/540ml H_2O), 1.5X (2255mg NaCl/540ml H_2O standard), 2.0X (4955mg NaCl/540ml H_2O standard) and 2.5X (7115mg NaCl/540ml H_2O standard). 1g/L of bacteria was then added to each sample followed by 21.6g molasses/100ml H_2O and 1ml of trace elements (see Center for Biotechnology Media Manual) to serve as nutrients for the bacteria. These samples were analyzed in a controlled environment in 3-4 day increments, using hydride generation atomic absorption spectrometry (HGASS) to determine parts per billion of selenate and selenite per ml of water present. It was found that the reduction potential of the algae and bacteria to reduce selenium remained the same regardless of the salinity concentration of the tested samples. This suggests that at low saline concentrations the bacteria are uninhibited by the changes to their environment and continue to remove selenium successfully, having no overall effect on the ABSR system.

Introduction

Selenium (Se) is a semi-metallic element that has been proven to be toxic when present in aquatic environments. The three most commonly found soluble forms of selenium are selenate (SeO_4^{2-}), selenite (SeO_3^{2-}) and selenide (Se^{2-}) (Dobbs 1997). According to the United States Environmental Protection Agency, the safe criterion level for chronic exposure of aquatic life to Se is limited to 5 $\mu\text{g/l}$ (Dobbs 1997). Selenium has become a focus contaminant because of its relation to malformations in embryonic waterfowl and other vertebrates (Oswald et al. 2000). In 1986, selenium-related birth defects were observed in wild fowl living among the outstretches of Kesterson Reservoir in California's San Joaquin Valley (Quinn et al. 1998). The problem arose from agricultural runoff which was heavily contaminated with selenium that drained into the reservoir, resulting in abnormal shell development and low hatchling survival. Today, most of this same agricultural drainage water in the western San Joaquin Valley remains contaminated with heavy anion concentrations of sulfate and chloride as well as selenate (50-1200 $\mu\text{g/l}$ as selenium) and nitrate (20-120 $\mu\text{g/l}$ as nitrogen), both of which have become focus contaminants over the past few years (Oswald et al. 2000). Aside from the problems caused by selenium, nitrates promote unwanted algae and weed growth within the aquatic system and interfere with the treatment process of selenium (Oswald et al. 2000). Currently, this agricultural drainage is either discharged into sloughs, which drain into the San Joaquin River and then into the delta or it is evaporated in terminal ponds, where the water is treated before release, presenting a less toxic threat to the environment (Oswald et al. 2000). Several research groups including the U.S. Bureau of Reclamation, the California Department of Water Resources and the U.S. Environmental Protection Agency have been seeking cost-effective methods to remove and/or reduce the presence of these elements (Oswald et al. 2000).

It has been found that certain forms of bacteria have the ability to reduce both selenium and nitrates into less toxic forms or into forms that can be more easily removed. (Losi and Frankenberger 1997). In a study done by R.M. Rael and W.T Frankenberger Jr (1994), it was discovered that a group of bacteria known as Aeromonads (*Aeromonas veronii*), commonly found in aquatic environments, have a tolerance to elevated levels of selenium because of their ability to assimilate various Se species (e.g. SeO_4^{2-} , SeO_3^{2-} , Se^{2-}) and produce volatile organic Se compounds. It is therefore believed that this organism has the potential to be used in the removal of Se from seleniferous agricultural drainage water (Rael and Frankenberger 1994). In another

study conducted by Masami K. et al. (2000), the bacterial strain was found to effectively reduce 20mM of selenate (most commonly found form of Se) to 2mM selenite and non-toxic insoluble elemental selenium in the presence of an appropriate carbon source and in the absence of oxygen. Since these previous studies, an Algal-Bacterial Selenium Removal System (ABSR) has been developed by Dr. William Oswald along with an environmental engineering group at UC Berkeley to utilize this phenomenon. Under this proposed method, agricultural drainage water enters the system, (made up of several treatment ponds) where it is treated with algae and bacteria in order to reduce both the nitrate and selenium content (Lundquist et al. 1994). Under this process heterotrophic bacteria are able to metabolize nitrogen and other elements by extracting oxygen from the nitrate to yield nitrite and eventually transform it into nitrogen gas. This nitrogen gas can then freely escape into the atmosphere. Once the nitrate source of oxygen is diminished, the bacteria utilize oxygen from any selenium present in the water. Under this process selenate is then reduced to selenite or to elemental selenium which is easier to remove (Oswald et al. 2000).

