Identification of Selenium reducing species within and suggested optimization of a passive biological reactor

By

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Abstract

As concern for the environment moves to the forefront of the issues considered most pressing by society, mankind is becoming increasingly aware of the harmful things it discharges into the ecosystem in the name of industry and progress. One of these compounds that is making itself increasingly known is selenium. This element is a metalloid that occurs naturally within the environment and is a necessary nutrient to many living organisms. The problem comes from human activity creating anthropogenic loadings of selenium species into the environment or accelerating the naturally process that otherwise introduce the necessary amount of selenium into the ecosystem. When selenium becomes too enriched in the environment the impacts upon animal populations can be drastic. It has been well documented, especially in bird and fish species, that exposure to high levels of selenium can impair reproductive processes and thereby have a detrimental impact of organism populations. One method implemented to remove selenium from wastewater streams in an attempt to prevent such things from happening is the use of a passive biological reactor. The type that this study pertains to are of a lagoon structure. These systems receive an organic carbon source for the microorganisms growing within the system, and generally get no additional input like aeration to enhance the process.

For this investigation we were tasked to examine such a process and report back with the requested results. The examined systems consisted of three lagoon type reactors operated in series. Each reactor received wood chips as the organic carbon source for the microorganisms present in the reactor. The task presented was to identify the species of microorganisms

responsible for the selenium reduction within the system as well as to suggest any means by which to optimize the process. The methods tested by which to potentially optimize the system fall into two categories. The first category consisting of altering the temperature within the reactor. The second category consisted of potential nutrient additives that could potentially increase the potential for and rate of selenium reduction. The additives tested were: Nitrogen, Phosphorous, Micronutrients, Molybdenum, Zinc, and Cobalt.

The identification of selenium reducers was performed at every sampling location within the reactors providing a profile for species present across the treatment process. The methods of optimization were tested by observing the rate of selenium reduction when different amounts of the proposed additives present within an isolated batch experiment. The same was done at varying temperatures to determine its effect on the process. Upon completion of the experiments, the rates of reduction were plotted according to the additive tested and the concentration of said additive corresponding to the observed rate of reduction.

After completion of the identification process, ten species of selenium reducers were positively accounted for. These species found are: *Bacillus Subtillus, Microbacterium aborescense, Enterobacter, Psuedomonas stutzari, Desulfomusa, Desulfomicrobacterium, Desulfovibrio desulfricans, Desulfobacterium, Geovibrio,* and *Shewanella putrifaciens*. These species were found to have changing prevalence from sampling location to sampling location across the process. After taking a closer look at data gathered while performing the species identification lab work it was concluded that the third reactor in process was operating under a surface treatment condition. This conclusion led to the first optimization suggestion which was to decrease the size of the wood chips added to that reactor thereby increasing the surface area at which selenium reduction occurs. Once collected, the data gathered from the procedures to

determine the influence of the presented variables for optimization were plotted and examined for the presence of an identifiable trend. The altering of the temperature at which reactor samples were held at, did display an observable impact on the reduction rate of selenium. This suggested that temperature could indeed be a means of process optimization, however the likely economic implications of temperature control for this process very likely make this an impractically means of optimization. Of the additives examined Nitrogen, micronutrients, and Molybdenum were successful in producing an observable impact on the reduction rate. Therefore it was suggested that the addition of these additives could be viable methods for process optimization.

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Chapter 1 Introduction

In a time where concern for the environment and mankind's impact on it is steadily on the rise, society is recognizing how many harmful compounds its' activities introduce to the environment as well as how harmful they can be. One of the things that man's actions discharge into the environment that is working its way into the limelight as one of the most pressing contaminants to address, is selenium. This element is a metalloid that is discharged into the environment largely through agricultural and power generation processes, amongst other avenues of loading. Selenium and can be very hazardous to wildlife when it inevitably makes its way into fresh water environments. Selenium can accumulate in the bodies of organisms that live in or depend on the contaminated waters for survival. Selenium primarily wreaks its havoc through disruption of the reproductive cycle of organisms, which can lead to devastating harm on populations. Now that the significance of selenium as a contaminant is being realized the next step to address the problem is to determine how to better treat water containing elevated levels of selenium.

One of the most promising forms of treatment being implemented and researched today is the use of a biological process to remove selenium from waters. Since past research has illustrated the ability of some strains of bacteria to remove selenium, biological treatment has been an area of great interest. For a microorganisms to survive and grow they depends upon the process of electron transfer from an electron donor to an electron acceptor. If an artificial

environment is properly constructed and maintained then the compounds, which are used in this electron exchange, can be controlled by promoting the growth of specific bacteria that utilize the contaminant of interest, which in effect removes it from the water being treated. In essence, the processes which treat the selenium in this manner are oxidation reduction reactions carried out and mediated by biological metabolic processes. (Sandy & DiSante, 2010)

The metabolic process that is utilized to treat contaminants is governed by the need of the microorganism to transfer electrons and gain energy. The electron donor is an energy source that can be either organic or inorganic in nature, while the acceptor of the electrons being transferred is whatever compound the bacteria uses as a means of respiration. Oxygen is the most widely used electron acceptor by microorganisms, however if oxygen is not present other oxidized inorganic compounds or oxyanions can be used instead. Microorganisms that can reduce selenium use organic sources to obtain their carbon and energy and can be classified as selenium and nitrate reducers. These bacteria follow a hierarchy of what compounds they use as electron acceptors, which begins with oxygen, then nitrate, and finally selenium. Because all of the available oxygen must be reduced before selenium is reduced a biological selenium reduction process operates under anaerobic conditions and reduce selenium only once there is no nitrate present in the system as well. It is helpful to note that the period resulting in Selenium reduction is the same as when Sulfur is reduced by the same mechanisms. So in order for a biological process to be successful it must be provided enough organic carbon to sustain the bacteria so that they can reduce all of the remaining quantities of compounds that are reduced before selenium (O_2 & NO_3^-) and sustain Selenium reduction as well. (Sandy & DiSante, 2010)

Engineered biological treatment methods fall under three categories which are bioreactors, constructed wetlands, and in-situ remediation methods. For the treatment of

discharge waters from any kind of industrial process, bioreactors are usually the method of choice for biological treatment. Bioreactors can be constructed as either an above ground tank or a lined in ground basin. There are a few ways of classifying these treatment process that must be explored. A bioreactor treatment process can either be a suspended growth system or an attached growth system. In a suspended growth system the microorganisms are kept in suspension within the water in the basin by mechanical means where they come into contact with the contaminant in question and react within. In an attached growth the microorganisms are grown on the surface of a support media and then the wastewater is passed through the media in a similar fashion to a filter. Attached growth systems are superior to suspended growth for removing low concentrations of a contaminant due to its increased contact time between the water being treated and the contaminant reducing bacteria. These types of reactors can also be configured and operated under a plug flow model. Another way of dividing treatment processes is by whether they are active or passive treatment processes. An active process receives input through chemical and mechanical means. These inputs intend to maintain optimal conditions so that the microorganisms are given the chance to remove the contaminant in question at their greatest rate. A passive system is constructed and then essentially just left to sit and do its' own thing. The most maintenance that passive systems receive is an occasional replenishment of necessary chemicals. (Sandy & DiSante, 2010) Typically the rate of contaminate removal is not at its highest achievable potential in a passive system.

Within these categories there are several types of biological treatment methods used for removing selenium, however there is one method that is particularly necessary to discuss. Passive biochemical reactors are a treatment method that is commonly implemented in the mining and power generation fields. In this method, a basin is dug and subsequently lined with a

method of choice for those implementing it. Water and discharged waste waters from the industrial activity are pumped into the created basin. This pond like structure then has large amounts of an organic substrate. This substrate can include but is not limited to things like wood chips, alfalfa, manure, mushroom compost, sawdust, straw, peat, and like organic materials. With such a large supply of a carbon source the microorganisms reduce all dissolved oxygen until anaerobic conditions are achieved and selenium reduction commences. This system is passive and only requires the replenishment of the carbon source once it is eventually exhausted. (Sandy & DiSante, 2010)

It is from here that the intent of this research endeavor will be explained. A passive biochemical biological reactor currently being utilized in the state of Alabama with the goal of removing high levels of selenium from power production waste. In this particular application three basins are being utilized in series with the organic carbon source being wood chips from hardwood tree species. To understand how this system achieves Selenium removal and if this process can be used in other locations, a research study was conducted. In this study a series of samples taken from several locations within each basin and analyzed. The goals of this research include developing a profile of identified bacteria within the samples and suggestions on how to optimize the operation. With a profile of the microorganism found in the basin, a better understanding of the intricate relationships between organisms in the basins can be better understood. By doing this we hope to take a step in the direction of improving the effectiveness and practicality of this method of selenium treatment and determining if the implementation of similar and equally successful systems can be extended to other locations.

Chapter 2 Literature review

2.1 Selenium in Nature

Selenium is an element that is found naturally in our world. It has been present as a part of our planet since its beginning and will remain as a vital constituent for life in our world. Through our ever growing demand for advancement and utilization of our planets resources, mankind's footprint has created new pathways for selenium to cycle within the ecosystem. These pathways are having an enormous impact on our environment and operate at a much higher rate than their natural counterparts. In order to determine how humans are impacting these natural ebbs and flows of selenium we must first examine how nature itself governs the pathways that selenium travels throughout our world.

The selenium cycle begins when the Earth was considerably younger and volcanic activity was shaping the ground we thrive on today. Selenium is found within molten lava found within volcanos. When volcanoes erupt, as they did in the Earth's infancy, selenium within this magma was brought to the surface of the planet. The release of selenium through volcanic activity can be characterized into one of three criteria. The first condition being that selenium can be volatilized and expelled directly into the atmosphere. In this circumstance, selenium can make up five percent of volcanic gasses. If the molten rock cools slowly enough to form igneous rock then some selenium can be trapped within these rocks before it have a chances to completely volatilize, resulting in the second circumstance. It is believed that in the slower cooling associated

with igneous rock formation, selenium can partition into sulfide minerals. These metallic sulfides that are derived from volcanic activity can contain concentrations of selenium that can reach as high as 1400 micrograms per gram. However in some circumstances, igneous sulfur deposits can contain up to 2000 micrograms per gram. Most commonly, however, the non-igneous sulfides contain a higher selenium concentration. This phenomenon is made possible by the similar atomic size and properties found in selenium and sulfur compounds, which allows selenium to be substituted for sulfur in commonly sulfurous compounds. The final condition entails ash expelled by volcanic activity can cool at a fast enough rate that it traps a larger concentration of selenium within this form than do the slower cooling igneous rocks. Volcanic activity has been largely credited with the beginning of the natural selenium cycle. (Presser, 1994) So much of the element is believed to have its origin in volcanic activity that it is postulated that 0.1 grams of selenium has been dispersed by volcanic activity for every square centimeter of the planet's surface (Fordyce, Selenium Deficiency and Toxicity in the Environment). Thus the original source of Selenium, volcanic activity, over an extensive stretch of time operated in this way bringing selenium to the surface of the Earth and depositing it in the three ways outlined. As time continued on these sources began to give way to new sources that are more influential in today's world. The igneous rocks through weathering relinquished their hold on the Earth's selenium. Geothermal heat also volatilized selenium from the igneous rocks. Through the much faster processes of dry deposition and wet deposition through rainfall, atmospheric selenium is brought back to the planet's surface. As geologic processes carried on shaping the Earth's surface this relocated selenium became a constituent to the sedimentary rocks that were being formed. (Presser, 1994) On average today sedimentary rocks average around 0.0881 milligrams of selenium per kilogram where igneous rocks average 0.35 milligrams per kilogram. (Fernandez-

Martinez & Charlet, 2009) Selenium is primarily concentrated with the clay fraction of sedimentary rocks and is found in greater concentrations within shales than limestones or sandstones. (Fordyce, Selenium Geochemistry and Health, 2007)

Considering the importance of coal to the objectives of this study, it requires a little additional attention. Selenium is said to be the single most enriched elements found in coal at the trace level. Apart from the original volcanic and weathering sources described previously, coal can be enriched in selenium through the organic matter in the ground that contributes to its formation as well as adsorbing it from any groundwater that may pass through it. Coal can contain selenium in a variety of different forms. Elemental selenium can be bound to organic matter within the coal, as well as having organic and inorganic compounds within its chemical matrix. Coal is rich in organic material, which is critically important in this discussion because organic matter has the ability to take in selenium and tightly hold on to it. This process will be discussed in greater detail later. In the United States the average concentrations of selenium in coal is approximately 1.7 parts per million. (Paschke, Walton-Day, Beck, Webber, & Dupree, 2014)

These diverse sedimentary rocks were weathered and eroded over immensely long periods of time giving way to the soils we are familiar with today. (Presser, 1994) Selenium salts can also result from the weathering of selenium bearing rocks, but these typically dissolve over time. (Paschke, Walton-Day, Beck, Webber, & Dupree, 2014) Considering that most of the Earth's soils originate from sedimentary rock, it is not surprising that the world average for soil selenium content, 0.05-0.09 milligrams per kilogram, is quite close to the average for the sedimentary rocks they originate from. This connection brings up a good point and general rule, a soil's concentration of selenium is reflective of the concentration of the type of rock from

which it is derived. The selenium concentration a soil is considered to be selenium deficient if it contains less than 0.1 milligrams of selenium per kilogram and is classified as high in selenium if it contains over 0.5 milligrams per kilogram of the element. From the world average above it is clear to see that soils falling into the selenium scarce category should be quite common where as soils excessively rich in the element are very rare. (Fernandez-Martinez & Charlet, 2009)

Next, we need to revisit the topic of atmospheric selenium. The amount of selenium that is released into the atmosphere through volcanic gasses alone is enormous. It is estimated that the amount of gaseous selenium emitted to the atmosphere volcanically is of the same order of magnitude as all other naturally occurring routes of selenium into the environment combined, which has been estimated at 4,500 tons per year. There are also a few other routes for selenium to find its way into the atmosphere. The first being spray from oceanic activity suspends sea salts in the atmosphere and these salts can have appreciable concentrations of selenium. Wind's influence on rock through weathering can also release selenium laden sediments into the air and suspending them there. The final means for selenium to become air born is through the life cycles of living organisms both on land and in water bodies. Living creatures can expel gaseous selenium into the atmosphere as a result of their metabolic processes. The most important aspect of atmospheric selenium is its deposition back to the ground. When selenium becomes airborne its flight patterns and destination are at the discretion of the wind, this can result in atmospheric selenium ending up great distances from its source and this movement of selenium can greatly affect the concentration of the soil it deposits the element upon. (Fernandez-Martinez & Charlet, 2009)

Now that our exploration has returned to the soil we can now discuss selenium's introduction into the food chain. As plants grow within the soil and soak up nutrients to fuel their

growth, they take in selenium along with all the other nutrients. This also is attributed to the similarities in selenium and sulfur and it is suggested that plants only take up selenium because it works its way into the place of sulfur in compounds that plants require. There has been no evidence to date that selenium plays any kind of vital role in the development of plants, it is simply worked into the plants structure. Once the selenium has been taken up by a plant it has entered the food web and is available to any animal that eats said plant. Once the animal consumes the selenium laden plants it progresses up the food web just as any other nutrient does. Uptake of selenium by a plant can be affected by a number of different soil conditions which include but are not limited to: pH, redox conditions, the form the selenium is in, soil texture, mineralogy, the presence of organic matter within the soil, and competitive compounds that may be taken up instead of the selenium. Another special facet of plant involvement in the selenium cycle is the correlation between predominant plant species and selenium concentration in the soil. Studies have shown there is a correlation that shows plant species with a greater affinity for selenium absorption tend to be the predominant types of species that grow on selenium rich soils while those that do not possess the ability to absorb as much tend to be found in higher quantities growing in soils with lower selenium concentrations. These plants with high affinities can accumulate enough selenium to bring the concentrations of selenium to as high as 1000 milligrams per kilogram, while those that do not absorb as much have concentrations of 50 milligrams per liter or lower. There is another way that selenium can enter the food web, and this pathway is more pertinent to the objectives of this study. When selenium bearing rocks and soils come into contact with water, the selenium within them can become depleted and enter the water. Typically natural concentrations of selenium in surface water rarely exceeds concentration over ten micrograms per liter. This average concentration does not apply to ground water.

Ground water is much slower moving than surface water which cause a much larger time for water and selenium to interact, and groundwater comes into contact with an immensely larger amount of rock and soil surface areas than do surface waters. Concentrations of selenium have been measured to be as high as 1000 micrograms per liter in ground water systems within the United States. The World Health Organization has set a standard of 10 milligrams per liter as an acceptable concentration of selenium for drinking purposes while the EPA has set a standard of 50 micrograms per liter is surface waters in attempts to limit environmental impact. As selenium finds its way into water source it is commonly absorbed by bacteria, which can bio accumulate a considerable concentration of selenium within themselves. Lower level consumers eat these bacteria and once again selenium has found its way into the food web. (Fordyce, Selenium Geochemistry and Health, 2007)

2.2 Speciation

To better development our knowledge of the flow of selenium through the environment the next topic that must be explored is the speciation of this element in the ecosystem. We have already discussed that selenium cycles and where it cycles within the world around us, but now we must delve deeper and examine the how behind the flow and in what forms selenium is found. Selenium exists in a number of different species in any compartment of the environment including rock, air, and aquatic environments. The importance of this topic is evident by the greatly contrasting influence different selenium species have on the ecosystem and the organisms within it. For example some forms are completely inert and harmless, yet others are highly toxic and warrant studies such as this one. “In nature selenium is found in four different oxidation states including: +VI, +IV, 0, and –II” (Belzile, Chen, & Xu, 2000). The corresponding species for these oxidation states are selenate (SeO_4^{2-}), selenite(Seo_3^{2-}), elemental selenium, and selenide

(Se^{-2}). Most often selenide tends to form an acid called hydrogen selenide. These four species are generally the most prominent forms selenium takes in aquatic environments but trace organic species can be expressed as well. The solubility of these selenium species is primarily controlled by redox/oxidizing conditions, pH, and adsorption. In waters that have a neutral or slight reducing redox potential, elemental selenium is most common and is very insoluble. This state of selenium precipitates out of solution and is virtually useless to organisms. In more highly reducing conditions selenide is the primary form selenium takes. This form is also quite insoluable due to its' affinity to adsorb to organic matter or trace metals. The exact mechanisms by which selenium adsorbs to organic matter is currently not thoroughly understood but there are three theories as to how this process takes places. (Fernandez-Martinez & Charlet, 2009) (Weres, Jaouni, & Tsao, 1989) (Johnson & Bullen, 2004) (Ryser, Strawn, Marcus, Johnson-Maynard, Gunter, & Moller, 2005) (Belzile, Chen, & Xu, 2000) (Liu & Narasimhan, 1994)

"One possibility is that Se is directly complexed with organic matter. The second hypothesis is that Se is indirectly adsorbed by OM-metal complexes to form a "Se-OM-metal" tertiary system. The third explanation centers around that Se(-II) production by microbes or plants can be incorporated into amino-, carboxyl-, or hydroxyl- groups present in organic matter." (Qin, Zhu, & Su, 2012)

These two oxidation states of selenium are generally attributed to anaerobic conditions. Most waters are however oxic and make selenate and selenite the most commonly encountered forms of selenium stemming from their origin in oxidative waters. Selenate is the most soluble of the two and is the form readily taken up by plants. It is the favorable of the oxidized species due to it having a completed valence shell of electrons. Selenite is much less soluable because it has a strong tendency to adsorb to iron or manganese ions and become unavailable for biologic use.

Selenite can find its way back to an elemental configuration as the compounds it forms with these metals can be reduced and release the selenium back into solution in an elemental form. Selenium can commonly flow between these species in the environment when acted upon by influencing factors like oxidation/reduction potential change and largely from microbial reductions. As has been pointed out previously, selenium is very similar in behavior to sulfur and commonly finds itself substituted into sulfur compounds. This can manifest itself again in more acidic waters where selenium can take the place of sulfur atoms in hydrogen sulfide and polysulfides. This particular substitution occurs with elemental selenium and predominantly selenide taking the place of sulfur in these compounds leading to another pathway for selenium to become soluble and available to biological roles. When this occurrence is a result of selenide interaction, it can show properties similar to that of elemental selenium. Increases of acidity to a high enough degree can destroy these complexes and result in precipitation of elemental selenium. Some studies have postulated that humic compounds are the responsible party for the adsorption of selenium upon organic matter. It is theorized that the humic compounds react with and reduce selenium as selenate or selenite to elemental selenium or selenide, which then become bound to organic matters and rendered immobile.(Fernandez-Martinez & Charlet, 2009) (Weres, Jaouni, & Tsao, 1989) (Johnson & Bullen, 2004) (Ryser, Strawn, Marcus, Johnson-Maynard, Gunter, & Moller, 2005) (Belzile, Chen, & Xu, 2000) (Liu & Narasimhan, 1994)
Selenium can also form allotropes comprised exclusively of selenium. These allotropes form geometric structures and can be classified by such. Some of the more well identified allotropes are: trigonal gray, rhombohedral, deep-red monoclinic forms, amorphous red, and black viterious. These allotropes differ by the number of selenium atoms in each molecule and by the

shape and structure of the allotrope crystals. The red and black allotropes are the forms commonly found in soils. (El-Ramady, Domokos-Szabolesy, Shalaby, Prokisch, & Fari, 2015)

Now that a brief overview of selenium speciation in aquatic environments has been covered, it would be beneficial to take a more thorough look at the most influential controlling factors on selenium speciation. Arguably the most influential factor on the speciation in the realm of interest of this study is adsorption. In aquatic conditions liberated selenium species have a tendency to adsorb to other minerals and compounds already occurring in the solution. "Adsorption highly depends on factors like ionic strength of the medium, which can reduce the adsorption properties of some minerals through the reduction of the size of the stern layer" (Fernandez-Martinez & Charlet, 2009). Competition from other ions in solution can also have a substantial effect on the degree to which adsorption takes place, this point is more thoroughly explained later when the effects of pH on speciation is discussed. The manner in which selenium is adsorbed to other compounds can be classified as either one of two mechanisms, these being outer sphere complexation or inner sphere complexation. Outer sphere complexation is a electrostatically driven process that is highly depend upon the surface change of the constituents involved and on the ionic strength of the solution. "Inner sphere complexes form when an ion is adsorbed specifically on a crystallo-graphic site, i.e. when covalent or ionic bonds are created with functional sites present on miner faces" (Fernandez-Martinez & Charlet, 2009). Two primary differences between inner and outer sphere complexations are that inner sphere complexes are much stronger bonds and that these complexes are associated with more permanent adsorption binding of ions. The kinetics of selenium adsorption, is not a very well researched field. Of the few studies that have been conducted it is agreed that the kinetics of selenium adsorption are very fast and that a state of equilibrium can be reached within a matter

of only a few hours. Of all the minerals and compounds in natural waters, oxides and hydroxides formed from iron, aluminum, and magnesium, carbonates, and organic matters are the primary species associated with selenium speciation. Due to its' dominance in the adsorption of selenium, interactions between selenium and iron species will be discussed in more detail in subsequent sections. Adsorption onto metal oxides and hydroxides are highly dependent upon pH, due to its ability to alter the surface charge of these molecules. This topic also is illustrated in greater detail in the subsequent section discussing how pH affects selenium speciation. (Fernandez-Martinez & Charlet, 2009) Adsorption can clearly have a significant role in selenium speciation. A number of common compounds have the potential to adsorb selenium. If each of these compounds has the potential to adsorb one or multiple selenium compounds, then the number of different potential species of selenium that can be present in the environment greatly increases.

Another major factor in selenium speciation is pH. This influences speciation greatly due to pH's ability to alter the surface charge on compounds that selenium reacts with and its' capacity to introduce competitive ions that could keep selenium from the reaction sites it would need to occupy in order to react. The commonly found selenium bearing ions in water carry a negative charge, therefore it must follow that they react with compounds that carry a positive charge. If the pH of the water body increases, then by definition the concentration of hydroxide ions follows the same trend. By increasing the hydroxide concentration in the water, ions that have the capacity to react with the same positively charged compounds that selenium would has increased. If the pH and hydroxide concentration increases to a drastic enough extent and the hydroxide molecules outnumber the negatively charged selenium molecules by a large enough margin, it would therefore be much more difficult for the selenium compounds to react due to the hydroxide ions having already filled the positively charged reaction sites. If the initially positive

ions were to react with enough hydroxide ions then the overall charge of that ion could possible change and lose the electrostatic driver that would have initially brought it and the selenium molecules together. The same thing can happen should the pH decrease enough and the abundance of protons do to the negatively charged selenium ions what the hydroxides do to the positively charged molecules the selenium compounds react with. pH clearly plays a big part in selenium speciation. By blocking some reactions pH can keep a certain species of selenium from forming, but at the same time can form a different species of its own when protons react with selenium ions. The ions that result from the reaction of selenium species with protons in acidic waters are biselenite ($HSeO_4^{4-}$) and selenious acid (H_2SeO_3). (Liu & Narasimhan, 1994)

The immediate external factor that influences selenium speciation in the environment is the oxidation or reduction potential of the environment. As we discussed earlier, selenium that finds its way in to the aquatic environment predominantly originates from rocks and sediments. When these two sources are weathered selenium is liberated and oxidized into one of two forms, either selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}). Depending on the potential for oxidation or reduction selenium can go in one of two directions from this point. If the environment is an oxidizing one, then these two species will stay as they are. However, if the environment is a reducing one then these two species will be reduced into either elemental selenium or selenide (-II). Which of these four species selenium finds itself in is not only a question of reducing or oxidizing conditions but also a question of the magnitude of the condition. If selenium finds itself in an oxidizing environment then it can become selenite if the system is mildly oxidizing or selenate if it is very oxidizing. The same type of situation can be found in reducing conditions where selenium can be reduced back to its elemental form in mildly reducing conditions or to

selenide in strongly reducing conditions. Each of these four possible paths for selenium lead to very different reactions and varying species. (Johnson & Bullen, 2004)

The adsorption of selenite and selenate onto iron molecules is an extremely important factor, not only in the speciation of selenium but for the selenium cycle as a whole. Selenium complexes with both iron and manganese in the same way. Due to iron accounting for an average 3.8% of soil mass and manganese only accounts for 0.085% iron has a much greater influence. (Chapman, et al., 2010) Iron occurring in the environment as a positive ion is the form of this element that reacts with selenium. Since the reacting iron is positively charged, only the negatively charged species selenate and selenite will react and be adsorbed. “The affinity of iron solids for selenate is is generally smaller than for selenite. This behavior may be related to the differences in the nature of respective surface complexes and to geometrical factors that may affect the extent of inner sphere complexation, although the exact coordination (mono- or bi-dentate) and species pronated or depronated depends on the structure and mineral surface charge” (Fernandez-Martinez & Charlet, 2009) due to this condition the rest of the iron adsorption discussion will center around selenite in the aquatic system.

“If iron oxide is present in the system, specific adsorption of selenium oxyanions takes place by ion exchange with surface *aquo, hydroxo, and ol* groups of the hydrous iron oxides and of hematite, which, in the preszence of water, readily develops a hydrous surface film with adsorptive properties of goethite.” (Howard, 1977) This process is so critical to the cycle of selenium as a whole that experimental work has produced findings that suggest iron can adsorb anywhere from 95-99% of the aqueous selenium in a body of water. This process acts as a natural barrier from the environment becoming too flooded with selenium from natural inputs.

Should the conditions of the system become too acidic, these compounds can be reduced giving way to elemental selenium which will precipitate out. (Howard 1977)

2.3 Agricultural loading

The first anthropogenic pathway that introduces Selenium to the environment that we will discuss is agriculture. As has been stated previously, plants do not require selenium in order to grow and maintain proper health, however that is not the case for humans or livestock. As crops are grown on farm lands they leech the selenium from the soil as they grow. When these crops are harvested and ingested, the organisms that eat these crops are provided with Selenium that they require to live, grow, and maintain proper health. As crops are repeatedly grown upon the land, harvested, and grown again a problem begins to arise. The rate at which the agricultural machine utilizes the land exceeds the rate at which the land itself can replenish the nutrients the plants take up for their own well being, as well as the nutrients they take up that the organisms that eat them need for their own well being. The result of this rate of crop growth is that the soil becomes deficient in many of these nutrients, including selenium. As research validated the necessity of selenium for not only human health, but the health of the livestock that also depend on these crops, agricultural practices sought to solve this issue. Selenium has been added to numerous chemicals and fertilizers for crops with the intention of providing adequate concentrations for human and livestock health. Selenium has been incorporated directly into feeds for livestock as sodium selenite and selenate, so that their resulting meats are rich enough in the element for human health. Another way that selenium has been added to agricultural chemicals is through fertilizers and top dressing. In some areas wastewater and sewage are applied to the land to try and accelerate the recharge of selenium in the farmland. This as well as use of selenium enriched fertilizers are common practices for selenium soil rejuvenation. An

especially interesting caveat into the agricultural use of selenium is its' incorporation into pesticides. Potassium ammonium sulfoselenide was one if the initial marketed selenium pesticides and was sold in the 1930's. However, as research found out, this chemical was highly toxic and today is restricted to non food crop use. Sodium selenate is a more modern incorporation of selenium. This chemical can be added to irrigation waters which when consumed by plants is volatilized in the leaves and exits as a gas that is poisonous to bugs and other harmful pests. (El-Ramady, Domokos-Szabolesy, Shalaby, Prokisch, & Fari, 2015) All of these products do just what they were engineered to do, however runoff can lead to these chemicals having unforeseen environmental impacts. This is especially the case for row crops, where runoff and soil erosion occur at a high rate.

Irrigation is the biggest player when it comes to mobilization of selenium and selenium containing chemicals into the environment as a result of agriculture. The irrigation of crops is commonly believed to be conducted in order to water plants during times of lesser rainfall, however there is another valuable result that comes from irrigation. As natural moisture in the soil evaporates salts tend to form in the root zone of the crops. These salts commonly inhibit the growth of the crops. The solution to this issue that farmers have arrived at is to over irrigate so that not only are the crops provided with water but these salts are flushed away as well. (Lemly, 2004)

Clay layers can also cause issues with salts in root zones that require flushing by irrigation means. Clay layers inherently have an incredibly low permeability and this can lead to a buildup of water in the root zone of the crops. As this immobilized water evaporates salts are once again left behind and require flushing. To combat this issue more aggressive methods are implemented to force this clay impeded water off of farm lands. Common methods include wells,

pumps, channels, and subsurface piping that assist the water in finding its way off of farm land. These measures are all effective in providing routes for waters to drain off instead of stagnating. (Lemly, 2004)

The over application of irrigation on farm lands while washing away harmful salts also washes away selenium sources that have been applied to the crops before they have a chance to be utilized by the crops. This results in large quantities of selenium, in many different forms, being washed from farm lands into streams, ponds, and tributary waters. Once it reaches these aquatic environments the selenium can begin to accumulate and cause devastating effects to the organisms living there. If the land from which the selenium being washed from is being used to cultivate row crops than the amount of runoff that can be seen can dramatically increase. The very nature of row crop agriculture increases issues associated with runoff. When the crops are cultivated in rows small channels exist between the rows. These convenient paths form an express route for runoff to travel. Considering these channels are primarily soil lined, there is little to even slow the flow of the runoff. These conditions illustrate how serious of a contributor to selenium pollution agriculture can be. (Potera, 2008)

The pollution of selenium is not limited to the cultivation of crops, animal agriculture also has the capacity to contribute to environmental loading of selenium. As previously stated, Selenium is commonly added to livestock feeds in an effort to increase the concentrations of selenium in meats. A higher concentration of selenium in food meats ensures that those whom consume these meats will not suffer from a deficiency of selenium in their diet and have the amount they need to maintain good health. As the livestock ingest their Selenium rich feed, the animal cannot process the full amount of the element they are fed. This in turn allows for some of the selenium to pass through the livestock's digestive track somewhat unscathed. The

selenium continues to follow the path of nature and finds itself in the excrement of the livestock. From here the selenium can leach its way into the soil or be carried into tributary water ways by surface runoff. The selenium does go through a change as it passes through the animal. The path through the belly of the beast as it were, alters the selenium into various organic compounds. This is especially a problem because organic selenium compounds have an increased bioaccumulation potential that is orders of magnitude greater than that of inorganic selenium compounds. (Lemly, 2004)

2.4 Mining

A major source of anthropogenic loading that must be explored is the environmental selenium loading that results from the process of mining. Taking into consideration the purpose of this research, mining with the intent of acquiring coal will be the focus of this analysis. In this day and age when technology is so prevalent and essentially everywhere around us, our society has a considerable appetite for electricity to power all of our gadgets and maintain the quality of life we have become so very accustomed to. Although we are trying to make a move to renewable forms of energy, our craving for electrical power is still heavily fulfilled by the burning of coal. It is hard to deny that our desire for electricity is only growing and so in tern must our consumption of coal. Coal mining has the ability to release immense amounts of selenium into our aquatic environment. The primary mechanism for this is how the mining exposes a much greater quantity of earthen materials to weathering conditions. This can be especially problematic in coal mining because coal is considerably enriched with selenium. Coal can be enriched tens of times greater than more common surrounding soils and minerals and selenium can even be the most concentrated trace element within coal. Rain water can work its way through the pore space in coal storage piles and leach out selenium. The same can happen

when the coal is washed before it is sent off to power plants to be used in electricity generation. (Lemly, 2004). The mining process is centered around excavating soil and rocks in order to find the desirable mineral the operation is focused on. The means of excavation employed in mining greatly increases the surface area of the materials being removed and brings them into contact with oxygen and water that they had not been exposed to before being disturbed. This increased exposure to oxygen and water primarily leads to all of the new surface area being weathered instead of the immensely smaller exposed faces that were present before excavation. By more of the mineral being weathered at once, the amount of selenium released through these means exponentially increases. When this happens it is obvious that the amount of selenium liberated into the environment rises drastically. Ecosystems are then directly impacted by this. The extra selenium is washed from the mining site by runoff waters and can find itself accumulating in the environment. Here the element can begin accumulating and have devastating impacts on the organisms that live in the areas it builds up. (Hendry et. al, 2015)

One of the largest causes of coal mining mobilizing large amounts of selenium into the environment is due to the logistics of the process used to mine coal in the first place. A relatively new procedure for mining coal, known as mountaintop mining, has risen to prominence in the last several years. This way of doing things is extremely invasive and harmful to the ecosystems linked to the mining sites, primarily due from the drastic alteration to the watershed that mountaintop mining creates. The United States EPA recognized the harmful potential of mountaintop mining and made two sobering predictions. What they foresaw was that, “these mines will have impacted 6.8% of the largely forested 4.86-million-hectare portion of the Appalachian Coalfield Region within West Virginia, Kentucky, Virginia, and Tennessee” (Lindberg, et al., 2011). They made a second observation, “based on complete utilization of fill

permits issued between 1992 and 2002, the EPA estimates that 1944 km of headwater streams were buried during this period and predicted that the extent of buried stream length will double to almost 4,000 km by 2012" (Lindberg, et al., 2011). This form of mining has been linked to elevated levels of certain elements including selenium in ecosystems downstream from the mining site. To appreciate how harmful mountain top mining is and how it releases selenium we need to take a more detailed look into how this mining procedure is carried out. (Lindberg, et al., 2011)

When one thinks of mining, likely the imagination pictures a hole in the side of a mountain like one might see in an old western. Mountain top mining is quite different from that type of mining practice. Instead of digging a mine shaft into the side of a mountain to try and find a coal seam, mountaintop mining excavates the top of the entire mountain with the intent of exposing the seam. Preparations of the mine site must be made before the operation can begin. Initially the site must be cleared of trees and vegetation as well as access roads be constructed to allow for large machinery to make it to the site. Topsoil is generally collected during this stage for intended reclamation uses later. Excavation is the next step in the process. Explosives and machinery are used to remove the top of the mountain and expose the coal seams. Anywhere up to 300 meters of depth can be removed during this stage. The next question is what to do with the material removed during the mining. Unfortunately the material cannot be replaced due to its volume increase from being broken up during excavation, it has also naturally lost most of its cohesiveness during excavation. Some of the material is placed back over the mined surface but the majority is dumped in valleys, hollows, and any other low lying areas near the mining site. This dumping is done without regard for any surface water that may be flowing through the dump site. Alteration of surface flow is one of the major impacts that works in conjunction with

other effects of the mining to liberate elevated amounts of selenium. By clearing of the foliage on the site initially, the flow of surface water is inherently altered. The operation of heavy machinery creates a well compacted, virtually impervious, layer over the site. This means that the runoff must be diverted away from the site through a number of artificial means. This water is guided off of the site and ultimately into the fill areas where the excavated material has been dumped. The directed water flows in very close proximity to the loose material. After it makes its' way through the fill areas, the water is diverted into a pond where solids may be settled out or any other conditions be altered. In an attempt to remedy the environmental travesty that has occurred, the mining operation will carry out a reclamation of the land that includes replanting trees and vegetation on the mining site and the fill areas. (Griffith, Norton, Alexander, Pollard, & LeDuc, 2012)

Mining results in the discharge of increased levels of selenium because as material is excavated its' surface area exponentially increases and all of this extra surface area is brought into contact with oxygen and water. This contact weathers the material, thus oxidizing selenium within the material and liberating it into the environment. This occurrence is made possible largely due to the heavy impact that the mining operation has on the flow of surface flow. Watershed impacts of mining can generally be broken into four means of impact. First when the land is cleared and all of the vegetation is removed, all of the natural barriers and obstacles that the flow had to originally overcome are removed. This means that the components of nature that slowed down the runoff are no longer present to retard the flow. This point also brings us to the second factor, as the runoff begins to travel over the land more quickly it does not have sufficient time to evaporate. As the rate of evapotranspiration falls, ultimately more water flows through the water shed, due to the lost mass outlet of evaporation. The third factor results from the

operation of heavy machinery on the mining site. Heavy equipment rolling around a cleared dirt site will inherently lead to a well compacted layer of topsoil. Compacted topsoil of course does not allow for much of any surface water to infiltrate, which results in more water flowing from the area rather than be retained in the soil of the operation site. Ground water location can also be influenced by mining operations, which leads to the final point. As the surface runoff flows through the fill zones the groundwater table rises into the fill material resulting in an unconsolidated aquifer. An increase in the flow across the loose fill material means that the oxidation of selenium from this material is increased which results in a greater amount of selenium being mobilized. The effect from the unconsolidated aquifer is the same because water being retained in the pore space of the fill means that the oxidation process is in constant motion regardless of the amount of runoff flowing through the fill. (Griffith, Norton, Alexander, Pollard, & LeDuc, 2012)

Up until this point we have explored a lot of information about how selenium is leeched from coal mining operations. To give this argument teeth data suggesting these claims are true is necessary. A study by Lindberg et al. presents data suggesting that mining operations do in fact influence the amount of selenium in surrounding surface waters. This study was performed along 14km of the Mud River in West Virginia as well as some of its tributaries that were upstream of, in, and downstream of mining impacted watersheds. Several environmentally hazardous elements and compounds were tested for, including Selenium. Concentrations of Selenium collected were then compared to the drinking water standard of 5 micrograms per liter, established the EPA. When the data was analyzed a concerning trend emerged, all samples taken upstream of mining activities were below detection limits while 43 of the 52 downstream samples exceeded the EPA's standard for drinking safety. The samples extended into ranges and

order of magnitude greater than the standard. Interestingly, constituents known to be mobilized by weathering were also found in elevated levels which supports the idea that increased weathering action is the cause of heightened levels of selenium. (Lindberg, et al., 2011) Hendry et al. conducted a study that went to the horse's mouth per say for a more direct analysis. This study was done on the drain structure for a $2x10^8 BCM^3$ fill area adjacent to a mining operation in Canada's British Columbia. The researchers measured the flow through the drain for selenium concentration over the course of several years. They concluded that annually this one fill site discharged 710 kilograms of selenium. Secondary in relevance was their observation that over the roughly ten years this study was conducted, the concentration within the fill material did not drastically decrease. The concern that stems from this is the possible timeline in which this source could continue to discharge large amounts of selenium. If ten years showed no significant change then one must ask just how long this and all other sources like it could dump Selenium into the ecosystem? A second question that comes to mind is what would even be a feasible course of action to remedy source that are so enormous in size? (Hendry, Biswas, Essilfie-Dughan, Chen, Day, & Barbour, 2015)

As it stands, the information readily available about mines' impacts on the environment is prominently analyses of how effluent pollutants harm ecosystems. The mechanisms by which mining practices release harmful compounds are largely unstudied. In an effort to remedy this deficiency of knowledge the team of Wellen, Shatilla, and Carey made an effort to use modeling programs in order to set in motion bridging this knowledge gap. In this study, the team used the SPARROW model to gain insight on what factors could influence the loading of selenium to the environment. "The SPARROW model is an empirical mass loading model, which uses spatial data describing watersheds to explain the spatial variability of their mass loading. Empirical

models extract patterns from datasets and help formulate hypotheses. The model relates observed annual loads of a constituent of interest measured at a number of points in space to the attributes of the watersheds draining to those stations" (Wellen, Shatilla, & Carey, 2015) This model used by the researchers is incredibly complex, which allowed for the analysis of numerous possible factors to be examined in how strongly they affect the liberation of selenium. In order to effectively collect enough information 33 locations were sampled and analyzed for selenium at regular intervals. To begin their study the researchers established five forms of selenium input, these included: the byproduct rock used as fill, rejected coal, and three classifications of unmined land which represented natural discharge. The changing volumes of waste rock were accounted for, as the mining operations continued, and adjusted as the study progressed. Thirteen variables that pertained to the conditions of the dumped waste rock were tested and altered to examine which ones had an influence of Selenium release. These parameters were then tested further to determine to what extent their impact reached. Once their analysis was completed, the team had identified three parameters that showed relevant impact. Age of the fill material was the least statistically significant of these parameters, however the statistics were strong enough to suggest its' influence is suggestible. Among the waste rock dumps used in the study the age of the fills ranged from around one year up to around thirty years in age. What the study found was that a fresh fill area will discharge slightly more Selenium than one that is decades old. This result implies that the dumps could theoretically over many decades exhaust its Selenium content. The second relevant parameter was the ratio of waste fill side area to top area. What the model showed was that as side areas of a fill increased relative to the top area of the fill, the release rates of selenium declined. The study could not identify what the exact mechanism is that resulted in this trend. The researchers did however formulate a hypothesis that could possibly

explain this phenomenon. They theorized that by increasing the relative side area meant that the flow paths to the outlets were shortened and that more precipitation would fall on and flow down the sides of the formation thereby shortening the water's contact time. The third and last parameter, which was the degree of fill site reclamation, was the most statistically significant. What the data showed was that as the fill sites are reclamated to a greater degree the amount of liberated selenium decreases. Although the study did not identify the exact mechanism for this trend either, an abundance of literature suggest that vegetation reduced the amount of water that penetrates into the waste material thereby reducing contact between water and the fill material. Another possible explanation is that increased vegetation increases the rate of evapotranspiration. This study could provide insight into specifically how mining processes discharge harmful Selenium and as to how these processes could be altered to limit this discharge.

2.5 Power Production

The final anthropogenic source of selenium that we will examine is the combustion of coal for the generation of electricity. Once coal is excavated from the ground it is transported to a power plant for use. Coal is burned in boilers to generate steam which power turbines and in turn produce the electricity that society craves. The cost of this method of electricity generation is that the burning of coal results in some incredibly harmful waste products that all too often impact negatively on the environment around us. Our reliance on coal to quench our thirst for electricity to power our lives makes this a very important issue to address. Coal is by far the most utilized fuel used to generate electricity. It alone is responsible for over half of our nation's electricity. (Rowe, Hopkins, & Congdon, 2002) This dependence on coal makes it clear that this source of Selenium pollution is here to stay, and is an issue that must be addressed. The methods of combustion waste disposal themselves are cause for concern due to the stringency of disposal

regulations falling under the domain of state regulation. This fact is well illustrated by the wide range of regulations involving surface impoundment ponds used for coal ash. “In some states measures like groundwater monitoring programs, leachate collection systems, and impermeable impoundment liners are not required” (Rowe, Hopkins, & Congdon, 2002). A survey of 259 power plants with capacities for production all exceeding 100 megawatts revealed a startling result of forty percent responding that they operate under no standards for preserving groundwater. (Rowe, Hopkins, & Congdon, 2002) As it stands there is a 5 microgram per liter of selenium standard to achieve fresh water requirements and the standard for human health is set at ten micrograms per liter. These standards have both been reviewed however due to concerns that they are not strict enough.

The waste from the combustion process is the beginning of the path that disperses Selenium into the environment as a result of electricity generation. “Because coal is itself a concentrated source of many trace elements, oxidation and loss of carbon from the solid substrate during combustion produces a residual ash material that is further concentrated in non-volatile elements. Addition of materials collected from boiler flues and air scrubbing units to bulk CCR [coal combustion residue] stream can return volatile components to the CCR stream which would otherwise have been lost during combustion” (Rowe, Hopkins, & Congdon, 2002)

This return of all of the undesirable waste compounds to a reduced mass means the waste of combustion is extremely concentrated in harmful materials, which makes a statement as to why these waste byproducts are such an area of concern. The typical combustion process produces four types of waste. Ash and FGD waste are the big players and warrant most of the attention. Fluidized bed combustion waste and coal gasification ashes are the other two types of

waste produced, however these two are not remotely as common or potent. Since general ash and FGD waste are so much more prominent they will be the focus of our exploration.

Even though fluidized bed combustion waste and coal gasification ashes will not be the focus of this examination it is not detrimental to the focus of the exploration to give a short description of what these wastes are. Fluidized bed waste is a byproduct of initially mixing coal with limestone before it is placed into the furnace for burning. Injecting air into the furnace creates a fluid bed on which this fuel is burned in. the resultant waste is a dry byproduct that is generally alkaline and high in concentrations of calcium. Coal gasification itself is a process technology in which engineered gas and liquid fuels are produced from coal. These man made forms are then burned instead of the common coal ore. This process produces an ash just as the tradition burning of ore does, the difference the physical characteristics of the ash produced. This form of ash is generally coarser. This kind of ash contains spherical particles like the ore combustion, but this ash also contains sharp edged rough particles. This waste is generally acidic and contains larger amounts of iron sulfides. (Carlson & Adriano, 1993)

Ash waste is split into two categories, there is fly ash and bottom ash. Fly ash is the residue from the combustion of coal ore that is buoyant and rises through the flue gas stream. Bottom ash is heavier and remains in the bottom of the boiler, from where it is later collected. (Carlson & Adriano, 1993) Fly ash is collected by mechanical means from the effluent gas stream. The methods employed are generally either electrostatic precipitators, fabric filters or wet scrubbers. Generally, one of these methods alone are not efficient enough to meet emission requirements, therefore generally two of these systems are run together in series to meet requirements. The amount of ash produced from the combustion of the ore is a property of the ore itself. This ash content can range from only a few percent of the weight of the ore all the way

up to around thirty-five percent of the ore weight. The distribution of this ash between bottom and fly ash is not a trait of the ore, it is dependent upon the type of boiler implemented in the electricity generation process. For example, “a pulverized coal fired boiler typically produce 80-90% of the ash as fly ash”. (Santhanam, et al., 1979) “In cyclone fired boilers the fly ash fraction is usually somewhat less, 65-80% of the total ash created.” (Santhanam, et al., 1979) Generally, fly ash is made up of glassy hollow rounded particles that can range in a hundredth of a micron to one hundred microns in size. The hollow trait is what allows these particles to be lifted up and out of the boiler through the effluent gas stream. Bottom ash is prominently made up of coarser particles that are generally denser and can exceed a few millimeters in size. This drastically increased particle sizes along with a greater density keeps these particles within the boilers during combustion. (Carlson & Adriano, 1993) “Fly ash is a complex heterogeneous material consisting of both amorphous and crystalline phases.” (Carlson & Adriano, 1993) Ash pH can vary widely; this range can extend from as low as 4.5 all the way up to 12. The fly ash has been found to be more enriched in trace elements than the bottom ash, and interestingly the smaller fly ash particles tend to contain the highest concentrations of these trace elements. (Carlson & Adriano, 1993) despite this disparity in trace element concentration, bottom ash and fly ash are generally made up of the same compounds. (Santhanam, et al., 1979) “Regardless of the type of ash (either fly ash or bottom ash), more than 80% of the total weight of the ash is usually made up of silica, alumina, iron oxide, and lime.” (Santhanam, et al., 1979) This breakdown is a general observation that first nearly all ashes, any variation between these compositions is a factor of the mineralogy of the coal itself. On that note, any differences in the compositions of the fly and bottom ashes are dependent upon the degree of pulverization and the boiler system employed in the process. (Santhanam, et al., 1979)

FGD waste is the second substantial form of combustion waste we will be examining. Before we discuss the characteristics of FGD waste, answering what a FGD system is would likely be a beneficial place to start. FGD stand for Flue Gas Desulfurization. This is a crucial step in the process for treating the flue gas stream before it is discharged into the environment. A FGD system is a wet scrubber that is the last means of mechanical waste removal in the treatment of the flue gas effluent. This system is a wet scrubber that has additives included in the scrubber water that results in a removal process honed in to a more specific contaminant. When discussing FGD scrubbers, the specific contaminant these mechanical removers are designed for is sulfur oxides. The additives to the scrubber water, which will be discussed later, are what makes this system especially effective at collecting sulfur oxides as well as other heavy metals volatilized during the combustion process. (Castaldi, Behrens, & Hargrove Jr.) As we have discussed earlier, selenium and sulfur have very similar atomic radii as well as chemical properties. These similarities make it a common occurrence for selenium to substitute itself into compounds in place of sulfur. Naturally then, one would expect that this waste would contain a substantial amount of the selenium released from the coal ore from combustion. The selenium captured in this particular scrubber will be present in the water as the common selenate and selenite ions. The concentrations that this waste can achieve range from 0.5 up to 2 parts per million. This concentration however, is dependent upon the characteristics and mineralogy of the ore being utilized in the generation process. (Castaldi, Behrens, & Hargrove Jr.)

“The treatment process uses the ferrous ion to reduce the oxy-anions of selenium to elemental selenium and co precipitates the selenium with the resultant ferrous and ferric hydroxide solids. However, the chemical reduction and co-precipitation of selenate-selenium is

inhibited by the presence of certain reduced sulfur compounds in the FGD scrubber waters." (Castaldi, Behrens, & Hargrove Jr.)