A pilot study is currently being conducted in the Panoche Water District of the San Joaquin Valley to test the efficiency of this system. Past studies have shown the system to be somewhat effective in the reduction of selenium, with certain drawbacks. One drawback is the ability to sustain an anaerobic (oxygen free) environment upon which the bacteria thrive as opposed to the natural environment which requires lengthy amounts of time for the processes to occur. Another is the variance in salinity concentrations of the drainage water resulting from changing weather patterns and evaporation of the already present water (Gerhardt 1991, Brent and Lundquist 1997). The objective of this project, therefore, is to determine the effectiveness of the ABSR system on the reduction of selenium by looking at varying salinity concentrations maintained in an anaerobic laboratory environment. It is believed that under these conditions the denitrification process of the bacteria will be accelerated, therefore resulting in a more efficient removal of both nitrates and selenium. The hypothesis for this experiment is that as salinity levels increase there will be a steady falloff of the overall effectiveness of the ABSR system. Rael and Frankenberger Jr (1994) showed in their study that as salinity levels increased *A. veronii* became inhibited, resulting in a decrease in their growth rate. It can therefore be assumed that as salinity levels increase the bacteria will become less effective, which will result in higher levels of both nitrogen and selenium present in the water.

Methods

Samples Water samples were collected weekly over a two month period from the Algal-Bacterial Selenium Removal System (ABSR) maintained in the Panoche Drainage District of San Joaquin Valley, California. These samples were returned to Lawrence Berkeley National Laboratories, where they were prepared for analysis. Each sample was evaporated to determine the initial salt content of the water. This was accomplished by removing 540ml of water and bringing it to a boil to speed up the evaporation process. The salt content was then weighed to determine a standard or Panoche influent. NaCl was then added to the collected water sample to establish three varying salt concentrations (1.5x, 2.0x and 2.5x) (Table 1).

Panoche Influent (PI)	4765mg NaCl/540ml H ₂ O
1.5X	2255mg NaCl/540ml PI
2.0X	4955mg NaCl/540ml PI
2.5X	7115mg NaCl/540ml PI

Table 1. Preparation of varying levels of salinity

1g/L (.054g/540ml) of bacteria collected from Red Rock Ranch in the Central Valley was then added to each of the samples. 21.6g molasses/100ml H₂O and 1ml/L of trace elements (see center for Biotechnology Media Manual) were also added to each of the prepared samples. These contents were used to supplement bacteria present in the water with a food source to aid in their reduction of selenium (Se). Seven sets of 100ml sample bottles were prepared (65ml in each) for each sub sample (PI, 1.5x, 2.0x and 2.5x), e.g. seven bottles of PI, seven of 1.5x etc for a total of 28 bottles. The samples were then subjected to an anaerobic environment (Sheldon Anaerobic Microenvironment) where upon Oxygen (O₂) was removed from the sample to accelerate the bacteria's reduction process of nitrate and selenium. The sample bottles were sealed with air-tight rubber stoppers and incubated at 35-36°C. The bottles were covered with aluminum foil to prevent photosynthesis by the bacteria and further regeneration of O₂. The samples (one from each sub sample) were then analyzed for Se in 3-4 day increments (E.g. day 1, day 4, day 8...day 18) to allow for sufficient reduction time by atomic absorption spectrometry as described below.

Filtration Upon removal of the samples from the incubator each was tested for pH of the water using a pH meter and dissolved oxygen content using a DO meter. The samples were then filtered to remove any suspended solids. 1.2 micron GF/C filters were used for the primary filtration process. These filters were cooked at 525° C to remove any contaminants present in the manufacturing of the filters. Approximately 50ml of each of the samples were then strained through the filters using a small vacuum pump, attached to a 250ml flask. The filters were then saved for later analysis of suspended solids to determine the amount of bacteria and other volatiles present in the sample. The filtered water samples were then re-filtered using a 0.22 micron glass filter to further remove any volatiles that passed through the larger filter. This was accomplished through the use of a “French press”, straining the water into two 25ml vials in preparation for analysis.

Digestion Upon completion of the filtration process the samples were digested on a heating block. In preparation for digestion, the heating block was warmed to 95°C (~45-60min). 16 test tubes were prepared by adding 2.5ml of sample to 11 tubes. The remaining five tubes were comprised of two separate (split) tubes, each prepared with a repeat of 2.5ml PI and 3 (spike) tubes, prepared with 2.25ml of sample (randomly selected) + .25ml of 1000ppb standard selenium stock solution. These last five test tubes were used to calculate quality assurance (QA) and quality control (QC) of the analysis of the samples to determine the accuracy and precision of the results. 2.5ml of HCl was added to each of the test tubes to allow for the testing of total soluble selenium. Two percent ammonium persulfate was then added to the samples in varying concentrations to assist in the digestion process. (Table 2). Ammonium persulfate levels that are too high or too low can have effects on the projected values of selenate and selenite and therefore these varying concentrations were used to establish a curve in the hydride generation atomic absorption spectrometry analysis (as described below), taking the highest projected number in the curve as the final value.

PI	0ml	0.1ml	
1.5x	0.1ml	0.2ml	0.3ml
2.0 x	0.1ml	0.2ml	0.3ml
2.5x	0.1ml	0.2ml	0.3ml
Split	0ml	0.1ml	
Spike	0.1ml	0.2ml	0.3ml

Table 2. Amount of 2% ammonium persulfate added to each sample

The tubes were lightly capped and allowed to digest in a fume hood for 35-40 minutes. After digestion the test tubes were removed from the heating block and allowed to cool.

Hydride Generation Atomic Absorption Spectrometry In preparation for hydride generated atomic absorption analysis, the hydride generated atomic absorption spectrometer (HGASS) was turned on and using the spectra aa program was optimized for 60 minutes. This allowed for the proper calibration of the selenium specific lamp for a stabilized output reading. After this time frame, the Acetylene gas regulator was opened to 11psi and the Argon gas regulator was opened to 54psi. The air switch was turned on and the flame was lit. Once the flame was lit the HGAAS was allowed to run for another 30 minutes to optimize the flames temperature. The flame should appear bluish in color to determine proper settings; an orange flame is indicative of problems.

Two separate solutions were prepared to maintain a constant gaseous mixture within the HGAAS. The first consisted of 6M HCl made from a 50:50 dilution of 250ml of HCl and 250ml DI water. The second was made from diluting 1.25g NaOH and 1.5g NaBH₄ with 500ml DI water. Three calibration standards (1, 5, 10 ppb) were also prepared using 100ml flasks. These were prepared by adding 0.1, 0.5, and 1.0ml of 1000 ppb standard selenium stock solution (maintained in the laboratory) to the flasks + 2.5ml HCl filled to 100ml with double deionized water (DDI water).

After the test tubes were allowed sufficient time to cool in the fume hood, the HGAAS was calibrated using the prepared standards. The samples were then analyzed. Each sample was

diluted using DDI water to establish proper readings. Dilutions consisted of 1 in 7, 1 in 16 or 1 in 40 depending on the initial reading of the HGAAS for each sample tested. (Table 3)

1 in 7 Dilution	0.50ml sample and 3.0ml DDI
1 in 16 Dilution	0.25ml sample and 3.75ml DDI
1 in 40 Dilution	0.25ml sample and 9.75ml DDI

Table 3. Dilutions used for varying samples to establish proper HGAAS readings.

A standard check was performed after every eight samples were run on the machine to assure there was no failure in the equipment and that the readings were accurate. From the samples tested the tubes with the highest value of selenium present were recorded for analysis. These final readings from the HGAAS were used to determine how many parts per billion of Selenium were present per ml of water tested.

Once the total soluble selenium test was completed, the remaining filtered sample was run straight without the addition of acid or 2% aluminum persulfate, to test for the presence of selenite (Se_4) a derivative of selenium. The same dilution and standard checks were performed as mentioned above. Again, the highest readings from each sample were recorded and used to indicate the parts per billion of selenite present per ml of sample.

Results

Due to time and budget constraints, this experiment was limited to a single sample made up of several replicates, conducted in a controlled laboratory environment. Upon analysis of the samples, a determination of the amounts of both total soluble selenium (toxic form) and selenite (reduced form) present within each sample of varying salinity were established. In both cases there was an initial reduction of selenium followed by a period of leveled consistency indicating the method taken from the ABSR system was successful.