FGD systems can be divided up into two categories of systems, either nonregenerable or regenerable. Nonregenerable systems produce waste that is only disposed of. Regenerable systems produce waste by products that can be utilized and sold such as sulfur or sulfuric acid. There are generally three types of FGD scrubbers: direct limestone scrubbing, direct lime scrubbing, and double alkali scrubbing. Most of the nonregenerable systems implemented in power plants today use the direct limestone and lime scrubbing approaches. In these systems limestone or lime are mixed into the FGD scrubber water until a slurry is formed. These additives make the scrubber water able to more effectively target sulfur oxides. Double alkali systems use a slurry of sodium salts with scrubber water to remove the sulfur compounds. This scrubber water is then introduced to lime outside of the scrubber to produce a dry waste product. (Santhanam, et al., 1979)

FGD waste is generally a slurry with ash being present to a varying degree. The amount of ash present depends on if the FGD scrubber is being used with the intent of capturing particulate or not. (Santhanam, et al., 1979) Generally the ash particulate that FGD scrubbers catch are on the finer end of the size spectrum considering these scrubbers are commonly the last step in the flue gas treatment process. The sizes of ash particles only range from a few microns in size up to approximately fifty microns. (Carlson & Adriano, 1993)the primary constituents of FGD waste are: calcium-sulfur salts, calcium sulfite, calcium sulfate, calcium carbonate, and any un-reacted lime or limestone. The degree of oxidation within the boiler system will determine the ration of calcium sulfate to calcium sulfite, while the quantity of un-reacted lime or limestone will depend upon the grading and properties of the materials used. This type of waste is where

the predominant amount of volatile trace elements found in the ore are captured after combustion. Some of the most important of these are arsenic, mercury, beryllium, chloride, fluoride, and especially selenium. As well as likely in the place of sulfur within compounds selenium can be released from the combustion process as elemental vapors which are then captured by the scrubber due to its' similarities to sulfur. These however are not as efficiently captured by the scrubber. (Santhanam, et al., 1979)

Now that we have discussed the different types of Selenium containing wastes produced by the generation of electricity and discussed briefly the characteristics of each, it is time to move our discussion to treatment methods. Even though the wastes discussed are removed at different stages of the generation process and have unique characteristics, these wastes are generally combined into one before being disposed of. A survey of 259 disposal sites showed that 91% of aquatic disposal sites receive mixed wastes, as does 75% of landfills. (Rowe, Hopkins, & Congdon, 2002) There are two common methods for disposing of coal waste and those are landfill dumping and aquatic surface impoundment.

Landfill disposal, since it is the most commonly used method, will be discussed first. In the recent decades regulations around aquatic surface impoundment have tightened which has made landfill disposal more desirable. Rather than risk having an issue with on-site disposal, plants can take their waste to the landfill and leave that responsibility to those overseeing the landfill. Considering liners for disposal areas are expensive, as are the means of measuring local water quality to assure there is no contamination, there is a large upfront cost that landfill disposal avoids. Despite this lack of upfront construction costs this method over time is the more expensive of the two considering any wet waste must be dried before it is taken to the landfill and then it must be trucked which can accumulate cost quickly. (Carlson & Adriano, 1993) Once

these waste arrive at the landfill they can either be mixed with soils or spread out as just ash and then compacted. (Santhanam, et al., 1979)

The next Ash disposal method we will discuss is the use of aquatic surface impoundments. In this method the wastes from the plant are combined and mixed into a slurry and then piped into a pond. Generally, there are multiple ponds in series and the water pumped in from the plant eventually flows through all of them. These ponds are established as a form of treatment and as the water progresses through the series more and more of the waste settles out. (Carlson & Adriano, 1993) Eventually the water is collected by weirs and can be handled in one of two ways. The water can at this point be released into surface waters or recycled and reused in the plant. (Santhanam, et al., 1979) Eventually the ponds fill with the ash and solid wastes from the plant. At this point the pond is either dredged and the process begins anew or the pond is decommissioned. If the decision is made to take the pond out of operation, it is allowed to dry and then covered with soil. At this point reclamation is also an option. (Carlson & Adriano, 1993)

These methods of disposal are generally well practiced and care is taken to try and prevent these waste from causing any harm to the environment, however no method is perfect. Accidents do happen and these waste, despite efforts to prevent them, do find their way into our environment. “Ash disposal in landfills and settling ponds can influence adjacent aquatic ecosystems directly, through inputs of ash basin effluent and surface runoff, and indirectly through seepage and groundwater contamination.” (Carlson & Adriano, 1993) There are a number of different ways that the release of effluent from disposals sites can impact waters: electrical conductivity, turbidity, temperature, pH, and elemental concentrations. one of the common elements released into the environment when these wastes make it past their disposal

barriers, is selenium. Accidental release through seepage or spillage is not the only way that selenium can be discharged from power production waste. As discussed previously, a surface impoundment pond can be allowed to dry and reclaimed once it fills with waste. As plants begin to grow on top of the former impoundment pond, they are exposed to and take up some of the selenium that is highly concentrated in the waste bellow. As the plants grow and continue to take in more selenium their own concentrations of the element increase. Then as animals eat these plants they acquire the selenium. It is through this pathway that the selenium in the waste can be introduced into the food chain and do harm to the surrounding organisms. (Carlson & Adriano, 1993)

2.6 Biological

The next mechanism by which Selenium that has been released into the environment is by biological processes. To begin this discussion, we must first examine the interactions of Selenium with those organisms that make up the base of the food web. Selenium in a very harmful and highly bio accumulated toxin when the concentrations an organism is exposed to becomes high enough. The base trophic level organisms of the food web that begin the process that leads to such bio-concentrations is a necessary place to begin. The primary organisms that make up the base of the food web and begin this process are plants, algae, and bacteria. These organisms absorb selenium species that have made their way into the water and incorporate them into their cellular structure making them available to subsequent organisms in the food web. This initial step in the food web also accounts for one of the largest steps in accumulation of the metalloid comparable only to phytoplankton and some other invertebrate species. These base organisms can accumulate selenium to concentrations in the range of six orders of magnitude greater than those of the waters from which the selenium is acquired. These organisms are also

capable of redistributing Selenium in organic forms when they die and decay, which diversifies the species of selenium in waters. These organisms can then also recycle the Selenium from other decaying organisms by uptake of the Selenium. This process returns Selenium to water bodies, therefore perpetuating the cycle. The species of selenium the decay process releases is generally organic selenides. The predominant anionic forms of selenium that these base organisms absorb are selenite and selenite, these species are then incorporated into the organisms structure by forming compounds that follow the same pathways as sulfur (Chapman, et al., 2010)

Higher order plants absorb selenium through their roots as they take up the water necessary for the biological processes they need to live. This route of selenium entry is highly influenced by the species present in the water, their concentrations, the redox conditions of the water, the water's pH, and the presence of any ions which might compete with selenium for absorption. As stated, selenium follows the same metabolic pathways in organisms. Higher order plants are no exception to this. Selenium acquired from waters are taken to shoots and leaves and subsequently distributed and utilized by the same pathways that process sulfur. Selenium compounds found in plants almost always have a sulfur analogue. Selenate distribution within plants mimics that of sulfate so therefore accumulation of this species can occur in areas in which sulfate is predominantly incorporated. These areas are: roots, seeds, leaves, and shoots. The age of the plant tissue can also play a role in the distribution of selenium species within the plant structure. It has been shown that older tissues tend to gather selenate, while younger tissues are prone to gathering organic forms. Younger tissues can even transform selenate into the organic forms they prefer before incorporating them into the plant structure. The accumulation of selenium by plants occurs when the rate of selenium absorption exceeds the rate of which the plants excrete selenium through pathways such as volatilization. As mentioned before, some

plant species have the ability to hyper accumulate selenium comparatively to other plant species. Non accumulators have been found to discriminate against selenium more efficiently and can better target the sulfur they intend to gain. On the other hand, species that can heavily accumulate selenium seem to prefer selenium over sulfur. The mechanisms by which this happens are still not understood. The absorption of selenate is an active transport process, meaning the plant must expend energy in order to acquire the selenate, which is carried against the concentration gradient by the same carriers responsible for the uptake of sulfate. Selenite however is brought into the plant by passive diffusion, which requires no energy input from the plant. The selenite uptake process can be inhibited by phosphates. Once inside the plant, the selenium is reduced from whatever species it entered as into selenide before being incorporated into amino acids. The two forms of amino acids selenium can take are selenocysteine and selenomethionine. (Chapman, et al., 2010) "The conversion of selenate to organo-Se compounds in plants is hypothesized to proceed through adenosine phosphoselenate, selenite, and reduced selenide, then to selenocysteine via synthase, from which selenomethionine is synthesized." (Chapman, et al., 2010) Once these two forms have been created they are incorporated into proteins. Selenomethionine is the most common of the two found in these food web base organisms. In the tissues of organisms, selenomethionine is the form that accumulates to higher concentrations. For this reason, this form is credited as the species that causes toxicity to organisms. (Chapman, et al., 2010)

Algae has unique characteristics when it comes to selenium which make it necessary to examine in separately from the general plant exploration. Where higher order plants show no real need for selenium in their everyday biological processes, that is not the case for algae. This category of organism needs selenium to maintain health. Algae absorbs selenium from the water

in which it lives through active means which require the expense of energy. Algae also have carriers that conduct the specific role of the acquisition of selenium. These active pathways allow for algae to accumulate immense concentrations of selenium even in very low ambient concentrations. These carriers that algae possess also allow these organisms to absorb a more diverse variety of selenium species that include both organic and inorganic selenium compounds. Although algae can absorb both inorganic and organic species, they show a preference to th organic selenmethionine when available. (Chapman, et al., 2010)

"Uptake of both selenate and selenomethionine shows saturation kinetics illustrating the involvement of specific transmembrane transport proteins. In contrast, uptake of selenite in freshwater green algae was found to be a linear function of ambient concentration, showing no evidence for carrier-mediated uptake." (Chapman, et al., 2010) +At the current state of our understanding, however, the specific identity of the carriers present in algae are yet to be known. It has been shown however that sulfate and phosphate can compete with selenium for incorporation into algae cells. Selenium can also find its way into algae cells by taking the place of sulfur just the same as in other plant species. Even though algae have means of actively taking in selenium even more can be incorporated through this pathway. Selenate is the primary species that gains access to algae cells in this way. This process is very heavily influenced by water chemistry and therefore the species of selenium present in ambient waters. Because algae utilizes carrier mediated uptake pathways, this absorption follows classic Michaelis Menten kinetics and does not show a linear relationship to the concentrations found in the waters in which the algae lives. This is especially true when the amount of selenium found in ambient waters saturates these carriers with the selenium they target. Because these selenium specific pathways exist for algae, the partitioning of selenium between the dissolved and particulate phases has no influence

on uptake by algae. Once absorbed, selenium is rapidly converted to organic selenium compound like those found in the higher order plants discussed previously. The fact that algae cells can regulate their uptake of selenium gives rise to a much greater ability to accumulate selenium within their structure. (Chapman, et al., 2010)

The final participant in the base of the food web that absorbs selenium from ambient waters is bacteria. Like algae, bacteria need selenium to perform their biologic processes necessary for life. Bacteria utilize selenium from the water generally in one of two ways. They can either uptake the selenium for use in respiratory reduction or by using it as an electron receptor in a similar respiration process. Some bacteria have demonstrated the ability to reduce selenium species. This is believed to be a sort of tolerance process. When Bacteria absorb selenate or selenite from waters they have the ability to reduce it down to either elemental selenium or selenides. A step by step process has been proposed that offers an explanation as to how bacteria go about this. The process is described as follows: seleniteis reduced to elemental selenium, then to selenide, then to methylselenide, and finally to dimethylselenide. These microbes can also absorb the major organic species of selenium from waters such as selenocysteine and selenomethionine. Bacteria have a very interesting potential for interaction with elemental selenium that is very unique. For the most part when elemental selenium is synthesized, it is highly unusable for organisms and almost always precipitates and settles into the sediment layer. Bacteria can reduce this elemental selenium into selenides and therefor bring it back into availability for utilization by other organisms. Bacteria can achieve some of the highest concentrations of accumulated selenium in the food chain, and seem to have a very high tolerance for the element. Unfortunately, this is one area of selenium in the food web is not well

researched. Bacteria's ability to so highly concentrate the metalloid wants its' importance and need for further research. (Chapman, et al., 2010)

2.7 Use of Selenium as an Essential Nutrient

Up to this point selenium has only been described as a harmful toxin to organisms within the environment. This is a very true statement. However, it must be noted that despite its toxicity, selenium is a necessary nutrient for organisms, besides higher order plants. Selenium is a key component of many proteins synthesized and utilized within the body known as selenoproteins. To date thirty of these proteins that require selenium are known and all are found throughout the worlds living creatures, additionally it is now scientific fact that twenty-five human genomes encode for these proteins. The discovery of selenoproteins began in 1973 when glutathione peroxidase in mammals and glycine reductase in bacteria. Glutathione peroxidase is a member of a larger protein family that acts as an antioxidant within living tissues. It was this discovered that initially proved the necessity of selenium within the body and identified a specific beneficial role that it plays. The selenium species selenocysteine, that we have examined quite extensively, is the twenty first natural amino acid. Proteins containing selenocysteine are found in all life regardless if it is vital for their survival or not. These proteins carry out vary necessary processes within an organism's body, all of which perform oxidoreductase functions. Even the formation of DNA is dependent upon selenium. When DNA is replicated, selenium acts as a catalytic site for thioredoxin reductases. Selenium can also activate or deactivate hormone produced in the thyroid, regulate how much selenocysteine is produced, or aid in the transportation of selenium throught the body. Some of the most notable roles selenium plays beneficially in the body are antioxidant properties, protein repair and immune system support. Slightly elevated levels of dietary selenium have even been shown to have anti-cancerous effects. (Chapman, et al., 2010)

These proteins can be classified according to three categories: “1) proteins into which Se is incorporated nonspecifically (mainly as selenomethionine), 2) specific selenium-binding proteins, 3)enzymes that incorporate selenocysteine into their active sites” (Chapman, et al., 2010). Additionally around 20 more selenoproteins have been identified, yet their functions remain largely a mystery. Selenium is a very beneficial nutrient to our diets, the problems arise when the concentrations within the body exceed the very low concentrations that are needed. In humans only around 0.6 micrograms or selenium per kilogram of weight are needed daily to maintain good health. Such a small requirement gives an idea on how little it would take to greatly exceed that dietary need and enter a thresh hold of harm. Naturally though, the figures for humans cannot be applied broadly, all species have their own tolerances and thresholds for harm. A good example would be algae and bacteria which show no real evidence of toxicity and even highly elevated levels. (Chapman, et al., 2010)

2.8 Within the Body

As stated previously, for non-food chain base organisms, selenium enters the body in appreciable amounts only through dietary ingestion. From species to species the efficiency by which selenium is absorbed and where it is ultimately partitioned is highly variable. Once selenium has entered the body, the process of homeostasis processes the selenium and differs excess to be excreted through exhalation and urine. Methylated selenium species are either excreted from the body or released through respiration. Urine is the primary exit pathway for selenium being released from the body, selenosugars are the species found in this pathway of release. Selenium that is ingested becomes incorporated in the body through the systematic reduction of the entering species, followed by incorporation into proteins and then subsequently distributed throughout the body. Once inside a cell, selenium is rapidly converted to organic

forms or poly peptides. Research indicates that Selenocysteine, selenate, and selenite have specific spots in proteins that they are only placed in when metabolized. This indicates that there are specific pathways that are intended to process these specific selenium species. This is not the case with selenomethionine, this species can find intself incorporated into proteins in no discernable structure. Such a lack in rigidity in the process of metabolizing this species shows that it has no designated pathway for processing within the body. Once processed, selenium is stored as either selenocysteine or selenomethionine within proteins sequestered within tissues. These forms are readily available for use by or harm to the organism. Once ingested, the route of transition selenium from the consumed food to within the cells of the body happens occurs through the cells of the intestines and kidneys. Studies have concluded that the absorption of selenium by these tissues can be efficient enough to gain in excess of eighty percent of the selenium stored within the food. In these tissues a b^o family of amino acids is responsible for the uptake of selenium. These carriers are highly efficient and can efficiently gather enough selenium to supply the body's needs even under lower selenium concentration diets. These carriers target selenium amino acids, yet do not show any attraction to any other selenium derivatives. (Chapman, et al., 2010)

2.9 Toxicity

The toxicity of selenium, as with most toxins, can be broken into two categories, they are acute and chronic toxicity. Selenium does have the ability to exert an acute toxicity on animals, and it has been observed before. This kind of effect is extremely rare because concentrations almost never get that high in a natural system. Many of the proteins that contain selenium are believed to be undiscovered, therefore the specific mechanisms that cause toxicity have yet to be known. However, what we do know is that selenium is almost always chronically toxic.

Selenium toxicity is nearly always manifested as reproductive impairment. A decrease in viability of offspring is one of the most difficulty identifiable results. Selenium toxicity is very well known to cause deformities in the off spring of organisms that have accumulated higher levels of selenium within their bodies. Egg laying organisms are the most prone to the ill effects of heightened selenium levels. This is due to the selenium being deposited in high concentrations in the egg yolks from the maternal tissues. The embryos then feed on this food source and suffer ill effects. Fish and birds are the classic examples and serve as the proverbial canary in the coal mine for a selenium contamination problem. If the offspring of contaminated creatures do make it to birth, the deformities they sustain make it impossible for them to survive. As this cycle continues entire communities are affected. As the birds and fish fail to produce viable offspring, their populations dwindle. Entire communities of species can be eradicated over the course of a few generations from the ill effects of selenium. Many cases have been seen where entire fish populations in water bodies have disappeared and thrown the entire ecosystems out of alignment which has drastic impacts on the other animals in the system. With one predator gone others may thrive or species that lose a food source suffer the same fate. The effect on entire populations is inherently more drastic for fish given their inability to escape from the waters that cause the problem. (Chapman, et al., 2010)

There are generally two schools of thought on how selenium causes these deformities. The first goes back to our understanding that the similarities between sulfur and selenium, both physically and chemically, cause selenium to be substituted for sulfur. This argument states that selenium likely takes the place of sulfur in structural and functional proteins. Since protein formation is dependent upon sulfur to sulfur linkages, the substitution of selenium in the place of a sulfur atom could result in a dysfunctional protein. An accumulation of these deformed

proteins could cause the birth defects and decreased hatchability of selenium laden offspring. (Chapman, et al., 2010)

The second way of thinking states that increased selenium can create oxidative stress on cells and cause ill effects. As previously discussed, selenium fosters the production of glutathione. This compound can then further react with selenium to synthesize selenopersulfides and thiyl radicals. These compounds then foster a superoxide ion that can potentially create great oxidative stress on cells, leading to deformities and issues hatching. (Chapman, et al., 2010)

Selenium can have disastrous effects on animals that consume too much of the element, But by far the most sensitive to its' effects are birds and fish. These two groups of animals are always the first to show any effects from environmental selenium contamination. These two groups are most impacted through reproductive impairment, since as previously stated the levels of selenium at which adult mortality is possible extremely rarely happen in the environment. In both groups selenium is passed on from the mother to her offspring through the incorporation of selenium into the food sources provided inside the egg for the embryo. Both groups exhibit teratogenic deformities, but birds often suffer from death before hatching. (Chapman, et al., 2010)

In fish, the component of the egg that houses the selenium contaminent is the phospholipooglycoprotein vitellogenin. This protein is made within the liver of the female and is then transported through the blood stream to the developing follicle within the ovary. Upon reaching the follicle it is morphed by enzymes into the main yolk proteins lipovitellin and phosvitin. Sulfur is a primary constituent of these proteins, so it is clear to see that selenium would be present in the place of sulfur atoms. This process occurs under the guidance of the hypothalamic-pituitary- gonadal- liver endocrine axis. The potential and timing for this cycle and

thereby the incorporation of selenium into the follicle is highly dependent on the species, specifically their frequency of spawning. To further describe the significance of this statement we must examine the two ends of the spectrum on spawning frequency. On one side we will consider fish that spawn once every year or any more infrequently, on the other end we will examine species that spawn multiple times, say three or more times, every year. If a species spawns on the infrequent end of the spectra, this species deposits selenium to its eggs that has been built up within its tissues over an extended period of time. The more frequently spawning fish however, incorporate selenium that has not had a chance to be stored within tissues for an extended period. In the first case, the selenium incorporated into the egg would have been ingested long before its development. Whereas in the second case, the diet of the fish very soon before the development of the egg would be the timeframe in which the selenium was ingested. Fish fry most always hatch when exposed to high levels of selenium in embryo. The manifestation of selenium contamination within this group of organisms is teratogenic deformities. Such deformities are generally not life threatening themselves, but make their ability to survive in the wild virtually impossible. There are three common deformities that are present in exposed fish. The first of which is a deformed head and mouth which makes feeding nearly impossible. The second is spinal deformity, which makes swimming much less efficient and therefore eliminates their ability to escape predators. The final common deformity is the presence of edema, which, similarly to the second deformity, is very restrictive in the young's movement. There are generally three methods employed when attempting to categorize teratogenic deformity presence in a fish population. The first method is to simply record the frequency of deformities present in fish fry populations. The second is to grade the severity of deformities on

a one to three scale of increasing severity. The final method is to collect the fry and conduct scientific measurements quantifying the extent of deformation. (Chapman, et al., 2010)

Unlike fish, In birds the primary storage of selenium within the embryo food supply in in the albumin instead of in the yolk. Because of this, bird chicks absorb the majority of the selenium before hatching and therefore are more susceptible to death before they make it to hatch. Some selenium is stored in the yolk as well, but the amount is greatly diminished from that in the albumin. Because the yolk sack is not fully utilized until after hatching, should the chick make it to hatch, it will then absorb that source of selenium and can suffer from impeded growth rates and acute mortality. Also unlike fish, birds do not pass on selenium that has been stored within tissues, instead they pass on selenium taken up immediately during ingestion. With this in mind, the concentrations of selenium imparted upon the developing chick are based from the diet of the mother immediately before and during the development of the embryo. The deformities suffered by baby birds are very similar to those found in fish fry. All of the deformities expressed in birds are not fatal themselves, but make survival impossible. Some of the common deformities observed in baby birds are a deformed beak, reduction or complete absence of eyes, and deformity of limbs. An especially interesting caveat in chick responses to selenium is that mortality without deformation happens at lower concentrations than chicks that hatch and exhibit deformities. (Chapman, et al., 2010)

2.10 Treatment

The next step in this exploration of selenium in the environment is to discuss some of the more popular treatment methods used to remove it from water. In this section we will explore a number of the more popular and classic treatment technologies. Before conclusions based on the research work within this paper can be presented, it is valuable to discuss what other options are

available. By doing so an informed decision can be made as to how effective the studied treatment in question is when compared to its utilized counterparts within the industry. In this section membrane filtration, ion exchange, and carbon filtration will be discussed, including how they work, and how effectively they operate for removing Selenium.