Figure 1 depicts the results for the reduction of total soluble selenium. This graph shows a consistent decline in the amount of total soluble selenium present for each of the samples (PI, 1.5X, 2.0X, 2.5X). Initially there was an increase in the presence of selenium content, indicative of incompleteness of the nitrate/nitrite removal process. In each of the four cases, however, the

selenium content was reduced from $\sim 310\mu\text{g/L}$ at day three to $50\mu\text{g/L}$ on day six, indicating that salinity had no effect on the reduction potential of the present algae and bacteria. After day six, the samples all maintained relative stability at $50\mu\text{g/L}$, indicating that there was only minimal reduction taking place.

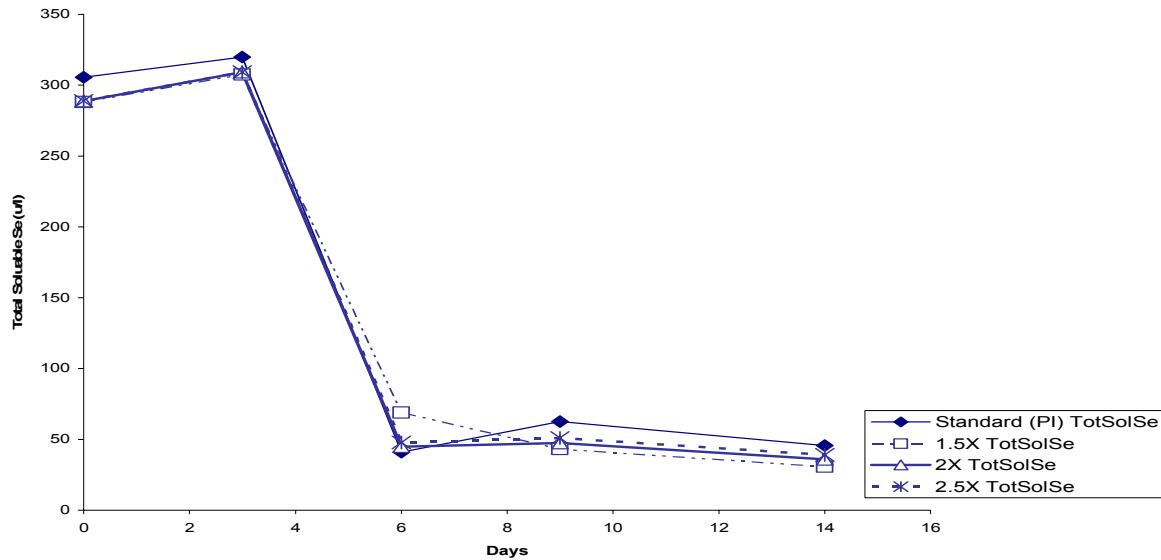


Figure 1. Total Soluble Selenium During Batch Bioassays of Nitrate and Selenate reduction

Figure 2 shows the conversion of total soluble selenium into Selenite. Initially, there were only minimal traces of selenite present within each of the samples because they had yet to be reduced from selenate (total soluble selenium.) This graph coincides with the first graph in that as total soluble selenium is reduced by the bacteria present in the water samples, an increase in selenite is observed. In the standard Panoche Influent selenite increased from $0\mu\text{g/L}$ - $\sim 60\mu\text{g/L}$ up until day three. This increase is also observed in the other samples. In 1.5X there's an increase from $0\mu\text{g/L}$ - $\sim 45\mu\text{g/L}$, for 2.0X an increase from $0\mu\text{g/L}$ - $\sim 35\mu\text{g/L}$, and in 2.5X an increase from $0\mu\text{g/L}$ - $\sim 30\mu\text{g/L}$. This is the result of the oxidation reduction potential of the bacteria. After day three, each of the samples' selenite content was again reduced with consistency, this time to $\sim 9\mu\text{g/L}$ leveling off from this point on. Salinity again was shown to have no direct influence on the reduction potential within the varying samples. The only variance seen was that in the standard (PI) sample an increase in selenite concentration was shown from day six till day fourteen from $10\mu\text{g/L}$ to $\sim 30\mu\text{g/L}$, indicative of the reduction of selenite after day six to a form of organic selenium which is the most stable of the three.