The first common technique for selenium removal that we will examine is membrane filtration. This method centers around a permeable or semi-permeable membrane. This membrane acts as a barrier through which water is forced through, leaving behind contaminants. The natural process of permeation by water is very slow, therefore energy must be imparted to the water to be treated forcing it through the membrane and accelerating the process. A pump is the means by which this energy is imparted on the water, whether it be used to pressurize the water to be treated or used to create a suction that draws the water across the membrane. This system allows water to pass through the membrane leaving behind any particulates, colloids, or soluble compounds. As the water passes through the membrane it is purified, this flow is called the permeate stream, leaving the contaminants behind on the initial side of the membrane. The undesirables are then removed from the system in what is called the reject stream. The amount of energy that must be applied to the system is highly dependent upon the physical and chemical characteristics of the wastewater stream. The energy applied is very dependent upon particle size and temperature. As the particle size increases, the particles more effectively clog up the pores in the membrane requiring greater force to push the wastewater stream through the membrane. The same dependence is seen in the temperature of the wastewater. As temperature increases the viscosity of the wastewater decreases, making it easier to pump across the membrane. As it follows, when the temperature of the wastewater decreases it becomes more viscous and therefore harder to pump across the membrane. A critical characteristic of the membrane itself

also plays a large role upon the pumping power required. As the pore size of the membrane decreases the forced used to push the water through inherently increases. The pH of the wastewater stream can also hinder the effectiveness of this system. If the pH becomes too strong on either end of the pH scale then the membrane will deteriorate, rendering the process less effective. (Sandy & DiSante, 2010)

A second form of pressure critical to the use of membrane filtration in the application of selenium removal is the osmotic pressure in the system. This pressure results from the concentration difference created across the membrane as the contaminants are not allowed to pass across the membrane with the water. As the concentration of pure water increases as it passes through the membrane and the concentration of contaminant builds on the initial side of the membrane, the natural process of osmosis creates a gradient that desires to drive the treated water back across the membrane and even out the concentrations. This creates a pressure that the system must exert additional for to overcome. This pressure is only large enough to be significant in membranes whose pore size puts them in the category of nanofiltration and reverse osmosis, which coincidentally are the only two categories that can effectively remove selenium. (Sandy & DiSante, 2010)

As previously discussed, the most common forms of selenium found in waters that would likely need remediation are selnite and selenate. These compounds both exhibit a size of approximately 2.4 angstrom. These very small size is the reason why only membranes with pore sizes small enough to be classified as nanofiltration and reverse osmosis can be used to remove them from water. This small pore size makes these filters very susceptible to clogging up during operation. Any appreciable size suspended particles within the wastewater stream will result in frequent clogging, the need for frequent membrane cleaning, and abrasion on the membrane that

will warrant more frequent membrane replacement. With this in mind pretreatment before a membrane filtration strategy is employed is a must. These small pored membranes are also susceptible to the very contaminates they are removing causing clogging problems. As the concentration of these removed compounds increase, they can easily approach or even reach their solubility point. If this is reached then the contaminants will precipitate out and cause clogging issues. (Sandy & DiSante, 2010)

Membranes that fall into the reverse osmosis category are the most widely used for this application and have shown an ability to effectively remove selenium to concentrations below the 5 milligrams per liter mark in the field. This method differs from other membrane filtration methods in the fact that these membranes are not permeable but only semi-permeable. A characteristic of these membranes, is that the required pumping power is much greater in these reverse osmosis systems than that of the other types of filtration that implement a permeable membrane. This type of membrane has pores that are so small it acts as if it is completely nonporous. This trait gives it the ability to remove contaminants as small as 0.0015 microns in size, these filters are even able to remove most dissolved salts. With these filters acting as nonporous membranes osmotic pressure is much more influential in this application. As the name implies, the action of the water flowing through the membrane is more like osmosis itself instead of a filter. (Sandy & DiSante, 2010)

Nanofiltration is the second category of membrane filter with the capability to remove selenium from solution. This system uses pores greater in size than that of reverse osmosis, which consequently leads to this method requiring only a third the pumping power that reverse osmosis requires. This method cannot remove salts and due to the pore size being closer to the size of the selenate and selenite ions, it is less effective at removing them. This larger pore size

however allows for less issue with clogging than that of reverse osmosis. As this method is utilized, the pores will become clogged which effectively decreases their size. As the pore size begins to decrease slightly the nanofilters ability to remove selenite and selenate increases. The biggest draw back to this system is that it has not been implemented in the field to a great enough extent to know how effective it is under real world conditions. (Sandy & DiSante, 2010)

This method of membrane filtration is an effective one in this application. Reverse osmosis removes an exceptional amount of selenium and nonofiltration is believed to also have promising ability. These systems are however expensive to operate. The need to large pumping power, the need to regulate pH and temperature, and the need for pretreatment are all very significant costs. This system however is not at all land dependent and can easily be removed after implementation is over. An important issue with this system is the state of the removed contaminant. This system removes and concentrates the harmful compounds it intends to remove, it does not make them any less harmful. If anything, it gives rise to a potentially more harmful entity considering the waste is just highly concentrated contaminant. The question then is how to safely dispose of what is removed. (Sandy & DiSante, 2010)

The second common selenium treatment method that we will examine is ion exchange. This system is widely implemented in drinking water treatment systems and shows an ability for the removal of selenium as well. The process of ion exchange remediation centers around what is called the ion exchange resin used in the system. This resin is a solid media that is often utilized in the granular form and organized in a filter bed configuration during use. These resins can be either artificially or naturally occurring compounds and their primary function is to foster an electrostatic interaction between them and the ions that are intended to be removed. These resins act like a magnet in a way, because these electrostatic interactions they create cause the ions in

the wastewater to stick to the resin and are thereby removed from the water. This interaction between contaminant and resin is a surface phenomenon between the two, therefore the capacity to which a resin can remove an ion is directly dependent upon the charge of both parties, the surface area of the resin, and the size of the ion itself. Pretreatment for this method is not absolutely necessary, but can easily become needed if certain conditions are met. Excessive suspended solids can clog the resin bed, so if the concentration of these becomes too large pretreatment will become necessary. The same can be said in regard to pH and temperature, Varying pH's will decrease the effectiveness of the system and along with increasing temperatures, can degrade the resin itself. (Sandy & DiSante, 2010)

This method has the potential to remove selenium effectively. Some real world applications have shown their ability to remove selenium below the EPA limit of 5 micrograms per liter. A large problem with this system is that its effectiveness is highly site specific, For it to work effectively the right resin must be acquired and the entire system fine tuned to the conditions at hand. With this method relying on electrostatic interactions for removal, this system can easily lose its' ability to remove selenium if the concentration of any other ion increases to the point that its competitive edge overwhelms selenium for resin surface sites. This process also shares the same issues with its removal stream that membrane filtration faces. This method concentrates an already harmful compound through its' removal process once again begging the question of what to do with it now. (Sandy & DiSante, 2010)

Filtration with activated carbon is one of the most common systems utilized in water treatment. This method is easily applicable and can serve as a means of complete treatment on its own or as a complimentary step paired with additional treatment options. Activated carbon filtration relies upon the surface interaction of adsorption between the activated carbon and any

contaminants present within the water stream. What makes activated carbon so prominent in this role is its' extensive surface area, all of this area along with its affinity for contaminant compounds makes activated carbon filtration an effective means for water purification. Activated carbon is implemented in either a granular or powdered form. Granular activated carbon is configured in a column filter bed arrangement through which water is passed, while the powdered state is added with the water to be treated into a completely stirred reactor and allowed to react with the contaminants present. Unfortunately, the typical activated carbon filtration system is ineffective at removing selenium species form water. (Sandy & DiSante, 2010)

Despite activated carbon's inability to remove selenium in its usual chemical form, researchers have worked to find a way to make this system effective and the future looks promising. A simple modification has been found to make activated carbon capable of adsorbing selenium, along with several other heavy metals, which also pays homage to a point previously discussed. As we have already examined iron has a considerable ability to adsorb selenium species from solution. Researchers have found that by subjecting activated carbon to an iron surface treatment, that the carbon became capable of adsorbing selenium. This treated carbon was observed to adsorb selenium effectively over a wide pH range that varied from 2 to 8. Once the pH exceeded this range the adsorption capability of the carbon significantly fell. The adsorption kinetics of this treated carbon was found to behave as a pseudo second order reaction. Kinetics were however slow. Complete equilibrium required nearly two days to achieve whereas approximately 90% removal required upwards of six hours of contact time. Experimentation showed that phosphate was very impactful on this process; a concentration of 5mmol/L rendered the carbon virtually useless for selenium. Interestingly enough, sulfate concentrations had no effect on the adsorption of selenium species. The capacity of this carbon was calculated to be in

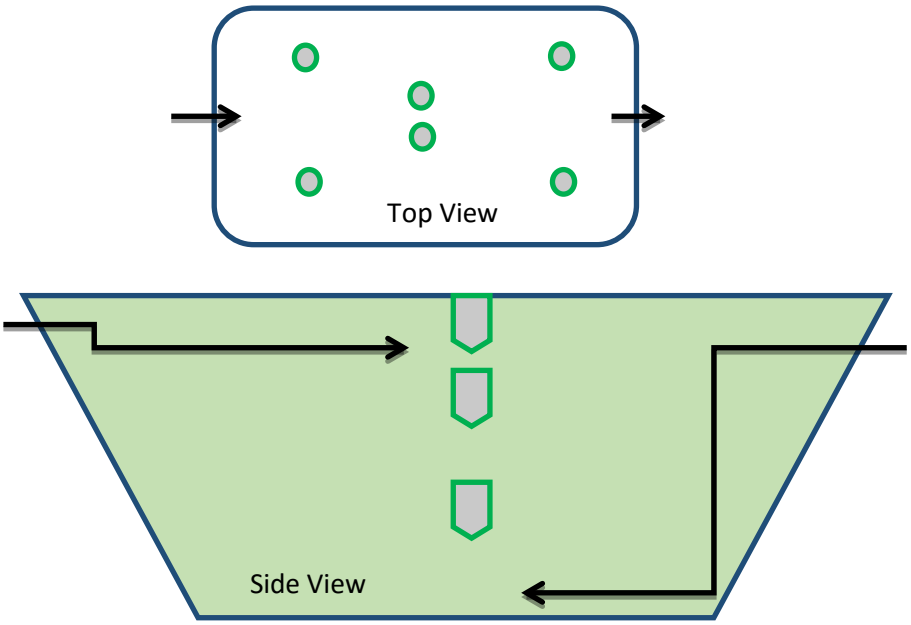
the range of 2.5-2.9 milligrams of selenium per gram of treated carbon. (Santos, Ungureanu, Boaventura, & Botelho, 2015)

Activated carbon in the traditional sense is of no use for this application of treatment, therefore there is no reason to argue its' pros and cons. On the other hand the potential for activated carbon to become useful is present thanks to possibilities such as the iron surface treatment. If this treatment could reach practicality this could be a highly useful source. Activated carbon is fairly inexpensive which is highly desirable from an economic standpoint, however without it being available the cost of iron treated carbon remains to be seen. For the time being activated carbon will remain on the sidelines in the fight against selenium contamination. However, the future may hold some use for it in the area yet.

Chapter 3 Methods

Samples were delivered from the power plant that implemented and maintained the treatment process being examined in this study. The company asked not to be identified in this document. These samples were taken in pairs from three strategic locations within the bioreactors. The system consists of three in ground basins configured to run in series. An image of one of these reactors can be seen below in Figure 1.Within each reactor three areas were sampled, one at the influent (IN), one in the middle of the reactor (MID), and one at the effluent (OUT). Within each of these areas two points were sampled. At each of these points three depths were sampled. A diagram of this can be seen below in Figure 1. Doing this resulted in six samples taken from each influent, middle, and effluent for each reactor. These six samples were then combined creating one inclusive sample for each area. These inclusive samples were then delivered to Auburn University for analysis. Therefore nine total samples were delivered for testing and they were: 2IN,2MID, 2OUT, 3IN, 3MID, 3OUT, 4IN, 4MID, 4OUT. These process was repeated four times and samples were delivered in May 2014, November 2014, May 2015, and November 2015.Due to this sampling procedure not being completed fully, the samples from May of 2014, and the data gained from this sampling, have not been included in the analysis presented thus forward

Figure 1 Image of Reactor and Sampling Points Location



Now that the layout of the process has been presented it is necessary to take a moment to discuss the effectiveness of this process. Along with the samples for analysis, readings of selenium concentrations at the influent point of the process and the effluents of each reactor were provided. The concentrations can be seen below in table 1. From this data it can be seen that the concentrations of selenium are drastically reduced through this process. With this evidence the ability of this process to treat selenium is evident.

Table 1 Process Selenium Concentrations

Selenium Concentrations				
	Influent Concentration ug/L	Reactor #2 Effluent ug/L	Reactor #3 Effluent ug/L	Reactor #4 Effluent ug/L
May 2014	2010	530	58	15
November 2014	2120	640	75	33
May 2015	1280	590	63	23
November 2015	2250	710	84	51

To begin the process of collecting data many agar plates containing different media to foster the growth of a specific subgroup of microorganisms to aid in the identification of what species of microorganisms were present within the process. The medias can be divided into ones that grow either anaerobic or aerobic microorganisms. The anaerobic medias consisted of: TG (thioglycolate liquid medium) to isolate the oxygen requirements of isolates, TGA (thioglycolate agar plates) for the isolation of facultative aerobes, TSA (trypticase soy agar plates) for the isolation of general anaerobic bacteria, SRA (sulfate reducer agar plates, medium based on DSMZ medium No. 63, modified) for isolation of sulfur reducing bacteria, MM (methanogenic Archaea liquid medium) for isolation of methanogenic bacteria, and AAM (autotrophic all-rounder liquid medium) for isolation of autotrophic bacteria. The aerobic medias consisted ofCZ (Czapek's Agar) for the isolation of fungi, ME (malt extract agar) for the isolation of yeast, CA

(chocolate agar) for the isolation of fastidious aerobic bacteria, KE (koenzyme enrichment, and NA (nutrient agar) for the isolation of non-fastidious aerobic bacteria.

Two kinds of samples were taken from the delivered samples from the reactors, one kind was for the growing of bacteria found within the water in the reactors and the other to sample the bacteria growing on the woodchips themselves. To sample the bacteria growing in the reactor water, samples of the reactor water were removed directly from the delivered buckets. The process for sampling the bacteria growing on the chips was not so straightforward. For this samplingwood chips were removed from their sample buckets and placed within glass bottles filled with a stock solution. These bottles were then sonicated to dislodge bacteria from the woodchips. Then sampling was done from the stock solution within the bottles which contained the woodchip bacteria. These two kinds of samples were taken and then subsequently diluted with the same stock solution. samples of these dilutions were then taken and used to inoculate plates representing all of the media described above. Once inoculated these plates were then incubated allowing the bacteria to grow and mature into colonies. Once this growth was achieved counts were taken of the colonies growing on the plates. Then a fragment of each different appearing colony on each plate was taken and introduced onto test tube slants containing nutrient agar for further growth and more long term storage of each colony sample.

Initially the isolates are partially identified by which media they are harvested from, considering the medias will only grow a specific class of microorganisms. For further characterization an API test system will be implemented. This system uses the metabolic capabilities of the microorganisms to identify them specifically. In this test pieces of the colonies that have been transferred to the agar tubes are once again mixed in stock solution and then samples of this mixture are added to the testing strips. The testing strips contain 20 bubbles that

house a specific media. By looking at the color change seen in the bubbles and referencing Bergey's Manual of Determinative Microbiology, the species of the microorganism can be identified. For the anaerobic microorganisms API 20E and 20NE strips will be used while only API 20A strips will be used for the aerobic organisms.

Testing of the ability of the isolates to reduce selenium will be conducted by inoculating them into a basal salts media. This media will be sterile and will contain a species of selenium. The media agar plates, upon inoculation will be incubated for a period of roughly four weeks. Once this period of growth is achieved the plates will be examined for an increase in turbidity and the presence of a red precipitate will indicate the ability for selenium reduction. The determination of specific nutrient requirements of the selenium reducing microorganisms will be determined by repeating the selenium reduction test with altered basal salts medias. The medias will have their concentration of essential nutrients altered to determine their impact on the rate at which the isolates can reduce selenium. The selenium reduction test will again be repeated, but at varying temperatures to determine the optimal temperature for selenium reduction.

Chapter 4 Materials

Stock Solution

TG (thioglycolate liquid medium)

- Peptone from casein (Becton Dickinson [BD], N……. 15g
- Yeast extract (BD)…… 5.0g
- d-(+)- glucose …… 5.5g
- NaCl…….. 2.5g
- Sodium acetate…….……. 3.0g
- Cysteine-HCl……..…… 0.5g
- Sodium thioglycolate…… 0.5g
- Sodium resazurin…….……. 0.001g
- Gas phase N2
- pH 7.1

TGA (thioglycolate agar plates)

- (TG medium plus agar 15g)
- TS (trypticase soy liquid medium) (TS broth [BD])…….. 30.0g
- Sodium resazurin……. 0.001g
- Sodium thioglycolate…… 0.5g
- Cysteine-HCl…… 1.0g

- Gas phase N2 [80%] and CO2[20%]

TSA (trypticase soy agar plates)

- TS medium plus agar……..……. 15g

SRA (sulfate reducer agar plates, medium based on DSMZ medium no. 63, modified)

- KH2PO4…….. 0.47g
- NH4Cl…….. 1.0g
- CaCl2*2 H2O……..…….. 0.1g
- Yeast extract (BD)…… 1.0g
- Na2SO4…….……. 1.0g
- MgSO4*7 H2O…….. 2.0g
- (40%) (wt/vol) 1-(+)-lactate…… 2.5ml
- FeSO4*7 H2O……. 0.004g
- Agar……. 10.0g
- Sodium resazurin…… 0.001 g
- Ascorbic acid…… 0.2g
- Sodium thioglycolate…… 0.2g
- pH7.0

MM (methanogenic archaea liquid media)

- NH4Cl……..…….. 0.5g
- KH2PO4…….. 0.4g

- MgCl2*6 H2O…… 0.15g
- CaCl2*2 H2O……. 0.05g
- Trace element solution [10X]…….……. 1ml
- Vitamin solution [10X]……. 1ml
- Sodium resazurin……..……. 0.001g
- Na2S…….……. 0.5g
- Cysteine-HCl……..…….. 0.5g
- H2 [80%] and CO2 [20%]
- BM (basal medium) (NH4Cl)…….…… 0.5g
- KH2PO4…….. 0.4g
- MgCl2*6 H2O……. 0.15g
- CaCl2*2 H2O…….. 0.05g
- NaHCO3……. 1.0g

AAM (autotrophic all-rounder liquid medium)

- KH2PO4……..…….. 0.4g
- CaCl2*2 H2O…….. 1.56g
- MgCl2*6 H2O……. 0.15g
- NaHCO3……. 1.5g
- Fe2O3*9 H2O……. 0.25g
- NaNO3……. 0.5g
- Na2S2O3*5 H2O…….…….. 1.56g
- Trace element solution [10X]……. 1ml

- Vitamin solution [10x]……. 1ml
- Sodium resazurin……. 0.001g
- Na2S……. 0.5g
- Gas phase N2[80%] and CO2 [20%]

CZ (Czapek's Agar)

- Sucrose…….. 30g
- NaNO3……..…… 3.0g
- KH2PO4…….. 1.0g
- MgSO4*7H2O…… 0.50g
- KCl……..…….. 0.5g
- FeSO4*7H2O…….. 0.01g
- Agar……..…….. 20.0g
- Distilled water…… 1L

ME (Malt Extract Agar)

- Malt Extract……...……. 10g
- Peptone……. 4.0g
- Agar……... 18g
- Distilled water…… 1L

CA (chocolate agar)

- Proteose Peptone…….. 15.0g
- Sodium Chloride…….. 5.0g

- Dipotassium Phosphate……..……. 4.0g
- Monopotassium Phosphate…… 1.0g
- Corn Starch…….. 1.0g
- Hemogloin, Bovine……. 10g
- KoEnzyme enrichment agar…… 10ml

KE (KoEnzyme Enrichment)

- Dextrose……...…… 10.0g
- L-Cysteine, HCl…….…….. 2.59g
- L-Glutamine…… 1.01g
- L-Cystine…… 0.11g
- NAD…….. 25mg
- Cocarboxylase……. 10.0mg
- Guanine Hydrochloride…….. 3.0mg
- Ferric Nitrate…….. 2.0mg
- P-Aminobenzoic Acid…… 1.3mg
- Vitamin B12……. 1.0mg
- Thiamine…… 0.3mg

NA (Nutrient Agar)

- Peptone……. 0.5%
- Beef extract/yeast extract…… 0.3%
- Agar…….. 1.5%
- NaCl…….. 0.5%

- Distilled water…… 1L

Basal Salts Media

- Ammonium sulfate…… 0.3g
- Calcium chloride dehydrate…… 0.2g
- Magnesium sulfate……..…… 0.07g
- Sodium chloride…… 5.58g
- Potassium phosphate…….. 0.1g
- Boric acid…….. 0.6mg
- Cobaltous sulfate……..…….. 0.11mg
- Cupric sulfate…… 0.08mg
- Manganous chloride…… 0.63mg
- Zinc chloride……. 0.22mg
- Carbon source (glucose, xylose, and lactate)…….. 2.0g
- pH adjusted to 7.5 with 1 normal NaOH

Chapter 5 Results and Discussion

In this study a biological treatment process implemented for the removal by reduction of selenium from power plant wastewater streams, generated by the burning of coal for electricity generation was examined. This process consists of three reactors numbered two three and four. These reactors are pond style reactors and operate in series. The ponds are filled with wood chips that were roughly one inch in size and were made from hardwood tree species. These wood chips provide the microorganisms within the reactors with a surface for colonization and a source of organic carbon. Each reactor was sampled in three locations, at the influent, the middle of the reactor, and at the reactor effluent. These samples were then delivered to the Department of Civil Engineering at Auburn University for testing and analysis. The defined objectives of this study, which the following results presented are intended to support, are to provide identification of the selenium reducing bacteria present within the process and to provide suggestions for the potential optimization of the process.

Once the samples provided were diluted, plated, and allowed to grow upon the various types of agar plates, the visible colonies of microorganisms were counted and the counts were recorded. The array of agars were selected to obtain counts of various bacterial types, including: anaerobic heterotrophs, sulfur reducing bacteria, methanogens, selenium reducing bacteria, aerobic heterotrophs, yeast, mold and fastidious aerobes. This process was done in triplicate for all nine sampling locations, for each set of delivered samples. Both liquid and wood chip samples were analyzed for the numbers of bacteria. The counts were then adjusted according to their dilution

and location so that all counts taken could be compared on an even field. The samplings taken from the liquid media (aqueous phase bacteria) in the delivered samples were multiplied by ten raised to the power of their dilution. This put all counts regardless of the dilution they were conducted at into standard units of number of colonies per liter of media. The samples taken from the surface of the wood chips (wood chip bacteria) were also multiplied by ten raised to the powerof their dilution, however because of their extraction from a mass of chips this left those counts in units of colonies per gram of wood chip. All work done to gain count data from the surface of the wood chips was done under the guidelines of having used five hundred and fifty grams of wood chips per liter of stock solution. By using this as a conversion factor and multiplying all wood chip counts by five hundred and fifty it was possible to get all counts in the units of colonies per liter and thus allow for comparison on an even field.

Individual colonies, that demonstrated the ability to reduce selenium, were obtained and transferred to agar slants for identification using ACI test strips. After being prepared the ACI test strips were incubated for a period of two days. After this period the test strips were evaluated, using keys found in Bergey's Manual to identify species of microorganisms present within the system capable of reducing the selenium species found in the wastewater stream. Upon classification, ten species of selenium reducers identified within the process. Some samples however, failed to be identified. This is not unexpected, since little research has been conducted to determine the diversity of species capable of selenium reduction. The ten species identified successfully were: *Bacillus subtillus, Microbacterium aborescense, Enterobacter, Psuedomonas stutzari, desulfomusa, desulfomicrobacterium, Desulfovibrio desulfricans, Desulfobacterium, Geovibrio, and Shewanella putrifaciens.*

Bacillus subtillus are rod shaped cells that are gram positive. This species can thrive under aerobic or facultative aerobic conditions and is capable of withstanding a wide range of pH, temperature, and salinity conditions. Similarly *Microbacterium aborescense* has the same rod like structure and is gram positive. This species is able to survive robustly under aerobic conditions, but can also survive under anaerobic conditions. This species thrives best at an approximate temperature of thirty degrees Celsius. *Enterobacter*, unlike the two species already discussed , is a gram negative microorganism. This species needs facultative aerobic conditions to live. This species can use either respiration or fermentation to metabolize its' food. Anywhere in a temperature range of twenty five to around forty degrees Celsius, this species can thrive. *Pseudomonas stutzari* is another gram negative species. This species, while facultative in nature, prefers anaerobic conditions and uses denitrification to metabolize substrate. All of these facultative organisms are capable of surviving in aerobic and anoxic conditions. However, selenium reduction can only occur in the absence of oxygen

Several species of sulfate reducing bacteria, capable of reducing selenium by a gratuitous pathway were also identified. The shape of *Desulfomusa* and *Desulfomicrobium* differs from the previous species discussed in that its' rod shape is curved. These species are sulfate reducers, and would likely contribute to selenium reduction. These two prefer a pH range from approximately six and a half to seven and a half and temperature range from thirty to forty degrees Celsius. *Desulfovibrio desulfricans* presents itself in spiral and vibrioid shapes. This species is a strictly anaerobic species and metabolizes sulfur compounds for energy. This microorganism shares the same preferred pH and temperature range and the two discussed before it. *Desulfobacterium* can also present a vibrioid shape in addition to oval or rod shapes. This species is anaerobic and commonly reduces sulfate species. It prefers similar ranges in pH as the other desulfo strains, but

it prefers a temperature range of twenty to thirty degrees Celsius. *Geovibrio* is an obligate anaerobic species that was identified in this study. This species uses sulfur compounds to reduce acetate and performs best at a temperature of approximately thirty five degrees Celsius. Another obligate anaerobe identified was *Shewanella putrifaciens*. This species is gram negative and has been observed to reduce metals. Both Geovibrio and Shewanella putrifaciens can reduce selenate directly, by using it as an electron acceptor for anaerobic respiration.

Delivery of samples taken from the reactor itself happened four times over the course of this research. Due to the first set being sampled from the reactor incorrectly from the reactor only the latter three rounds of delivered samples will be examined in this research. The three utilized sets of delivered samples will be delineated and examined by the month and year in which they were delivered. These three sampling sets are: November 2014, May 2015, and November 2015.

November 2014

In order to understand how the profile of microorganisms changed along the process the average number of bacteria yielded from each of the agars used were plotted in spatial sequence from the beginning of the process to the end. All of this data was not shown in one graph, the data was divided up between bacterial counts obtained from the wood chips and from the liquid media, and then further divided between anaerobic and aerobic species. The graphical forms of this data can be seen below in figures 2-5. The data used to produce these figures is located in Appendix A.

The first area of interest is the behavior of the aerobic population of microorganisms along the process. Although the process by which the desired reduction and thus removal of Selenium occurs under only anaerobic conditions, the trend shown by the aerobic populations

provide us insight into how the dissolved oxygen within the system is depleted and maintained at an essentially zero across the process. When the populations of aerobic organisms are at a high we can conclude that the dissolved oxygen is also present at that point in the process. In these locations, the aerobic population consume organic substrate and reduce the oxygen levels. This conclusion can also be applied to when aerobic populations are at a low and say that the dissolved oxygen is low or absent. This is insight into the change in the dissolved oxygen is important because the dissolved oxygen content can have an impact on the redox conditions within the reactor and a negative impact on selenium reduction.. If we examine the aerobic population behavior over the process for both in the liquid media and on the surface of the wood chips, the similarity in the behavior is very clear. In both graphs the populations start out at a high at the beginning of the first reactor and proceed to drop across this reactor. This is likely because as the wastewater enters the first reactor it no longer is allowed the extra oxygen introduced by any aeration efforts. So as the aerobes utilize what dissolved oxygen they enter the reactor with, the amount of dissolved oxygen falls because the natural diffusion of oxygen cannot keep up with the metabolic processes of the microorganisms. Once the populations reach a low when they arrive at the beginning of the middle reactor, the populations hold constant across this reactor. Finally in the last reactor the aerobic populations begin to rebound. This is because the substrate that the anaerobes are utilizing should be coming to its lowest concentration across this reactor. This insight into the process can be seen in the aerobic populations plots produced from all delivered set of examples. Two additional trends can also been seen in this data. The number of bacteria present on the wood chips exceeds that found in the liquid media. It can also be seen that the behavior of the aerobic populations on the wood chips and in the media follow very similar trends across the process. Due to the relevance of the

anaerobic populations exceeding that of the aerobic populations and their role in Selenium Reduction we will now focus all of our attention on the anaerobes within the process and the data acquired relative to them.

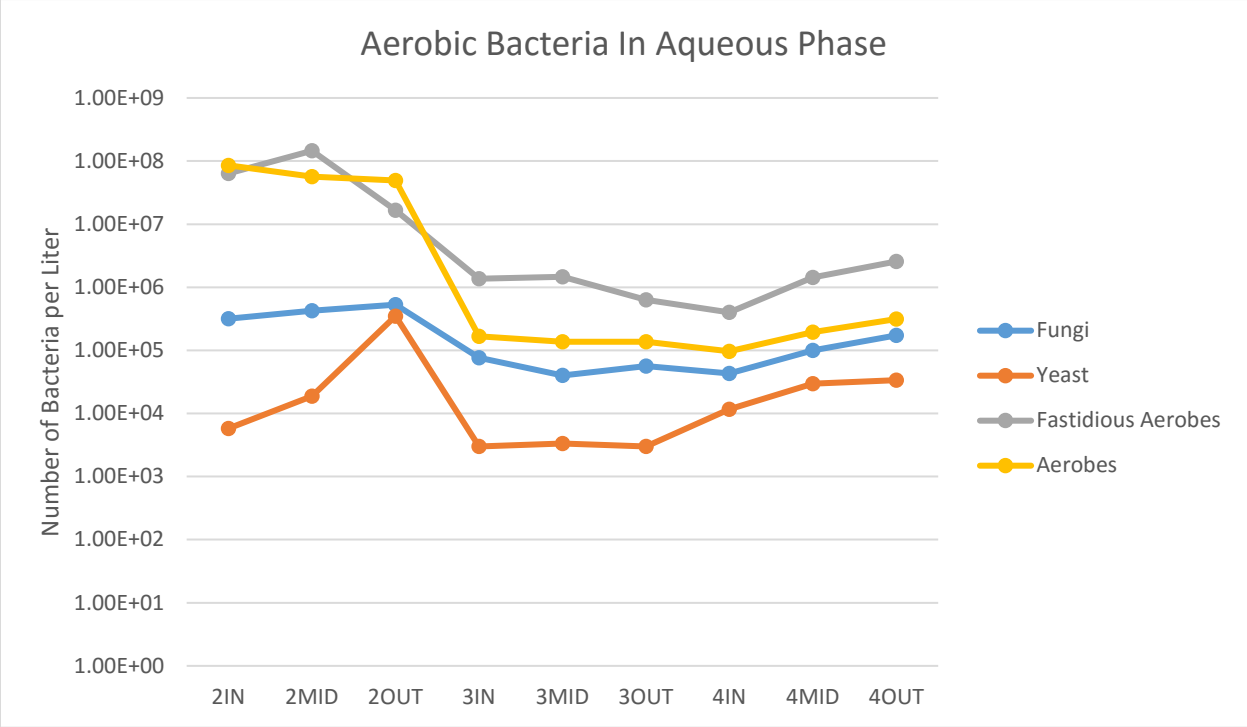


Figure 2 Aerobic Bacteria In Media 11/14

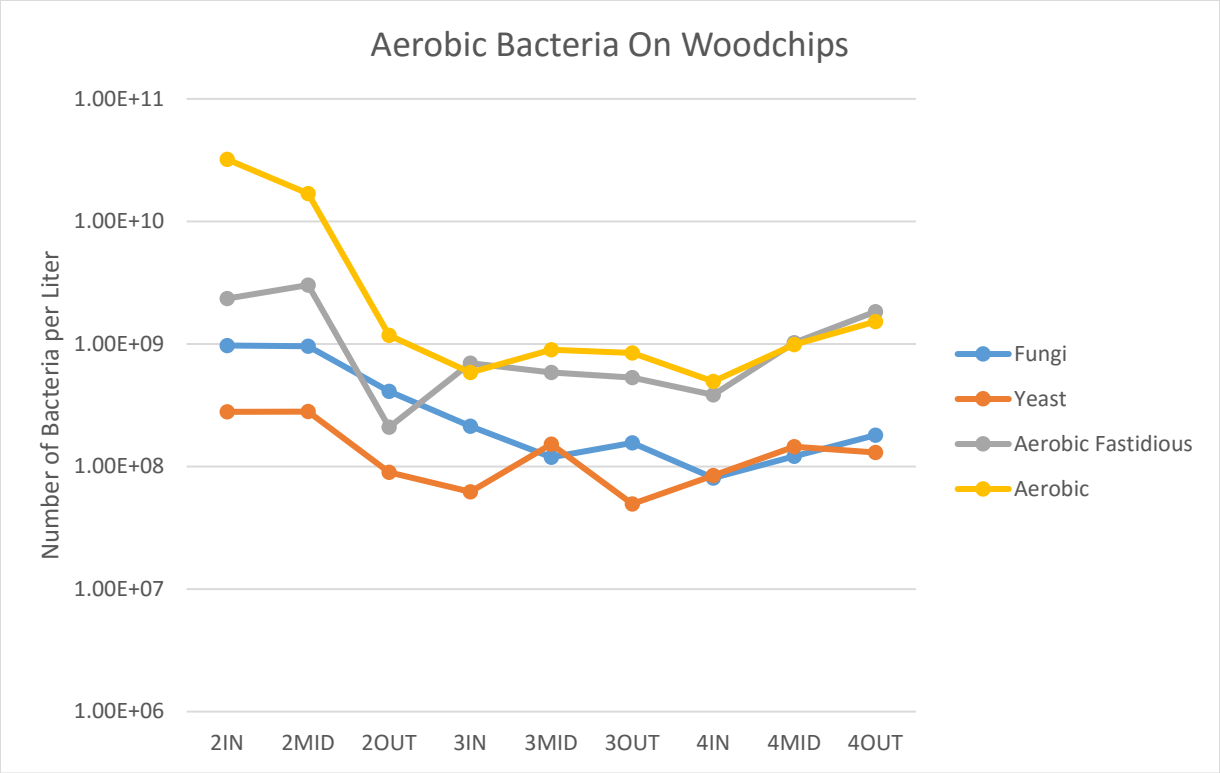


Figure 3 Aerobic Bacteria on Woodchips 11/14

Shown in Figure 4 is the plot of various types of anaerobic bacteria across the process in the aqueous phase, Figure 5 on the other hand, shows the same types of anaerobes that are present as a biofilm on the surface of the wood chips. The first observation made was that the number of bacteria on the wood chips exceeds the number within the liquid media by orders of magnitude for each type of anaerobic bacteria. With this in mind it can be concluded that the biofilm on the surface of the wood chips is much more influential over the gratuitous and bacterial mediated Selenium reduction. This conclusion is supported by the trend shown that the number of sulfur and selenium reducers, which are the group responsible for the desired reduction of Selenium in the process, are both greater for the surface of the wood chips that for suspended in the water.

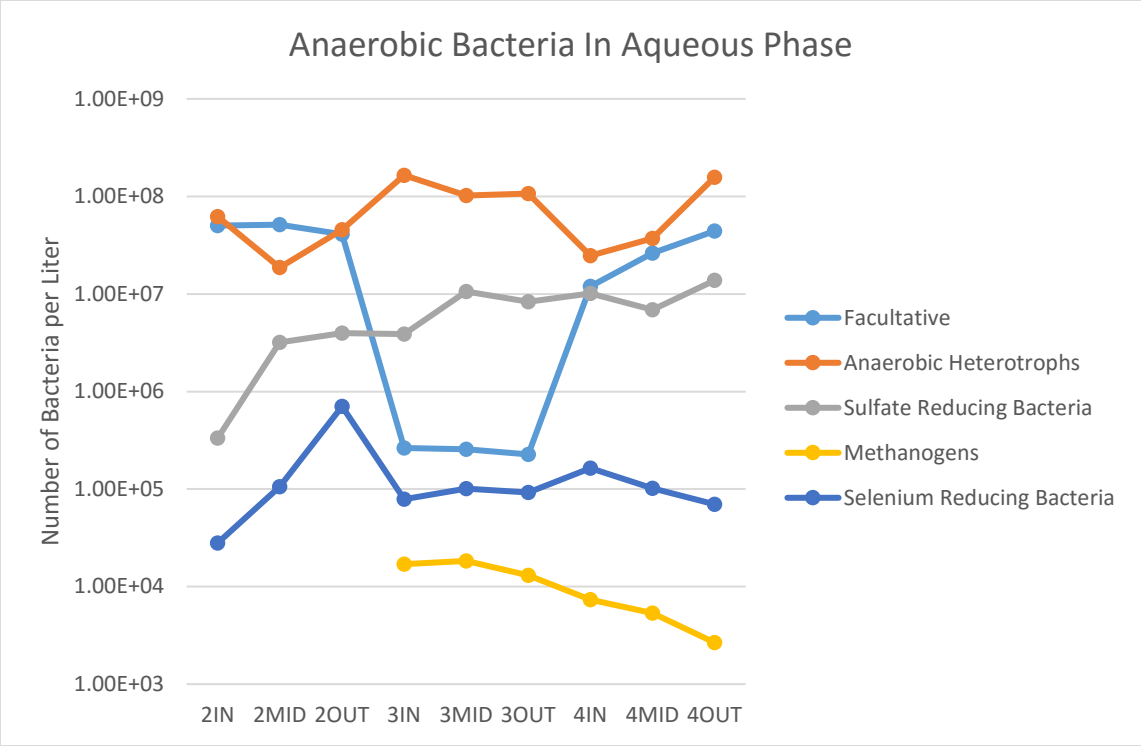


Figure 4 Anaerobic Bacteria in Media 11/14

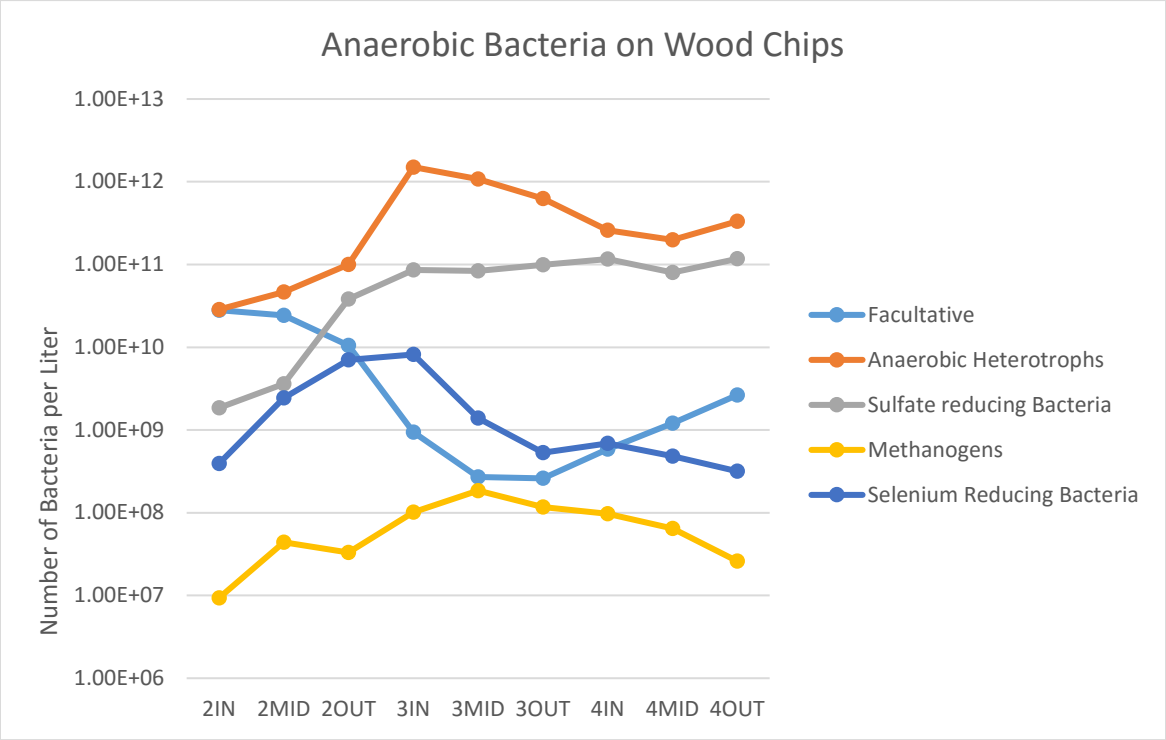


Figure 5 Anaerobic Bacteria on Woodchips 11/14

The anaerobic selenium reducing populations on the wood chips are close to 10^9/liter for both the sulfur reducers and direct selenium reducers at the beginning of cell 2. While this number seems high, there are not enough selenium reducers to achieve a significant degree of selenium reduction at this point. This is probably due to the presence of mildly aerobic conditions at this sample point. By the mid-point of Cell 2, the numbers of sulfur reducers and selenium reducers have increased by half of an order of magnitude, indicating more favorable growth conditions exist, and that selenium reduction would be several times higher at this location than at the front of the cell. By the exit of Cell 2, the sulfur reducers have increased by an additional order of magnitude, and are now present at almost 10^{11}/L, which represents a large potential for selenium reduction via the gratuitous pathway. At the end of Cell 2, the selenium reducing population has increased to 10^{10}/L, which means that a large degree of direct selenium reduction would also be possible. However, the sulfur reducers are present at ten times higher numbers, and gratuitous selenium reduction is likely to be the predominant mechanism. The sulfur reducing bacteria were present at near 10^{11}/L across Cell three. This indicates that these bacteria are present in numbers that would allow large amounts of gratuitous selenium reduction to occur in this part of the system. The direct selenium reducers declined in number across cell 3. This is likely due to the depletion of selenate, which serves as their electron acceptor for anaerobic methabolism. This indicates that direct selenium reduction is likely to be less important in this part of the system. The numbers of sulfur reducers remained relatively stable across Cell 4, while the direct selenium reducers continued to slowly decline.

The identification data of Selenium reducing species is presented in table 2. The species highlighted in yellow are species of Sulfur reducers. Although the Sulfur reducers can carry out

their metabolic processes under either aerobic or anaerobic conditions, they reduce Selenium under anaerobic conditions only. These species are vital to this investigation due to their ability to gratuitously reduce selenium under anaerobic conditions as previously discussed. The four species identified that are listed above the highlighted region are species of selenium reducers that are facultative microorganisms. This means that these species can conduct their metabolic processes under either aerobic or anaerobic conditions. The two species listed below the highlighted region are strictly anaerobic species. The selenium reducing species reduce the element through direct reduction whereas the sulfur reducers perform the same task through indirect reduction. All species identified are documented Selenium reducers and can therefore all be concluded to be partially responsible for the desired treatment. This fact is further supported by considering they were isolated on a media that demonstrates their ability to reduce Selenium. It can be seen that the distribution of species changed from more facultative at the beginning of the process to more anaerobic in the middle of the process. There is clearly a diverse group of species that are capable of caring out the desired Selenium reduction within the system. This diversity means there is a diverse set of mechanisms present that can carry out the desired process. By having this, the system is more robust and can carry out the treatment process under varying conditions. All of the bacteria present are common in the environment, this means that should this process be recreated elsewhere there would likely be no need for special seeding procedures to prepare the reactors.

Table 2 Species Identification 11/14

	Bio 2 Inlet	Bio2 Middle	Bio 2 Out	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	9	8	5	2	2	3	2	6	7
Microbacterium arborescense	5	4	1	0	0	0	0	0	1
Enterobacter sp.	7	3	3	0	0	0	0	0	0
Psuedomonas stutzari	9	6	4	1	2	1	1	2	3
Desulfomusa spp.	0	2	3	6	8	5	2	3	1
Desulfomicrobium (norvegicum?)	0	1	4	7	9	7	3	1	1
Desulfovibrio desulfricans	2	3	7	8	11	14	8	6	3
Desulfobacterium spp.	3	4	11	14	16	17	11	8	5
Geovibrio spp.	0	3	2	2	1	0	6	7	9
Shewanella putrifaciens	3	5	3	4	0	0	6	8	7
Unidentified Se Reducers	12	11	7	6	1	3	11	9	13
Total Selenium Reducers Tested	50	50	50	50	50	50	50	50	50

May 2015

Seen below in Figures 6-7 are the plotted average number of anaerobic bacteria for each sampling location for both the aqueous phase and the wood chips' biofilm respectively. It can clearly be seen that the number of bacteria on the wood chips exceeds the number of bacteria in the liquid media by orders of magnitude. This paired with the observation that the sect of microorganisms within the system responsible for the desired reduction, Sulfur and Selenium reducers, are both in greater numbers on the wood chips suggest that the surface of the chips is when most of the gratuitous and bacterial mediated reduction occurs. These observations suggest that the process is a biofilm type removal process.

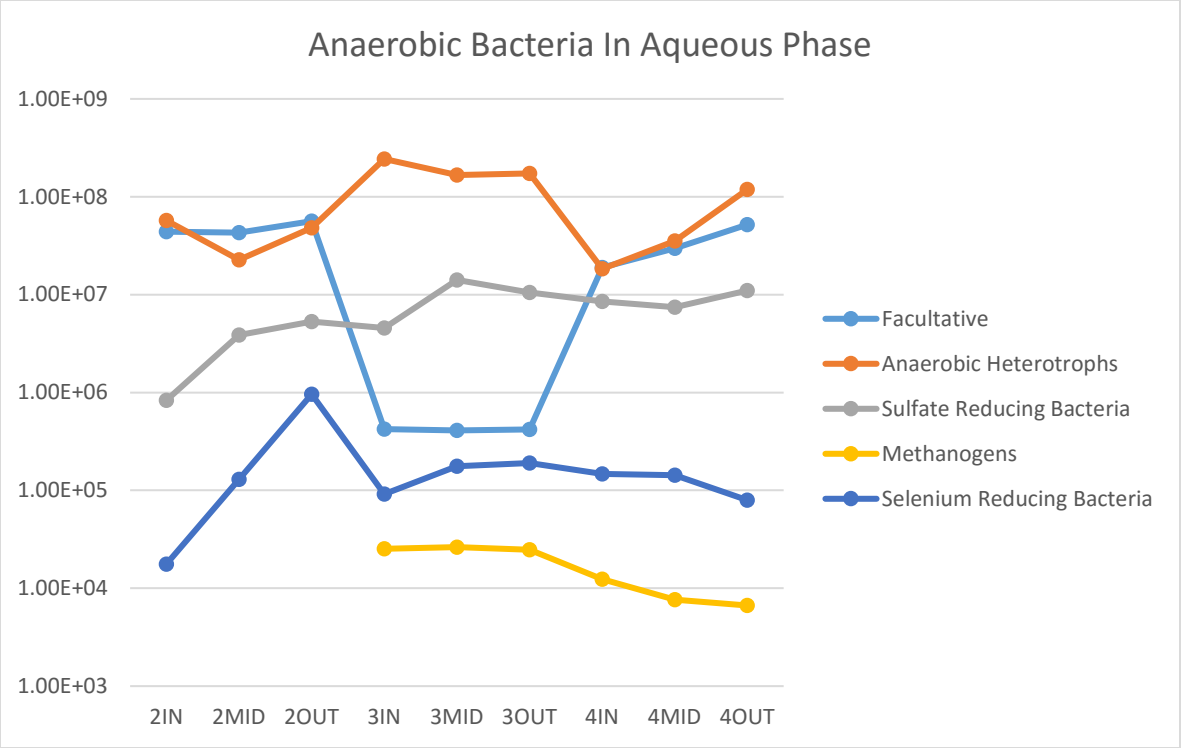


Figure 6 Anaerobic Bacteria in Media 5/15

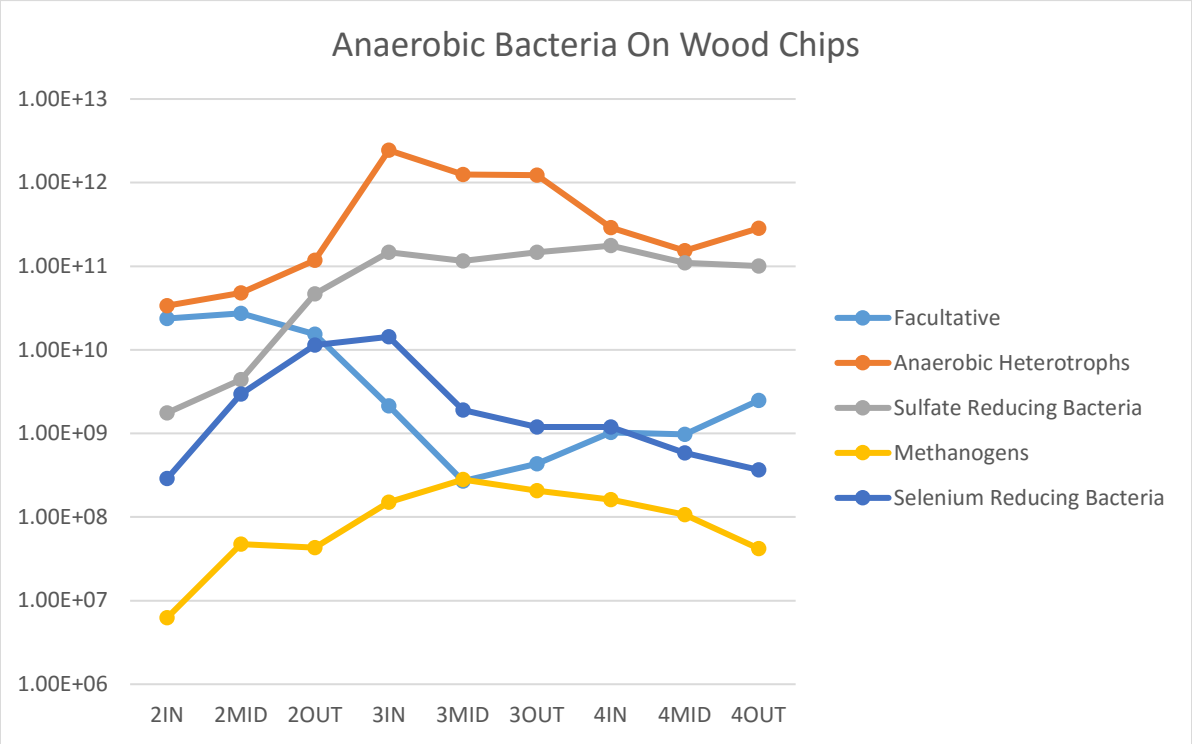


Figure 7 Anaerobic Bacteria on Woodchips 5/15

At the beginning of the process the populations for both anaerobic selenium and sulfur reducers are close to 10^9/ Liter. Although this number can give the impression that there are enough selenium reducers to achieve a high level of selenium reduction, this is not the case. This observation is likely due to the lack of anaerobic conditions at this point in the system, however at the midpoint of the first reactor the increase in the populations of these microorganisms suggest that is changing. By the end of the second reactor in the series the population of sulfur reducers is up to around 10^{11}/Liter while the selenium reducer population is around 10^{10}/ Liter. Although there are many selenium reducers at this point, the significantly higher number of sulfur reducers means that the mechanism by which they reduce selenium is the one of prominence at this point. Across the third reactor in the series the number of sulfur reducers stayed constant at around 10^{11}/Liter, while the number of direct selenium reducers fell. This is likely due to a decline in the selenium concentration across this reactor.

The species identification data is presented below in table 3. The species highlighted in yellow are Sulfur reducing species of bacteria that were identified in this study. Although aptly named, under anaerobic conditions these species can gratuitously reduce selenium through an indirect reduction process. The four species listed above this highlighted region are facultative species of bacteria that reduce selenium through a bacterial mediated direct reduction process. These species are classified as facultative because they can continue to carry out their metabolic process in either aerobic or anaerobic conditions. The two species listed below the highlighted region are species of anaerobic bacteria that also are also classified as Selenium reducers and also perform a bacterial mediated process to reduce the selenium. One can see from the species distribution that the predominant form of species in the process changed from more facultative to more anaerobic at the beginning of the middle reactor. All of these species are proven Selenium

reducers because all were isolated on a media that validated their ability to do so. It can clearly be seen that there is a diverse group of species present capable of performing the desired reduction of Selenium. With this diversity of species there is also a variety of different mechanisms by which Selenium is reduced. This means that the desire process that results in the treatment of the wastewater stream can continue under a number of different conditions. It also makes the process very robust. All of the species identified in this study are common in nature, meaning that duplication of this process would be easily done.