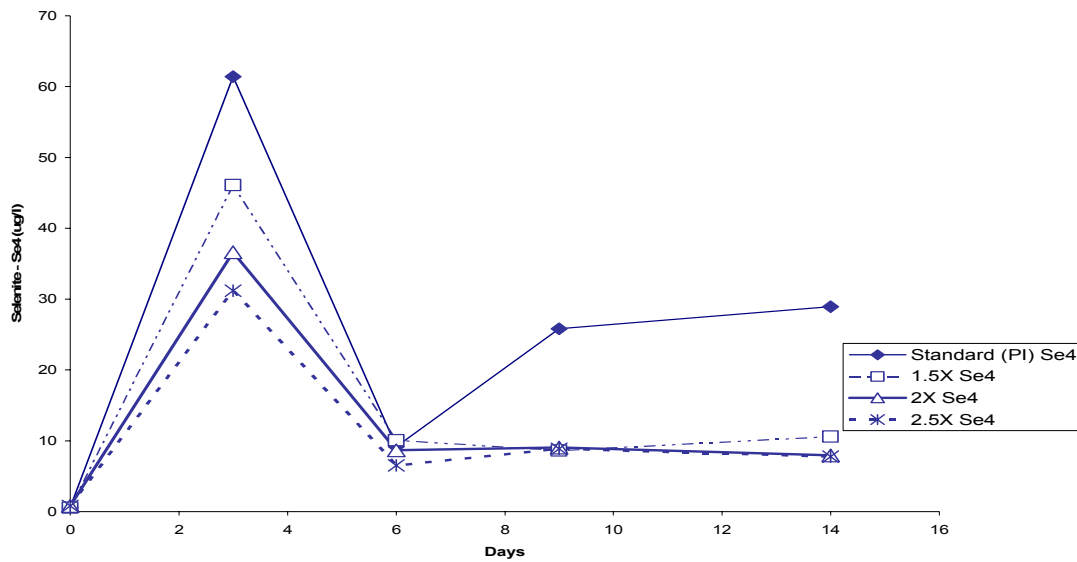


Figure 2. Selenite (Se₄) During Batch Bioassays of Nitrate and Selenate reduction

Figure 3 shows the difference of the reductions of total soluble selenium and selenite to infer the total overall total soluble selenium content after completion of the entire reduction process. Starting at day zero total soluble selenium was reduced from the initial 300 μ g/L as seen in figure 1. to ~275 μ g/L at day three. This again was followed by a swift reduction down to ~25 μ g/L on day six followed by leveled consistency up until the final day.