Table 3 Species Identification 5/15

	Bio 2 Inlet	Bio 2 Middle	Bio 2 Outlet	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	11	9	4	1	2	2	1	4	6
Microbacterium arborescense	9	5	1	0	0	0	0	0	1
Enterobacter sp.	5	2	3	0	0	0	0	0	1
Psuedomonas stutzari	7	5	4	1	2	0	3	3	2
Desulfomusa spp.	0	2	4	5	8	4	1	2	1
Desulfomicrobium (norvegicum?)	0	0	1	8	9	8	2	3	1
Desulfovibrio desulfricans	1	6	9	15	11	16	9	5	2
Desulfobacterium spp.	2	3	14	14	16	19	16	9	4
geovibrio spp.	0	1	1	1	1	0	4	6	8
Shewanella putrifaciens	6	4	4	2	0	0	5	9	9
Unidentified Se Reducers	9	13	5	3	1	2	9	9	15
Total Selenium Reducers Tested	50	50	50	50	50	51	50	50	50

November 2015

Presented below in figures 8-9 are the populations of anaerobic bacteria from the liquid media within the process and from the wood chips within the reactor. What can immediately be seen from these two displays of data is the rather large gap between the number of bacteria in the media and on the surface of the wood chips. By a margin of orders of magnitude, the number of bacteria on the wood chips far exceeds that of in the liquid media for ever sect of microorganisms. This observation leads to a very important conclusion that most of the Selenium

being reduced is being done so on the surface of the wood chips. This means the bacteria at the surface of the wood chips play the largest role in gratuitous and bacterial mediated reduction.

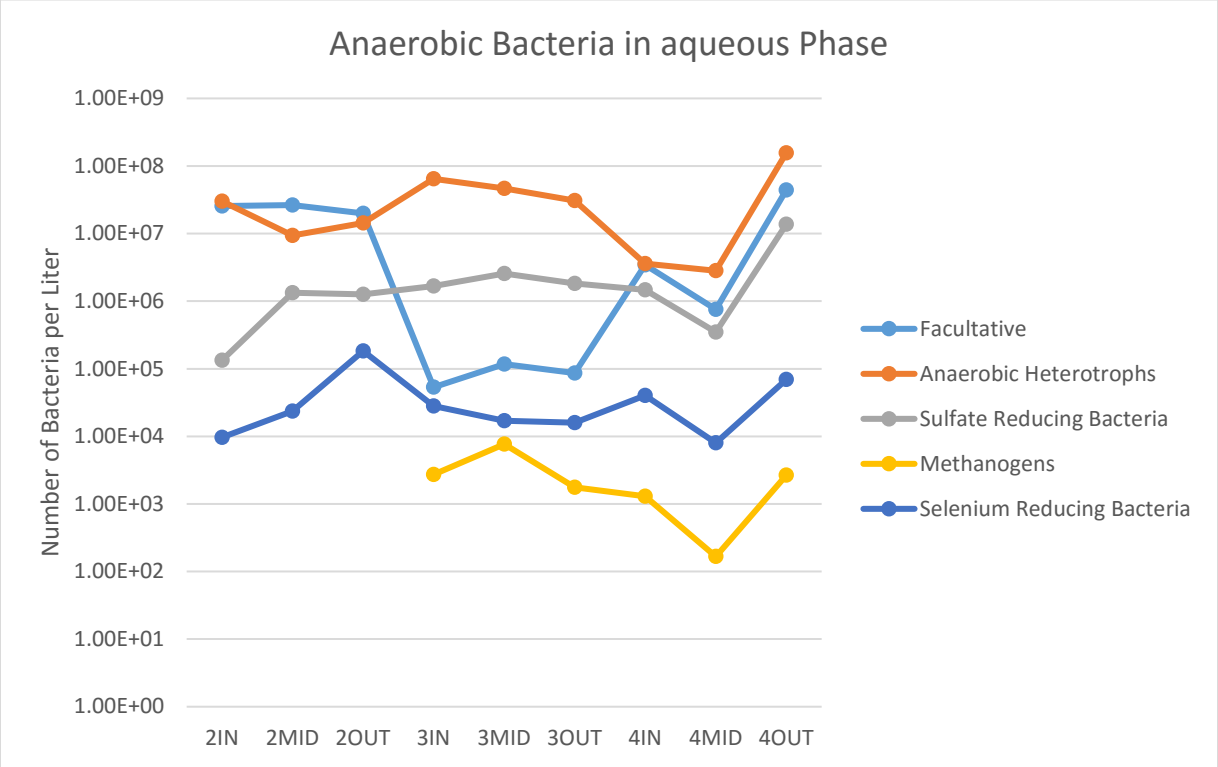


Figure 8 Anaerobic Bacteria in Media 11/15

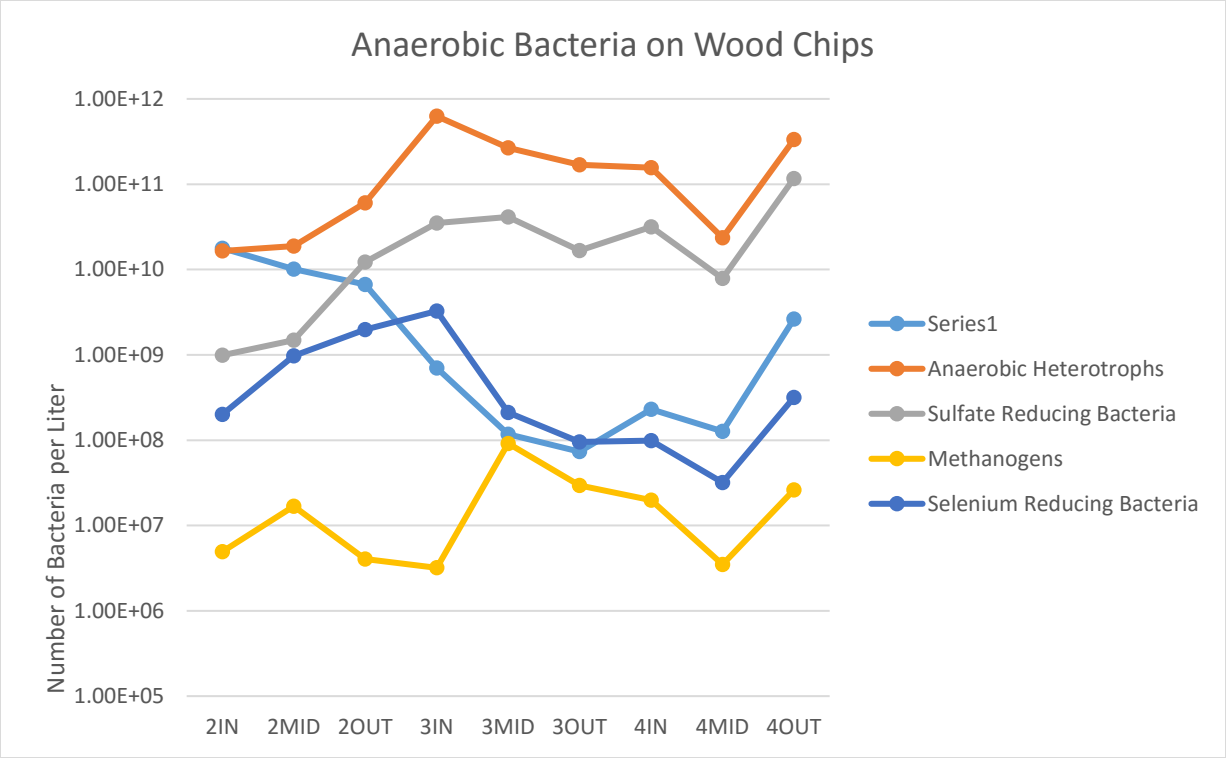


Figure 9 Anaerobic Bacteria on Woodchips 11/15

Upon examination of the populations, it could be seen that the population for anaerobic selenium reducers and sulfur reducers on the wood chips was around 10^9/Liter at the starting point of the process. This relatively low number of bacteria is likely a manifestation of the likely still aerobic conditions at this point in the process. However, across the first reactor in the series, the number of these bacteria continued on a rising trend, indicating the development of anaerobic conditions. As the population advances to the middle reactor the number of sulfur reducers hold constant at almost 10^{11}, while the number of selenium reducers begins to fall off. A likely cause for this is a decreasing amount of dissolved selenium, therefore leaving the selenium specific reducers without their preferred substrate.

Presented below in table 4 is the data pertaining to the identification of species capable of reducing selenium. The species highlighted in yellow are Sulfur reducing bacteria. These

bacteria reduce selenium indirectly through a gratuitous reduction process. Although these species can survive under either aerobic or anaerobic conditions, they can only reduce Selenium under anaerobic conditions. The four species listed above the highlighted ones are all facultative selenium reducing species of microorganisms. These species can also survive in both aerobic an anaerobic conditions, hence the facultative classification, however they can reduce Selenium under both of these conditions. The two species listed below the four highlighted ones are Selenium reducers that are strictly anaerobes. All species identified were isolated using a media that proved their ability to reduce selenium. The anaerobic species as well as the facultative species of Selenium reducers do so under direct bacterial mediated processes. The sulfur reducers however, do so by an indirect gratuitous process. As it can be seen from the presented data there is a wide variety of species present within the process capable of reducing Selenium. This means that there is a myriad of different mechanisms by which Selenium can be reduced. Because of this diversity this process is capable of carrying out its' desire function under a range of different conditions. It can also be seen that the population of microorganisms changed from predominantly facultative at the beginning of the process to anaerobic at the midpoint of the process. All species identified are common species easily found in nature. The implications of this are that replication of this process would be relatively easy.

Table 4 Species Identification 11/15

	Bio 2 Inlet	Bio 2 Middle	Bio 2 Outlet	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	5	6	6	1	1	2	1	4	6
Microbacterium arborescense	4	2	1	0	0	0	0	0	1
Enterobacter sp.	6	2	2	0	0	0	0	0	0
Psuedomonas stutzari	4	4	1	0	0	1	0	1	3
Desulfomusa spp.	0	1	1	4	3	4	1	2	0
Desulfomicrobium (norvegicum?)	0	0	2	3	7	5	3	1	0
Desulfovibrio desulfricans	3	1	4	4	8	8	5	4	2
Desulfobacterium spp.	2	2	6	8	9	10	8	3	3
geovibrio spp.	0	2	1	2	1	0	3	4	6
Shewanella putrifaciens	1	3	2	3	0	0	2	5	4
Unidentified Se Reducers	5	7	4	5	1	0	7	6	6
Total Selenium Reducers Tested	30	30	30	30	30	30	30	30	31

Upon examination of the bacterial counts data it became evident that more bacteria growing on the surface of the wood chips than in the liquid media became a consistent observation. To determine if there was any merit to this observation a series of students' t tests were performed to determine if the difference between the counts of bacterial growth from the wood chips and from the liquid were statistically significant. This examination was performed on counts obtained from plates utilizing thee agars in particular. The agars in question are Tryptocase Soy agar, Sulfate reducing agar, and the Selenium reducing agar. The bacterial counts for each coinciding sampling location within the reactors from all three sets of delivered samples data was combined to strengthen the statistics performed. For example the bacterial counts from the 2 Influent sampling location from the liquid media stemming from delivered dates of November 2014, May 2015, and November 2015 were combined into one series of data. The same was done for the bacterial counts obtained from the surface of the wood chips for the 2 influent sampling locations, combining all similar counts from the November 2014, May 2015,

and November 2016 data sets. This method of data merging was performed for all reactor sampling locations. After doing this the counts from samples taken from the media were compared to the counts from the surface of the wood chips. This was done for each sampling location across the process. This method of analysis was performed in the same way for all of the agars being examined by the t test. To determine if the difference in the counts was significant a confidence interval was calculated. The calculated confidence interval was obtained under the criteria necessary to satisfy the requirements to be ninety five percent confidence interval. If the confidence interval does not contain zero then it can be said that the differences in the means is statistically significant. If however the confidence interval should contain zero then it cannot be said that the difference in the means is statistically significant. A confidence interval highlighted in green denotes a confidence interval that does not contain zero and can therefore show that the difference in means is significant. A confidence interval highlighted in red denotes a confidence interval that does contain zero and therefore shows that the difference in means is not statistically significant. The results of these tests have implications as to the kind of treatment process the reactors examined operate as. If the results prove that more bacteria grow on the surface of the wood chips than in the media and that the treatment process is an anaerobic one, the results suggest that more selenium reduction is occurring at the surface of the chips rather than in media.

The first type of bacteria examined by these series of Students' t tests were those growing on Tryptocase Soy Agar. This agar is able to promote the isolated growth of anaerobic heterotrophs. Because the treatment process under examination operates under anaerobic conditions, this agar is especially well suited to provide insight about the diverse population of microorganisms within the reactor that contribute to establishing anaerobic redox conditions. The results of the Students' t test performed on the counts for this agar can be seen below in table 5.

The results show that the observation that the surface of the wood chips produced more bacterial counts than the liquid media was significant at all sampling locations within the process. These results tell us that the population of anaerobes is in fact greater on the surface of the wood chips than the population of anaerobes within the liquid media.

Table 5 Students' t test on Tryptocase Soy Agar

liquid		2in	2in	2mid	2mid	2eff	2eff	3in	3in	3mid	3mid	3eff	3eff	4in	4in	4mid	4mid	4eff	4eff
solid	november 2014	9.9E+07	1.9E+09	1.4E+07	9.1E+07	2.3E+07	1.6E+08	1.7E+08	2.7E+09	9.3E+07	1.9E+09	7.8E+07	1.4E+09	2.1E+07	3.9E+08	5.1E+06	3.1E+08	1.5E+08	5.7E+08
		8.7E+07	1.9E+09	1.9E+07	6.7E+07	4.3E+07	1.3E+08	1.8E+08	3.4E+09	8.7E+07	1.9E+09	1.5E+08	1.1E+09	2.9E+07	4.8E+08	4.5E+07	3.4E+08	1.9E+08	6.9E+08
		1.2E+08	2.1E+09	2.3E+07	8.8E+07	7.1E+07	2.6E+08	1.5E+08	2.1E+09	1.2E+08	2.1E+09	8.9E+07	9.7E+08	2.5E+07	5.4E+08	6.1E+07	4.3E+08	1.3E+08	5.6E+08
	may 2015	6.5E+07	5.9E+07	1.8E+07	8.9E+07	3.1E+07	1.7E+08	2.8E+08	4.8E+09	1.3E+08	2.1E+09	1.2E+08	2.2E+09	1.7E+07	4.9E+08	5.8E+06	2.7E+08	1.1E+08	5.2E+08
		5.5E+07	6.7E+07	2.3E+07	7.8E+07	5.2E+07	1.8E+08	2.4E+08	4.5E+09	1.8E+08	2.5E+09	1.9E+08	1.9E+09	1.9E+07	5.5E+08	5.1E+07	2.5E+08	1.4E+08	5.4E+08
		5.2E+07	5.8E+07	2.7E+07	9.4E+07	6.2E+07	2.9E+08	2.1E+08	4.0E+09	1.9E+08	2.2E+09	2.1E+08	2.6E+09	1.9E+07	5.4E+08	4.9E+07	3.2E+08	1.1E+08	4.9E+08
		2.4E+07	2.3E+07	8.0E+06	4.1E+07	1.1E+07	1.2E+08	5.5E+07	1.3E+09	4.3E+07	4.6E+08	2.4E+07	1.9E+08	3.5E+06	7.3E+08	4.5E+06	5.8E+07	1.5E+08	5.7E+08
	november 2015	3.2E+07	4.4E+07	9.0E+06	2.4E+07	8.0E+06	9.0E+07	6.7E+07	1.1E+09	3.5E+07	2.1E+08	4.3E+07	2.8E+08	4.2E+06	8.7E+07	3.2E+06	2.8E+07	1.9E+08	6.9E+08
		3.4E+07	2.3E+07	1.1E+07	3.8E+07	2.4E+07	1.2E+08	7.2E+07	1.0E+09	6.1E+07	7.9E+08	2.5E+07	4.5E+08	3.1E+06	3.4E+07	7.6E+05	4.3E+07	1.3E+08	5.6E+08
		2in	2in	2mid	2mid	2eff	2eff	3in	3in	3mid	3mid	3eff	3eff	4in	4in	4mid	4mid	4eff	4eff
	average	6.32E+07	6.85E+08	1.69E+07	6.86E+07	3.61E+07	1.68E+08	1.58E+08	2.77E+09	1.05E+08	1.57E+09	1.04E+08	1.22E+09	1.55E+07	4.27E+08	2.50E+07	2.28E+08	1.44E+08	5.77E+08
	standard deviation	3.31E+07	9.60E+08	6.77E+06	2.76E+07	2.22E+07	6.70E+07	8.00E+07	1.48E+09	5.56E+07	8.47E+08	6.95E+07	8.63E+08	9.55E+06	2.27E+08	2.55E+07	1.47E+08	2.78E+07	6.93E+07
	n	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00
	SEM	1.10E+07	3.20E+08	2.26E+06	9.19E+06	7.41E+06	2.23E+07	2.67E+07	4.93E+08	1.85E+07	2.82E+08	2.32E+07	2.88E+08	3.18E+06	7.55E+07	8.49E+06	4.91E+07	3.27E+06	2.31E+07
	SE		3.20E+08		3.46E+06		2.35E+07		4.34E+08		2.83E+08		2.83E+08		7.56E+07		4.99E+07		2.49E+07
	delta		-6.22E+08		-5.17E+07		-1.32E+08		-2.61E+09		-1.47E+09		-1.12E+09		-4.11E+08		-2.03E+08		-4.32E+08
	t 95		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00
	t*SE		7.25E+08		2.14E+07		5.33E+07		1.12E+09		6.40E+08		6.53E+08		1.7E+08		1.13E+08		5.63E+07
	CI high		1.03E+08		-3.03E+07		-7.87E+07		-1.49E+09		-8.26E+08		-4.68E+08		-2.40E+08		-8.98E+07		-3.76E+08
	CI Low		-1.35E+09		-7.31E+07		-1.85E+08		-3.73E+09		-2.1E+09		-1.77E+09		-5.82E+08		-3.15E+08		-4.89E+08

The second type of bacteria subjected to this examination were those growing on the Sulfate reducing agar. This agar is able to promote the isolated growth of bacteria that are classified as sulfur reducers. Sulfur reducers are important to this study because they perform a very special kind of Selenium reduction. These bacteria reduce selenium through an indirect gratuitous Selenium reduction process. These bacteria release electrons into solution which creates a reducing environment that results in the reduction of Selenium. The results from the t tests performed on the bacterial counts from sulfur reducing agar plates can be seen below in table 6. As we can see the observation that the number of bacterial counts from the surface of the wood chips were higher than those from the liquid media was indeed statistically significant. In fact, it was significant at all sampling locations within the process. These results tell us that more treatment of selenium due to the efforts of sulfur reducing bacteria is occurring on the surface of the wood chips than within the surrounding liquid media.

Table 6 Students' t Test on Sulfur Reducers

liquid		2in	2in	2mid	2mid	2eff	2eff	3in	3in	3mid	3mid	3eff	3eff	4in	4in	4mid	4mid	4eff	4eff
solid	november 2014	3.0E+05	3.2E+06	2.1E+06	5.9E+06	3.8E+06	5.3E+07	3.2E+08	1.4E+08	8.7E+06	1.1E+08	7.6E+06	1.8E+08	8.9E+06	2.0E+08	7.6E+06	1.5E+08	1.5E+07	2.3E+08
		3.0E+05	4.1E+06	3.4E+06	7.4E+06	4.7E+06	6.7E+07	3.9E+08	1.7E+08	9.8E+06	1.6E+08	8.4E+06	1.8E+08	9.3E+06	2.2E+08	6.8E+06	1.5E+08	1.3E+07	2.0E+08
		4.0E+05	2.8E+06	4.1E+06	6.3E+06	3.4E+06	8.9E+07	4.5E+08	1.5E+08	1.3E+07	1.9E+08	8.9E+06	1.8E+08	1.2E+07	2.1E+08	6.3E+06	1.3E+08	1.3E+07	2.1E+08
	may 2015	8.0E+05	3.0E+06	2.6E+06	6.8E+06	4.6E+06	9.8E+07	4.1E+08	2.6E+08	1.1E+07	2.3E+08	9.5E+06	1.8E+08	7.8E+06	2.3E+08	6.8E+06	1.8E+08	1.1E+07	1.8E+08
		1.1E+06	3.2E+06	4.3E+06	8.3E+06	6.1E+06	7.4E+07	4.7E+08	2.3E+08	1.4E+07	2.1E+08	1.1E+07	2.3E+08	8.5E+06	3.9E+08	7.5E+06	2.3E+08	1.2E+07	1.7E+08
		6.0E+05	3.4E+06	4.7E+06	8.2E+06	5.2E+06	8.2E+07	4.9E+08	3.1E+08	1.8E+07	1.9E+08	1.1E+07	2.8E+08	9.3E+06	3.4E+08	8.1E+06	1.9E+08	1.0E+07	2.0E+08
	november 2015	1.0E+05	2.3E+06	1.1E+06	2.4E+06	1.3E+06	1.4E+07	1.3E+08	6.9E+07	3.2E+06	6.1E+07	2.1E+06	2.9E+07	2.1E+06	3.9E+07	3.8E+05	1.3E+07	1.5E+07	2.3E+08
		2.0E+05	1.9E+06	1.3E+06	2.6E+06	1.5E+06	2.1E+07	1.6E+08	5.4E+07	2.4E+06	8.9E+07	1.5E+06	2.8E+07	1.4E+06	5.8E+07	2.4E+05	2.1E+07	1.3E+07	2.0E+08
		1.0E+05	1.2E+06	1.6E+06	3.1E+06	1.0E+06	3.2E+07	2.1E+08	6.8E+07	2.1E+06	7.5E+07	1.9E+06	3.4E+07	9.0E+05	7.6E+07	4.3E+05	9.0E+06	1.3E+07	2.1E+08
		2in	2in	2mid	2mid	2eff	2eff	3in	3in	3mid	3mid	3eff	3eff	4in	4in	4mid	4mid	4eff	4eff
	average	4.33E+05	2.79E+06	2.80E+06	5.73E+06	3.51E+06	5.89E+07	3.37E+08	1.62E+08	9.09E+06	1.46E+08	6.88E+06	1.59E+08	6.70E+06	1.96E+08	4.91E+06	1.20E+08	1.28E+07	2.03E+08
	standard deviation	3.39E+05	8.68E+05	1.37E+06	2.46E+06	1.85E+06	3.06E+07	1.38E+08	9.07E+07	5.57E+06	6.26E+07	3.94E+06	1.05E+08	4.11E+06	1.22E+08	3.46E+06	8.38E+07	1.78E+06	1.93E+07
	n	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00
	SEM	1.13E+05	2.89E+05	4.57E+05	8.19E+05	6.18E+05	1.02E+07	4.60E+05	3.02E+07	1.86E+06	2.09E+07	1.31E+06	3.49E+07	1.37E+06	4.06E+07	1.15E+06	2.79E+07	5.94E+05	6.43E+06
	SE		3.11E+05		9.37E+05		1.02E+07		3.02E+07		2.10E+07		3.50E+07		4.06E+07		2.80E+07		6.46E+06
	delta		-2.36E+06		-2.93E+06		-5.54E+07		-1.53E+08		-1.37E+08		-1.52E+08		-1.89E+08		-1.15E+08		-1.90E+08
	t 95		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00
	t*SE		7.03E+05		2.12E+06		2.31E+07		6.84E+07		4.74E+07		7.91E+07		9.19E+07		6.33E+07		1.46E+07
	CI high		-1.65E+06		-8.13E+05		-3.23E+07		-9.05E+07		-8.92E+07		-7.30E+07		-9.75E+07		-5.16E+07		-1.75E+08
	CI Low		-3.06E+06		-5.05E+06		-7.85E+07		-2.27E+08		-1.84E+08		-2.31E+08		-2.81E+08		-1.78E+08		-2.05E+08

The final type of bacteria examined using a series of Students' t tests were those growing on the Selenium reducing agar. This particular agar is able to promote the specific growth of bacteria with the ability to directly and intentionally reduce selenium. Keeping in mind that the reduction of Selenium, which causes the precipitation of selenium, and therefore removes Selenium from the water being treated, these are the most desirable bacteria we want to know about. The results from the Students; t tests for the bacterial counts data grown on Selenium reducing agar can be seen below in table 7. As the analysis shows, the observation that more bacterial counts were recorded from agar plates inoculated with samples from the surface of the wood chips rather than from the liquid media is statistically significant. It also shows that this difference in statistically significant throughout the entirety of the process. This data tells us that more of the selenium reduction that can be accredited to selenium reducing bacteria occurs at the surface of the wood chips rather than in the liquid media.

Table 7 Students' t Test on Selenium Reducers

liquid		2in	2in	2mid	2mid	2eff	2eff	3in	3in	3mid	3mid	3eff	3eff	4in	4in	4mid	4mid	4eff	4eff
solid	november 2014	1.6E+04	5.7E+05	1.5E+05	5.1E+06	7.4E+05	9.9E+06	7.8E+04	1.2E+07	8.7E+04	1.9E+06	8.8E+04	1.2E+06	1.7E+05	1.5E+06	1.1E+05	8.9E+05	8.7E+04	5.7E+05
		3.5E+04	8.2E+05	8.7E+04	4.4E+06	5.9E+05	9.4E+06	7.2E+04	1.8E+07	3.5E+04	2.9E+06	1.0E+05	8.7E+05	1.5E+05	1.3E+06	1.0E+05	9.8E+05	6.7E+04	6.5E+05
		3.1E+04	7.5E+05	8.1E+04	3.8E+06	7.8E+05	1.9E+07	8.6E+04	1.5E+07	1.2E+05	2.8E+06	8.7E+04	7.3E+05	1.7E+05	9.8E+05	8.9E+04	7.8E+05	5.5E+04	5.1E+05
	may 2015	9.0E+03	4.8E+05	1.3E+05	6.4E+06	9.1E+05	2.2E+07	9.5E+04	2.0E+07	1.4E+05	3.8E+06	1.6E+05	1.8E+06	2.5E+05	2.0E+06	1.4E+05	9.0E+05	9.2E+04	6.2E+05
		2.1E+04	5.6E+05	1.5E+05	5.2E+06	8.9E+05	1.6E+07	8.3E+04	2.6E+07	1.8E+05	3.9E+06	2.1E+05	2.1E+06	2.1E+04	1.9E+06	1.8E+05	1.1E+06	7.8E+04	7.1E+05
		2.3E+04	5.3E+05	1.1E+05	4.5E+06	1.1E+06	2.4E+07	9.7E+04	3.2E+07	2.1E+05	2.7E+06	2.0E+05	2.6E+06	1.7E+05	2.6E+06	1.1E+05	1.2E+06	6.9E+04	6.7E+05
	november 2015	6.0E+03	2.5E+05	1.7E+04	2.4E+06	2.2E+05	2.7E+06	2.8E+04	8.1E+06	2.1E+04	4.3E+05	1.1E+04	1.6E+05	2.5E+04	2.1E+05	1.1E+04	5.4E+04	8.7E+04	5.7E+05
		8.0E+03	4.2E+05	2.1E+04	1.7E+06	1.5E+05	3.6E+06	2.4E+04	5.4E+06	1.4E+04	3.3E+05	2.3E+04	1.5E+05	5.3E+04	1.7E+05	7.0E+03	7.0E+04	6.7E+04	6.5E+05
		1.5E+04	4.2E+05	3.3E+04	1.2E+06	1.8E+05	4.5E+06	3.2E+04	4.3E+06	1.6E+04	3.9E+05	1.4E+04	2.1E+05	4.3E+04	1.6E+05	6.0E+03	5.0E+04	5.5E+04	5.1E+05
		1.84E+04	5.33E+05	8.66E+04	3.86E+06	6.16E+05	1.23E+07	6.61E+04	1.56E+07	9.82E+04	2.13E+06	9.33E+04	1.10E+06	1.17E+05	1.20E+06	8.43E+04	6.67E+05	7.30E+04	6.07E+05
	average	1.73E+05	5.31E+04	1.75E+06	3.51E+05	8.16E+06	2.97E+04	9.37E+06	7.19E+04	1.44E+06	7.69E+04	9.00E+05	8.26E+04	8.90E+05	6.23E+04	4.74E+05	1.38E+04	7.07E+04	
	standard deviation	1.02E+04	1.73E+05	5.31E+04	1.75E+06	3.51E+05	8.16E+06	2.97E+04	9.37E+06	7.19E+04	1.44E+06	7.69E+04	9.00E+05	8.26E+04	8.90E+05	6.23E+04	4.74E+05	1.38E+04	7.07E+04
	n	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00	9.00E+00
	SEM	3.38E+03	5.77E+04	1.77E+04	5.82E+05	1.17E+05	2.72E+06	9.88E+03	3.12E+06	2.40E+04	4.79E+05	2.56E+04	3.00E+05	2.75E+04	2.38E+05	2.10E+04	1.59E+05	4.59E+03	2.36E+04
	SE		5.78E+04		5.82E+05		2.72E+06		3.12E+06		4.79E+05		3.01E+05		2.38E+05		1.59E+05		2.40E+04
	delta		-5.15E+05		-3.77E+06		-1.17E+07		-1.55E+07		-2.03E+06		-1.00E+06		-1.08E+06		-5.83E+05		-5.34E+05
	t95		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00		2.26E+00
	t*SE		1.31E+05		1.32E+06		6.16E+06		7.06E+06		1.08E+06		6.81E+05		6.74E+05		3.60E+05		5.43E+04
	CI high		-3.84E+05		-2.45E+06		-5.57E+06		-8.47E+06		-9.43E+05		-3.20E+05		-4.08E+05		-2.22E+05		-4.79E+05
	CI Low		-6.46E+05		-5.09E+06		-1.79E+07		-2.26E+07		-3.11E+06		-1.68E+06		-1.76E+06		-9.43E+05		-5.88E+05

One of the most influential goals of this study was to examine if there is an effective way in which to optimize this biological Selenium removal process. In order to determine if this was possible a several methods of altering the conditions within the reactor were tested. These methods consisted of altering of the temperature at which the process was allowed to run and the addition of several additives that when added to the system would provide the microorganisms with nutrients that could potentially improve their ability to treat the wastewater in the desired fashion. The essential element additives tested were: Nitrogen, Phosphorous, micronutrients, Molybdenum, Zinc, and Cobalt. In order to examine the results obtained from the testing procedure, the data was essentially tested for a relationship that could be identified through an attempt to model the data points. Remembering that all optimization experiments were performed in triplicate, all data points were plotted on a graph together while making no connection between points from the same batch of testing. The graphs created were then visually inspected for the presence of any relationship that would suggest the potential for optimization.

The first of the additives tested was nitrogen. It was tested in added concentrations from none to 20 mg/L. The behavior of the rate of selenium reduction in response to this additive was very interesting. The addition of just a small portion led to a drastic increase in the rate of reduction, but as the doses increased the rate of selenium reduction was met with diminishing returns. This shape appeared to resemble that of a saturation curve. At around five added mg/L the reduction rate began to show an asymptotic relationship. Once the amount added had reached approximately this concentration the rate of reduction just began to bounce back and forth around 2.4 mg/L/hr. Based on the shape of this graph it can be inferred that this process is deficit in nitrogen and therefore addition of this could lead to system optimization. This data is presented in Figure 10 below.

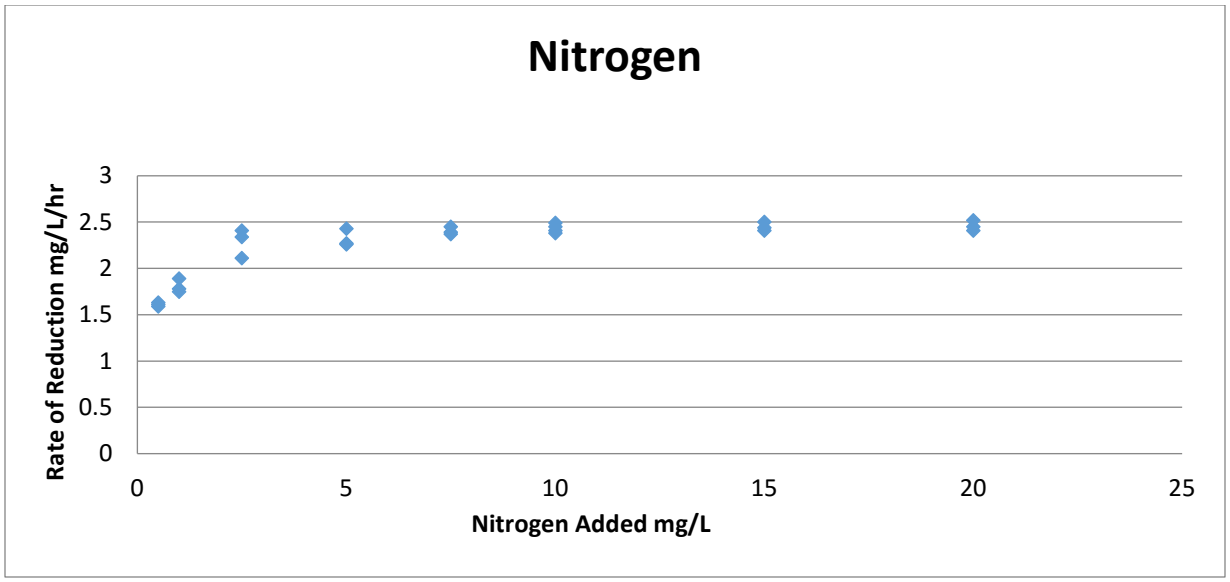


Figure 10 Nitrogen vs. Rate of Reduction

The next additive tested was phosphorous. This nutrient was added to the testing batch in concentrations ranging from zero to ten milligrams per liter. Although the initial addition showed a slight increase in the rate of reduction observed, all subsequent higher doses of phosphorous yielded no appreciable increase in the rate of reduction. The shape of this graph showed no relationship between phosphorous and the rate of selenium reduction. Based on this observation it can only be concluded that the addition of Phosphorous has no appreciable influence over the observed rate of reduction. This data is presented in Figure 11 below.

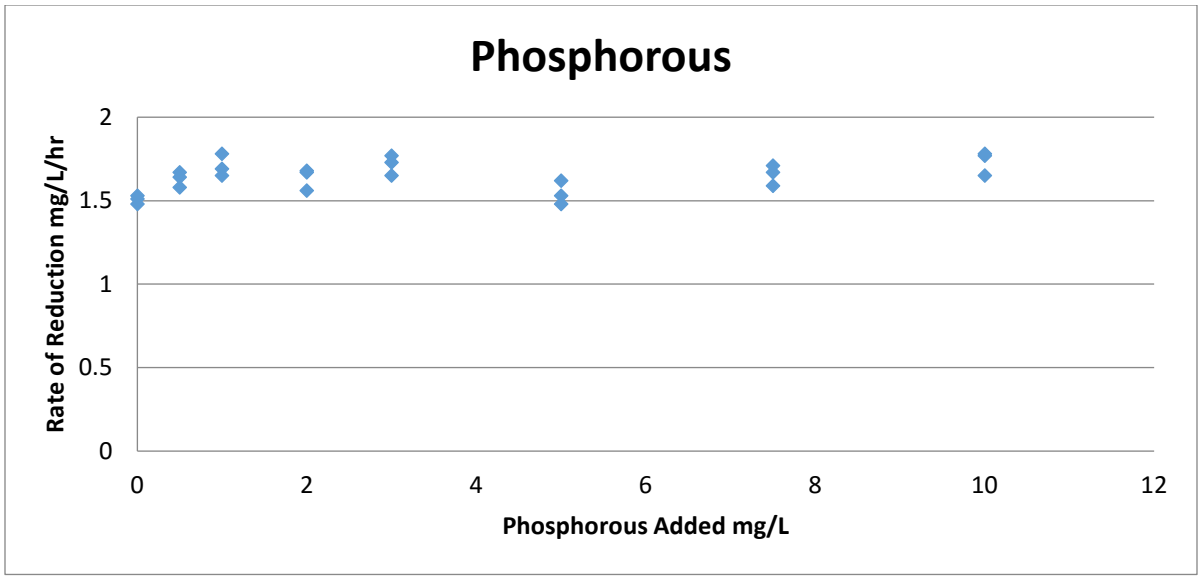


Figure 11 Phosphorous vs. Rate of Reduction

The next additive on the list is the combination micronutrient product, which was a conglomerate of molybdenum, copper, nickel, zinc, cadmium, and cobalt. All of these essential elements were combined at a concentration of one hundred micrograms per liter. This additive was tested in a range of concentrations that spanned from zero to ten milliliters per liter. The response by the rate of reduction to the addition of this solution was drastic at first and then began to diminish in the magnitude of its response as greater amounts of the solution were introduced. The response to the addition of this solution was at its' most extreme from zero to one milliliter per liter. From that point on the responses to more of the solution were smaller. The rate of reduction appeared to reach its' zenith at an added amount of seven and a half milliliter per liter. It would appear that this added concentration of seven and a half milliliter per liter is the optimum concentration for this solution. The shape of this graph also resembles that of a saturation curve. Based on the observed behavior of the rate of selenium reduction it can be concluded that the micronutrients blend could potentially be used to optimize the system. The

response by the rate of reduction in conjunction with this additive can be seen below in Figure 12.

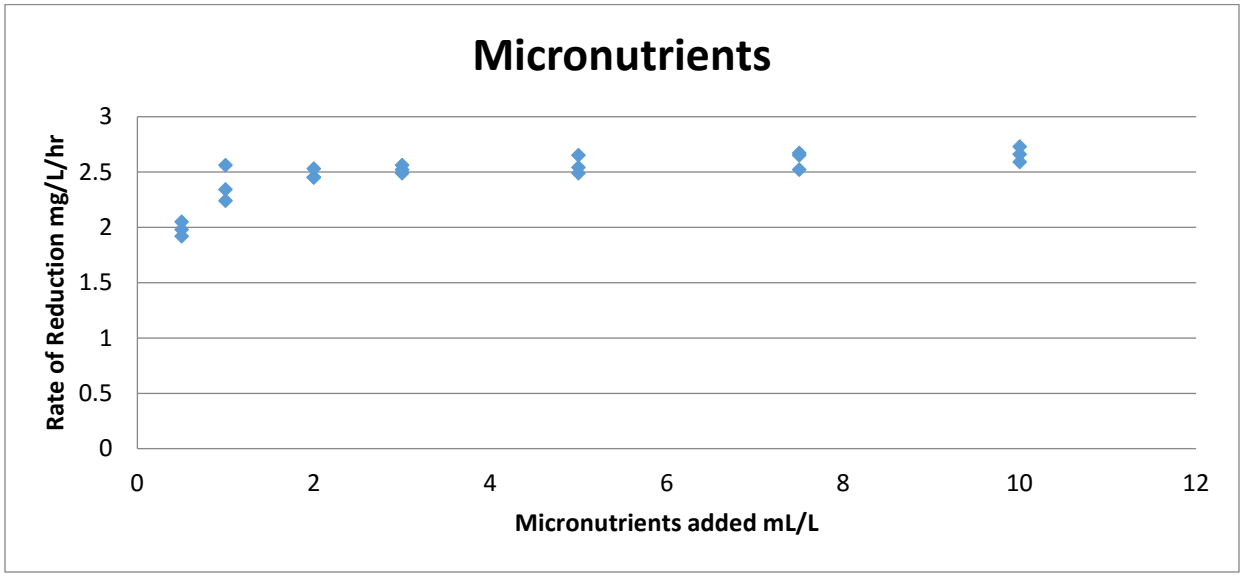


Figure 12 Micronutrients vs. Rate of Reduction

Molybdenum was the next additive in which the process was subjected to in order to see if it had any influence over the rate of Selenium reduction within the process. The results for this additive can be seen below in Figure 13. This additive was tested in a concentration range of zero micrograms per liter up to forty micrograms per liter. The rate of Selenium reduction increased at a very gradual rate in response to this compound. A gradual rise in the rate of reduction was observed before somewhat leveling off at an added concentration of approximately twenty micrograms per liter. This indicates a small deficiency and adding approximately twenty micrograms per liter would contribute to process optimization. From that concentration on the rate of reduction bounced between a range of approximately 2.7 and 2.8 milligrams per liter per hour. At an added concentration of thirty micrograms per liter the rate of reduction exhibited its tightest group in this range of highest exhibited rates of reduction. With that in mind a concentration of thirty micrograms per liter appears to be the optimum added concentration of

Molybdenum. The shape of this graph resembles that of a saturation curve. Due to the response of the rate of reduction behaving in such a desirable fashion it can be concluded that Molybdenum could potentially be used as a means for optimization.

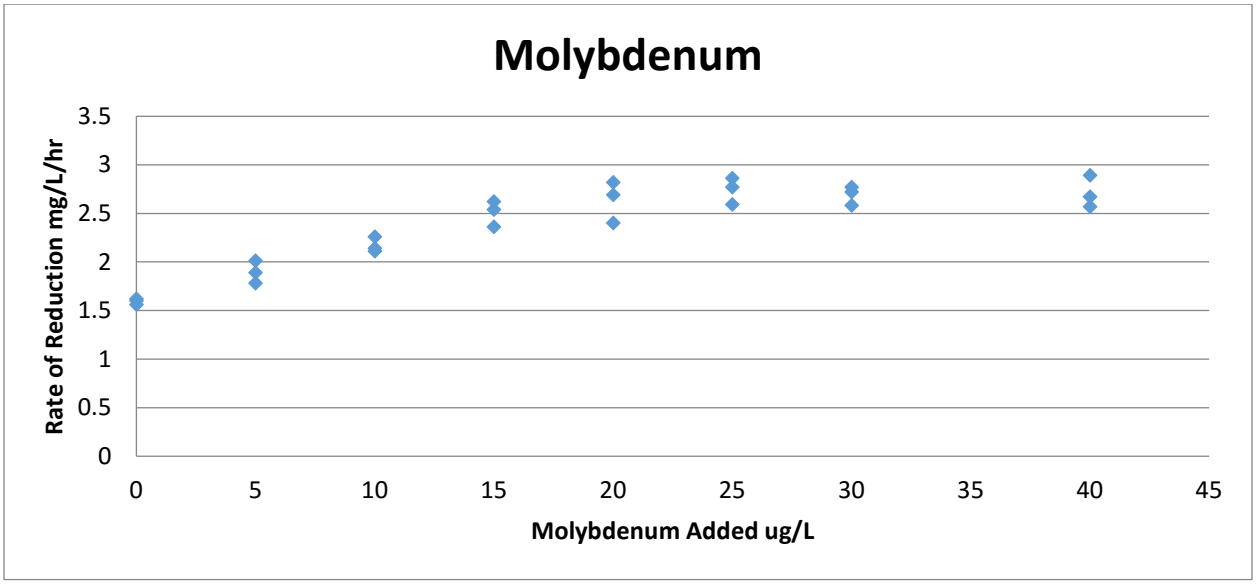


Figure 13 Molybdenum vs. Rate of Reduction

The next additive on the list of tested specimen to discuss is Zinc. Zinc was tested in a range of added concentrations that spanned from zero to forty micrograms per liter. The rate of reduction responded to the inclusion of this additive by increasing a very slight amount after the initial addition but then only proceeded to jump back and forth between a range of rates of reduction that achieved a high of 2.01 and a low of 1.53 milligrams per liter per hour. The shape of this graph did not fare so favorably. The rate of reduction did not exhibit any significant improvement from the addition of zinc. Based on the observed shape of this curve it cannot be concluded that zinc would be a potential means of system optimization. The results from this additive can be seen below in Figure 14.

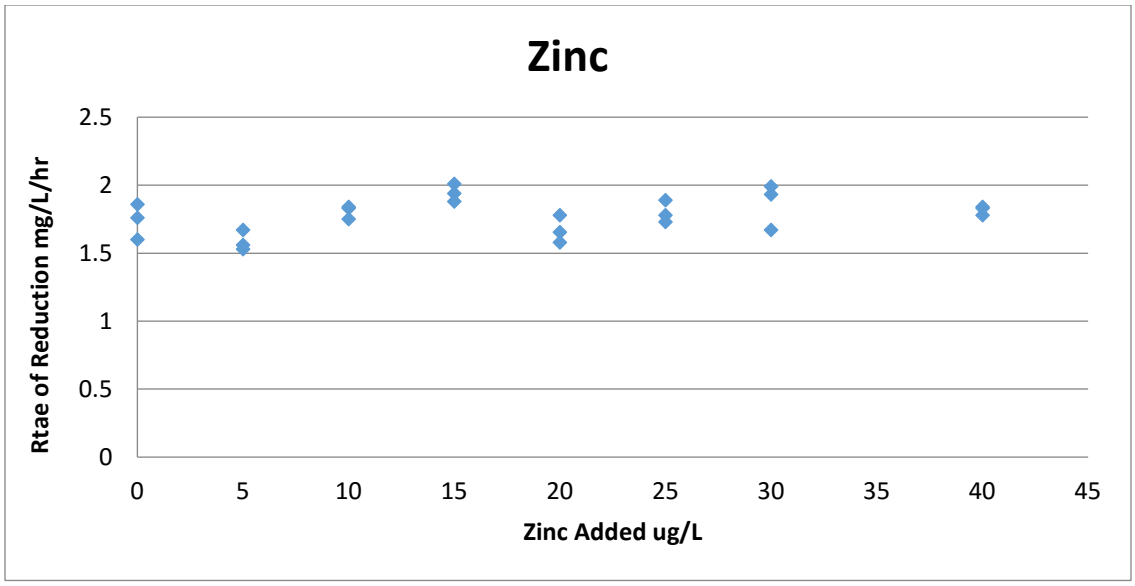


Figure 14 Zinc vs. Rate of Reduction

The final additive used to test for system optimization was Cobalt. This potential nutrient was tested in a range of zero to forty micrograms per liter added to the testing batches. The results have been graphed and can be seen below in Figure 15. With the initial inclusion of Cobalt the rate of reduction responded with a slight increase. All subsequent addition resulted in rates of reduction that appeared to have no discernible pattern. The rate of reduction achieved a high of 1.99 and a low of 1.56 milligrams per liter per hour. The shape of the graph suggest that cobalt had no appreciable influence over the rate of selenium reduction. The rate of reduction shows an overall horizontal shape that suggest that cobalt had no substantial impact on the rate of selenium reduction. Based on this observation it can be concluded that cobalt would not yield any potential for selenium reduction.

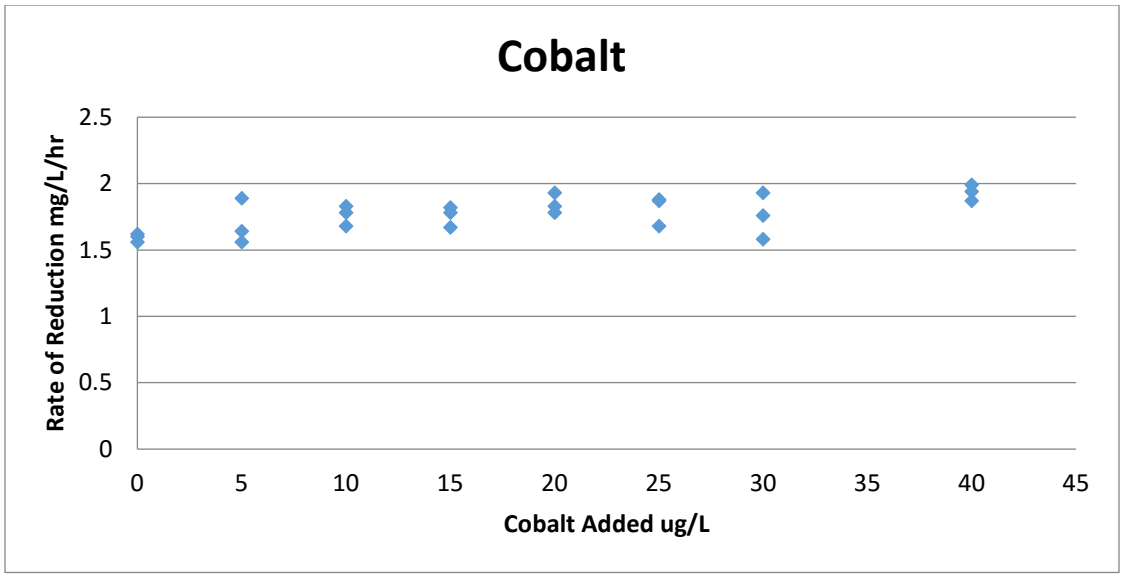


Figure 15 Cobalt vs. Rate of Reduction

The only non-chemical parameter tested for its' impact on the rate of Selenium reduction within the process was temperature. To analyze how the temperature at which the process is held affects the rate of Selenium reduction, the testing samples were held at temperatures that varied from five to forty five degrees Celsius. The rate of reduction responded in a parabolic shape that rose to its zenith at thirty degrees and a achieved a maximum rate of reduction at 2.45 milligrams per liter per hour. This temperature of thirty degrees Celsius was clearly the optimal temperature for the process. This graph took a parabolic form. This tells us that the range of temperatures tested was broad enough to encompass the point where the rate of selenium reduction met with diminishing returns as the temperature increased. This graph shows that the rate of selenium reduction responds favorably until around thirty degrees Celsius, after which the rate of reduction declines. Based on these observations it can be concluded that temperature could potentially be used as a means for optimization. The results for this series of tests can be seen below in Figure 16.