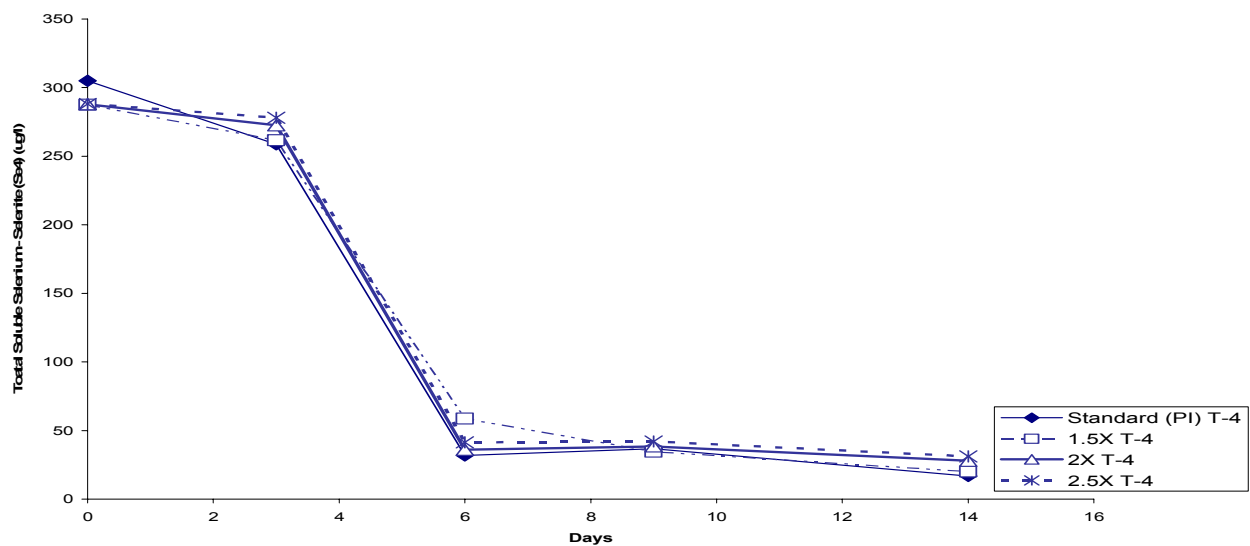


Figure 3. Total soluble selenium content after selenite reduction.

Discussion

In past studies, increased salinity levels have been shown to influence and denature the efficiency of certain bacteria. In a study conducted by R.M. Rael and W.T Frankenberger Jr (1994), increasing salt content revealed that the specific growth rate of *A. Veronii* was highly sensitive to raised salinity levels. In another study conducted by A.R. Dincer and F. Kargi (1999) it was found that biological processes used for the nitrification and denitrification process of saline wastewater results in low treatment efficiencies because of plasmolysis or loss of activity of organisms in the presence of salt. Although the effects of salt inhibition on the removal of selenium are unknown, the results of this study show that under low salinity concentrations there is no effect on the overall removal of total soluble selenium or selenite.

The results revealed that even at salinity concentrations 2.5x (250%) that of the standard influent, the bacteria were still able to reduce the waters selenium content with the same efficiency as all the other concentrations (PI, 1.5x, 2.0x). In each of the cases studied (removal of total soluble selenium and selenite), it was found that there was no variability in any of the samples and that they all followed the same patterns and amounts of reduction. For all the samples, total soluble selenium showed a steady decline after 3 days of incubation dropping from ~300 µg/L on day three to 50µg/L on day six. This resulted in the increase of selenite present within the samples. Once the bacteria began reducing the total soluble selenium, the selenite content also showed a decline from ~60µg/L on day three down to 10µg/L on day six. Overall the toxic form of selenium (total soluble selenium) present within the samples was reduced from ~300µg/L down to nearly 25µg/L over the course of the fourteen day period. Although the EPA's standard for safe levels of selenium are maintained at 5µg/L (Dobbs 1997), which is lower than our projected results, this study shows that low levels of salinity (between 8.8g/L (P.I.) and 22g/L (2.5x)) have no effect on the Algal-Bacterial Removal System's methodology. If some effect were present, variability would be seen in the graphing of the individual samples tested. This would result in graphs with different slopes of reduction, indicating a more gradual slope for higher concentration of salinity and a steeper slope for that of the Panoche influent.

This study was conducted under controlled laboratory conditions which could be under representative of the natural environment. Using the Sheldon Anaerobic Microenvironment to accelerate the removal of oxygen is one of the primary factors that could have influenced the

results. In the field, the algae and bacteria within the water are exposed to a constant inflow of oxygen from the surrounding environment which could slow their reduction potential of nitrate to nitrite and selenate to selenite, therefore allowing extended exposures to the present salinity concentrations. Another influencing factor could be that of the variability of salinity concentration throughout the year. It was found from the sample collected that the influent water had a salinity concentration of approximately 8.8g/L H₂O; however, this could be varied based on the amount of rainfall and/or erosion present in the area. Future studies might therefore look at concentrations of salinity at different locations within the environment of the Panoche Water District to determine actual salinity patterns present over the course of a year. Upon finding these values, field studies could then be conducted to determine if these salinity levels had an overall effect on the reduction potential of the bacteria. If redone under the same laboratory conditions it would be recommended that a baseline be determined of the concentration at which the bacteria are affected and/or if an effect is present at all. From this study it can be concluded that at low salinity concentrations that there was no obvious effect on the removal of removal of selenium and therefore, future experiments might consider testing higher saline concentrations, possibly: 26.8g/L (3.0x), 35.2g/L (4.0x), 44g/L (5.0x) and 52.8g/L (6.0x), to determine if there is a present effect at higher level concentrations which could effect the overall outcome of the efficiency of the Algal-Bacterial Removal System.

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