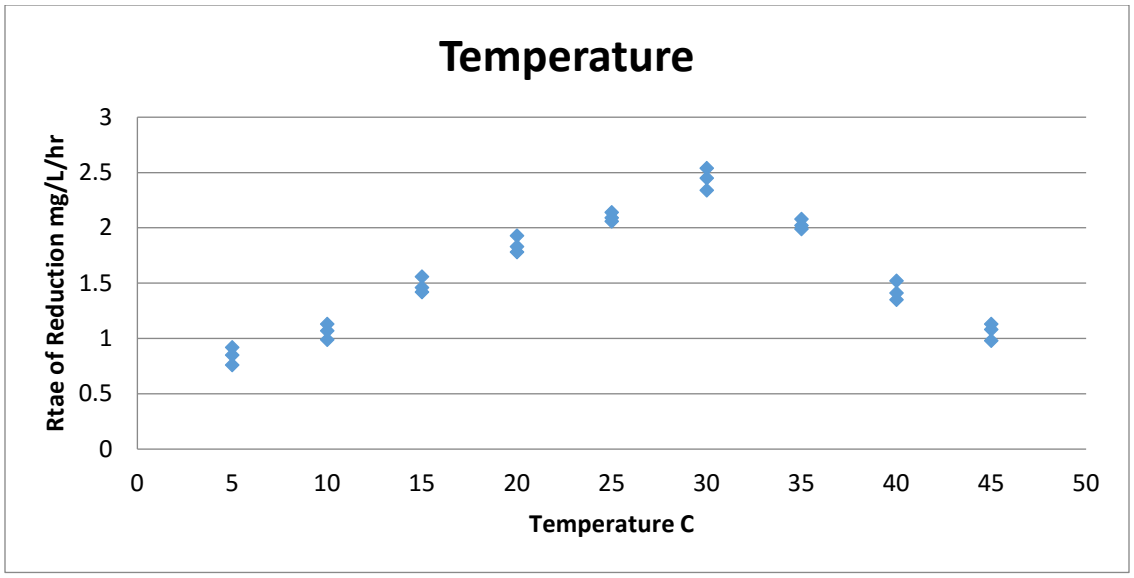


Figure 16 Temperature vs. Rate of Reduction

Additional analysis was performed on the wood chips themselves in order to examine their release of sugars into the reactor water. It is important to understand this behavior because the simple sugars released by the wood chips serve as a carbon source as well as an energy source for the microorganisms within the reactor. Because these wood chips provide these things to the bacteria in the reactor, redox potential is driven down and conditions become anaerobic which is necessary for Selenium reduction to occur. Figure 17 shows the amount of soluble sugars released from ten grams of woodchips over the course of one hundred and fifty days. What the behavior shown on this graph tells us is that the chips initially release a substantial amount of sugars at first. This means that when the wood chips are initially added to the system they immediately drive down the redox potential of the system. After this initial behavior the chips then slow their release of sugars into the system. This slowed release helps to maintain the anaerobic conditions created by the initial rapid release behavior. Figure 17 depicts the wood chips' percent composition of three of these key sugars. The takeaway from this graph is the relatively nonexistent change in wood chip composition over the course of the one hundred

and fifty day testing period. This shows that the content of sugars in the wood chips is essentially unchanged over the testing period. The ramification of this is that the wood chips can continue to act as a carbon and energy source well into the foreseeable future.

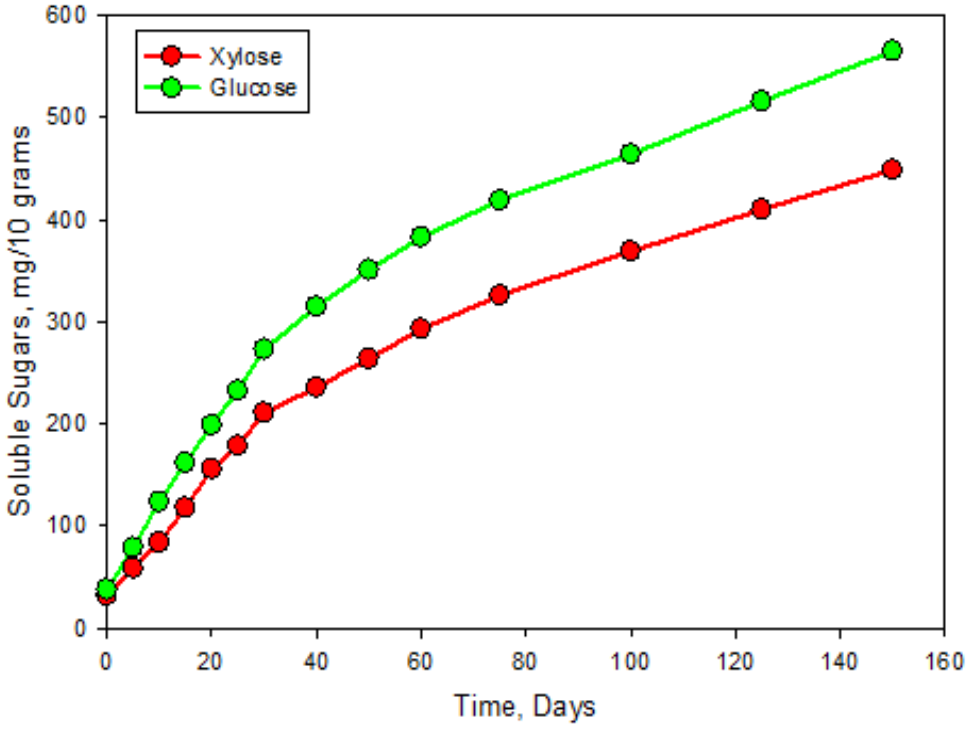


Figure 17 Cumulative Sugars Leached From Wood Chips

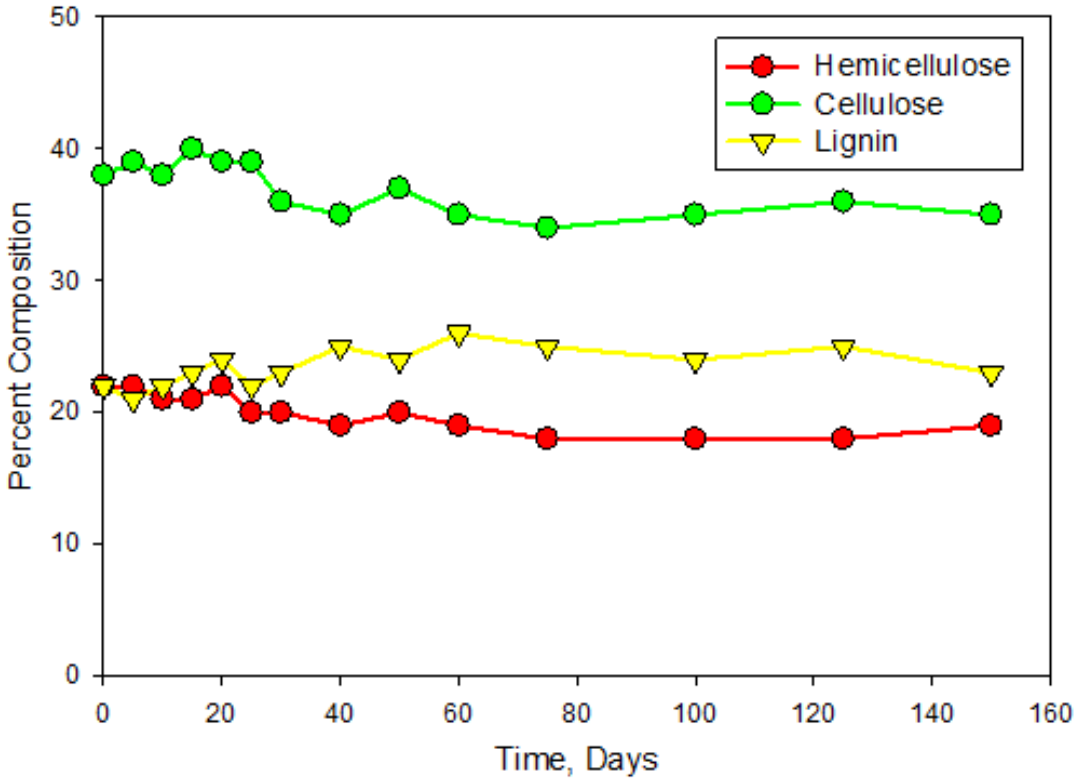


Figure 18 Percent Sugars Remaining in Wood Chips After 150 Days

Chapter 6 Conclusions

The first major conclusion that can be drawn from this research deals with the very nature of the process being examined. Personnel at the power plant where this process is located believed that this process could possibly be strictly a chemical precipitation. The results of this research suggest that there is a strong likelihood that the selenium removal observed is influenced by biologic activity. In this study species of microorganisms that directly reduce selenium were identified. This tells us that there is indeed selenium treatment taking place due to the lifecycles of microorganisms, however based off this research no comments can be made as to the magnitude of biologic selenium reduction or what percentage of the selenium treatment can be attributed to microorganisms. The notion that this treatment process could potentially be a chemical precipitation is not entirely implausible, In fact the data obtained from this study supported the presence of a chemical precipitation removal process. The precipitation displayed in this study however was mediated by microorganisms. The sulfur reducers found in this process treat the selenium found in the solution through a precipitation process. By creating a reducing environment around them that results in the reduction of selenium, these microorganisms create a localized precipitation reaction.

The second conclusion that can be drawn from the data present can be drawn from the results of the series of Students' t tests performed on the populations of anaerobic organism present within the reactors in the liquid media and on the wood chips. In all cases examined the difference between the number of bacteria in the liquid media and on the wood chips was

determined to be statistically significant. This difference was often as great as several orders of magnitude. With this in mind it can be definitively said that more microorganisms can be found on the wood chips than in the liquid media. The ability to say this allows this process to be classified as a fixed film treatment process. Due to this classification a conclusion can be drawn for a method of optimizing this process. Because more Selenium is being reduced and effectively at the surface of the wood chips, decreasing the size of the individual chips and thereby increasing the surface area of the total wood chips added could optimize the process. By increasing the surface area the Selenium would be exposed to more bacteria that have the potential to reduce and thereby treat it resulting in a more effective process

The third conclusion that can be drawn deals with the test performed to determine what nutrient additives could potentially influence the rate of selenium reduction. As discussed earlier, the graphs showing the response by the rate of selenium reduction to the additions of these chemicals were visually examined for a relationship that would suggest a potential for optimization. One type of desirable shape was exhibited by the chemical additives. This shape was one that resembled that of a saturation curve. With that being said Nitrogen, micronutrients, and molybdenum were the three tested additives that had an impact on the rate of selenium reduction. It is therefore suggested that the addition of these chemicals to the process could potentially increase the performance of the overall process.

The final conclusion pertains to the temperature of the process. The data for this test was performed just like that of the additive nutrients and the data gathered was analyzed the same. The graph for this tested optimization method manifested a parabolic shape. This shape was desirable and suggested that temperature could be used as a means of optimization. By examining the data plotted, the highest rates of reduction occurred when the process was held at

thirty degrees Celsius. It can be inferred then that keeping the entire process at approximately thirty degrees Celsius would contribute to the optimization of the process. However, since heating water is likely to not be a cheap expense, this optimization technique may not be economically feasible.

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Appendix A Bacterial Count Data

August 7th 2014

BIO 2 INFLUENT			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	45	6	37	6	55	6	4.5E+07	3.7E+07	5.5E+07	**4.6E+07**	#/L
		solids	23	5	34	5	5	6	2.3E+06	3.4E+06	5.0E+06	**3.6E+06**	#/gram
									1.3E+09	1.9E+09	2.8E+09	**2.0E+09**	
Anaerobic Heterotrophs	Tryptocase Soy Agar		16	6	24	6	18	6	1.6E+07	2.4E+07	1.8E+07	**1.9E+07**	#/L
			39	6	55	6	141	5	3.9E+07	5.5E+07	1.4E+07	**3.6E+07**	#/gram
									2.1E+10	3.0E+10	7.8E+09	**2.0E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar plates		5	5	8	5	6	5	5.0E+05	8.0E+05	6.0E+05	**6.3E+05**	#/L
			21	5	16	5	13	5	2.1E+06	1.6E+06	1.3E+06	**1.7E+06**	#/gram
									1.2E+09	8.8E+08	7.2E+08	**9.2E+08**	
methanogens	methanogenic archaea		0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
			24	3	59	3	98	2	2.4E+04	5.9E+04	9.8E+03	**3.1E+04**	#/gram
									1.3E+07	3.2E+07	5.4E+06	**1.7E+07**	
Selanate Reducing Bacteria			15	3	99	3	21	3	1.5E+04	9.9E+04	2.1E+04	**4.5E+04**	#/L
			39	4	67	4	50	4	3.9E+05	6.7E+05	5.0E+05	**5.2E+05**	#/gram
									2.1E+08	3.7E+08	2.8E+08	**2.9E+08**	
fungi	Czapek's Agar		75	4	18	4	28	4	7.5E+05	1.8E+05	2.8E+05	**4.0E+05**	#/L
			12	5	21	5	89	4	1.2E+06	2.1E+06	8.9E+05	**1.4E+06**	#/gram
									6.6E+08	1.2E+09	4.9E+08	**7.7E+08**	
Yeast	Malt Extract Agar		10	3	79	2	85	2	1.0E+04	7.9E+03	8.5E+03	**8.8E+03**	#/L
			33	4	27	4	190	3	3.3E+05	2.7E+05	1.9E+05	**2.6E+05**	#/gram
Aerobic Fastidious	Chocholate Agar		94	6	120	6	89	6	9.4E+07	1.2E+08	8.9E+07	**1.0E+08**	#/L
			18	5	37	5	7	6	1.8E+06	3.7E+06	7.0E+06	**4.2E+06**	#/gram
Aerobic Non Fastidious	Nutrient Agar		150	6	132	6	109	6	1.5E+08	1.3E+08	1.1E+08	**1.3E+08**	#/L
			56	6	33	6	28	6	5.6E+07	3.3E+07	2.8E+07	**3.9E+07**	#/gram
									3.1E+10	1.8E+10	1.5E+10	**2.1E+10**	

BIO 2 EFFLUENT			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	31	6	16	6	19	6	3.1E+07	1.6E+07	1.9E+07	**2.2E+07**	#/L
		solids	48	5	92	5	79	6	4.8E+06	9.2E+06	7.9E+07	**3.1E+07**	#/gram
									2.6E+09	5.1E+09	4.3E+10	**1.7E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	24	6	42	6	48	6	2.4E+07	4.2E+07	4.8E+07	**3.8E+07**	#/L
		solids	97	6	16	7	21	7	9.7E+07	1.6E+08	2.1E+08	**1.6E+08**	#/gram
									5.3E+10	8.8E+10	1.2E+11	**8.6E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar plates	liquid	18	5	21	5	16	5	1.8E+06	2.1E+06	1.6E+06	**1.8E+06**	#/L
		solids	38	6	41	6	62	6	3.8E+07	4.1E+07	6.2E+07	**4.7E+07**	#/gram
									2.1E+10	2.3E+10	3.4E+10	**2.6E+10**	
methanogens	methanogenic archaea	liquid	0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
		solids	37	3	120	3	130	3	3.7E+04	1.2E+05	1.3E+05	**9.6E+04**	#/gram
									2.0E+07	6.6E+07	7.2E+07	**5.3E+07**	
Selanate Reducing Bacteria	senanate reducing bacteria	liquid	49	4	38	4	54	4	4.9E+05	3.8E+05	5.4E+05	**4.7E+05**	#/L
		solids	67	5	82	5	11	6	6.7E+06	8.2E+06	1.1E+07	**8.6E+06**	#/gram
									3.7E+09	4.5E+09	6.1E+09	**4.7E+09**	
fungi	Czapek's Agar	liquid	42	4	27	4	31	4	4.2E+05	2.7E+05	3.1E+05	**3.3E+05**	#/L
		solids	45	4	59	4	62	4	4.5E+05	5.9E+05	6.2E+05	**5.5E+05**	#/gram
									2.5E+08	3.2E+08	3.4E+08	**3.0E+08**	
Yeast	Malt Extract Agar	liquid	46	4	51	4	37	4	4.6E+05	5.1E+05	3.7E+05	**4.5E+05**	#/L
		solids	21	4	18	4	21	4	2.1E+05	1.8E+05	2.1E+05	**2.0E+05**	#/gram
									1.2E+08	9.9E+07	1.2E+08	**1.1E+08**	
Aerobic Fastidious	Chocholate Agar	liquid	21	6	18	6	25	6	2.1E+07	1.8E+07	2.5E+07	**2.1E+07**	#/L
		solids	59	4	46	4	68	4	5.9E+05	4.6E+05	6.8E+05	**5.8E+05**	#/gram
									3.2E+08	2.5E+08	3.7E+08	**3.2E+08**	
Aerobic Non Fastidious	Nutrient Agar	liquid	54	6	63	6	44	6	5.4E+07	6.3E+07	4.4E+07	**5.4E+07**	#/L
		solids	35	5	12	6	49	5	3.5E+06	1.2E+07	4.9E+06	**6.8E+06**	#/gram
									1.9E+09	6.6E+09	2.7E+09	**3.7E+09**	

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BIO 2 INFLUENT													
			Count	Dilution	Count	Dilution	Count	Dilution					Average Number	UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	52	6	46	6	53	6		5.2E+07	4.6E+07	5.3E+07	**5.0E+07**	#/L
		solids	41	6	45	6	67	6		4.1E+07	4.5E+07	6.7E+07	**5.1E+07**	#/gram
										2.3E+10	2.5E+10	3.7E+10	**2.8E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar		62	6	59	6	65	6		6.2E+07	5.9E+07	6.5E+07	**6.2E+07**	#/L
			51	6	59	6	46	6		5.1E+07	5.9E+07	4.6E+07	**5.2E+07**	#/gram
										2.8E+10	3.2E+10	2.5E+10	**2.9E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar	plates	3	5	3	5	4	5		3.0E+05	3.0E+05	4.0E+05	**3.3E+05**	#/L
			32	5	41	5	28	5		3.2E+06	4.1E+06	2.8E+06	**3.4E+06**	#/gram
										1.8E+09	2.3E+09	1.5E+09	**1.9E+09**	
methanogens	methanogenic archaea		0	0	0	0	0	0		0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
			13	3	22	3	16	3		1.3E+04	2.2E+04	1.6E+04	**1.7E+04**	#/gram
										7.2E+06	1.2E+07	8.8E+06	**9.4E+06**	
Selanate Reducing Bacteria			18	3	35	3	31	3		1.8E+04	3.5E+04	3.1E+04	**2.8E+04**	#/L
			57	4	82	4	75	4		5.7E+05	8.2E+05	7.5E+05	**7.1E+05**	#/gram
										3.1E+08	4.5E+08	4.1E+08	**3.9E+08**	
fungi	Czapek's Agar		43	4	23	4	29	4		4.3E+05	2.3E+05	2.9E+05	**3.2E+05**	#/L
			19	5	13	5	21	5		1.9E+06	1.3E+06	2.1E+06	**1.8E+06**	#/gram
										1.0E+09	7.2E+08	1.2E+09	**9.7E+08**	
Yeast	Malt Extract Agar		68	2	57	2	48	2		6.8E+03	5.7E+03	4.8E+03	**5.8E+03**	#/L
			45	4	49	4	58	4		4.5E+05	4.9E+05	5.8E+05	**5.1E+05**	#/gram
										2.5E+08	2.7E+08	3.2E+08	**2.8E+08**	
Aerobic Fastidious	Chocholate Agar		68	6	55	6	67	6		6.8E+07	5.5E+07	6.7E+07	**6.3E+07**	#/L
			29	5	43	5	56	5		2.9E+06	4.3E+06	5.6E+06	**4.3E+06**	#/gram
										1.6E+09	2.4E+09	3.1E+09	**2.3E+09**	
Aerobic Non Fastidious	Nutrient Agar		95	6	83	6	79	6		9.5E+07	8.3E+07	7.9E+07	**8.6E+07**	#/L
			66	6	62	6	47	6	750000	6.6E+07	6.2E+07	4.7E+07	**5.8E+07**	#/gram
										3.6E+10	3.4E+10	2.6E+10	**3.2E+10**	

BIO 2 middle													
			Count	Dilution	Count	Dilution	Count	Dilution					Average N	UNITS
Facultativ	Thioglycolate Media													
		liquid	37	6	53	6	64	6		3.7E+07	5.3E+07	6.4E+07	**5.1E+07**	#/L
		solids	31	6	45	6	56	6		3.1E+07	4.5E+07	5.6E+07	**4.4E+07**	#/gram
										1.7E+10	2.5E+10	3.1E+10	**2.4E+10**	
Anaerobic	Tryptocase Soy Agar		14	6	19	6	23	6		1.4E+07	1.9E+07	2.3E+07	**1.9E+07**	#/L
			98	6	67	6	88	6		9.8E+07	6.7E+07	8.8E+07	**8.4E+07**	#/gram
										5.4E+10	3.7E+10	4.8E+10	**4.6E+10**	
Sulfate Rd	sulfate reducing aga		21	5	34	5	41	5		2.1E+06	3.4E+06	4.1E+06	**3.2E+06**	#/L
			59	5	74	5	63	5		5.9E+06	7.4E+06	6.3E+06	**6.5E+06**	#/gram
										3.2E+09	4.1E+09	3.5E+09	**3.6E+09**	
methanog	methanogenic archa		0	0	0	0	0	0		0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
			58	3	92	3	89	3		5.8E+04	9.2E+04	8.9E+04	**8.0E+04**	#/gram
										3.2E+07	5.1E+07	4.9E+07	**4.4E+07**	
Selanate Reducing Bacteria			15	4	87	3	81	3		1.5E+05	8.7E+04	8.1E+04	**1.1E+05**	#/L
			51	5	44	5	38	5		5.1E+06	4.4E+06	3.8E+06	**4.4E+06**	#/gram
										2.8E+09	2.4E+09	2.1E+09	**2.4E+09**	
fungi	Czapek's Agar		65	4	32	4	31	4		6.5E+05	3.2E+05	3.1E+05	**4.3E+05**	#/L
			25	5	14	5	134	4		2.5E+06	1.4E+06	1.3E+06	**1.7E+06**	#/gram
										1.4E+09	7.7E+08	7.4E+08	**9.6E+08**	
Yeast	Malt Extract Agar		23	3	155	2	180	2		2.3E+04	1.6E+04	1.8E+04	**1.9E+04**	#/L
			45	4	56	4	52	4		4.5E+05	5.6E+05	5.2E+05	**5.1E+05**	#/gram
										2.5E+08	3.1E+08	2.9E+08	**2.8E+08**	
Aerobic Fa	Chocholate Agar		134	6	158	6	145	6		1.3E+08	1.6E+08	1.5E+08	**1.5E+08**	#/L
			48	5	39	5	78	5		4.8E+06	3.9E+06	7.8E+06	**5.5E+06**	#/gram
										2.6E+09	2.1E+09	4.3E+09	**3.0E+09**	
Aerobic N	Nutrient Agar		67	6	54	6	48	6		6.7E+07	5.4E+07	4.8E+07	**5.6E+07**	#/L
			32	6	41	6	19	6		3.2E+07	4.1E+07	1.9E+07	**3.1E+07**	#/gram
										1.8E+10	2.3E+10	1.0E+10	**1.7E+10**	

BIO 2 EFFLUENT			Count	Dilution	Count	Dilution	Count	Dilution					Average Number	UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	45	6	41	6	37	6	4.5E+07	4.1E+07	3.7E+07		**4.1E+07**	#/L
		solids	55	5	67	5	45	6	5.5E+06	6.7E+06	4.5E+07		**1.9E+07**	#/gram
									3.0E+09	3.7E+09	2.5E+10		**1.0E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	23	6	43	6	71	6	2.3E+07	4.3E+07	7.1E+07		**4.6E+07**	#/L
		solids	155	6	128	6	26	7	1.6E+08	1.3E+08	2.6E+08		**1.8E+08**	#/gram
									8.5E+10	7.0E+10	1.4E+11		**1.0E+11**	
Sulfate Rducing Bacteria	sulfate reducing agar plates	liquid	38	5	47	5	34	5	3.8E+06	4.7E+06	3.4E+06		**4.0E+06**	#/L
		solids	53	6	67	6	89	6	5.3E+07	6.7E+07	8.9E+07		**7.0E+07**	#/gram
									2.9E+10	3.7E+10	4.9E+10		**3.8E+10**	
methanogens	methanogenic archaea	liquid	0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00		**0.0E+00**	#/L
		solids	48	3	68	3	65	3	4.8E+04	6.8E+04	6.5E+04		**6.0E+04**	#/gram
									2.6E+07	3.7E+07	3.6E+07		**3.3E+07**	
Selanate Reducing Bacteria	senanate reducing bacteria	liquid	74	4	59	4	78	4	7.4E+05	5.9E+05	7.8E+05		**7.0E+05**	#/L
		solids	99	5	94	5	19	6	9.9E+06	9.4E+06	1.9E+07		**1.3E+07**	#/gram
									5.4E+09	5.2E+09	1.0E+10		**7.0E+09**	
fungi	Czapek's Agar	liquid	58	4	49	4	52	4	5.8E+05	4.9E+05	5.2E+05		**5.3E+05**	#/L
		solids	78	4	68	4	78	4	7.8E+05	6.8E+05	7.8E+05		**7.5E+05**	#/gram
									4.3E+08	3.7E+08	4.3E+08		**4.1E+08**	
Yeast	Malt Extract Agar	liquid	31	4	40	4	34	4	3.1E+05	4.0E+05	3.4E+05		**3.5E+05**	#/L
		solids	16	4	18	4	15	4	1.6E+05	1.8E+05	1.5E+05		**1.6E+05**	#/gram
									8.8E+07	9.9E+07	8.3E+07		**9.0E+07**	
Aerobic Fastidious	Chocholate Agar	liquid	18	6	15	6	17	6	1.8E+07	1.5E+07	1.7E+07		**1.7E+07**	#/L
		solids	44	4	34	4	36	4	4.4E+05	3.4E+05	3.6E+05		**3.8E+05**	#/gram
									2.4E+08	1.9E+08	2.0E+08		**2.1E+08**	
Aerobic Non Fastidious	Nutrient Agar	liquid	68	6	48	6	32	6	6.8E+07	4.8E+07	3.2E+07		**4.9E+07**	#/L
		solids	24	5	21	5	19	5	2.4E+06	2.1E+06	1.9E+06		**2.1E+06**	#/gram
									1.3E+09	1.2E+09	1.0E+09		**1.2E+09**	

Bio 3 Influent			Count	Dilution	Count	Dilution	Count	Dilution					Average Number	UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	21	4	42	4	16	4	2.1E+05	4.2E+05	1.6E+05		**2.6E+05**	#/L
		solids	24	5	155	4	120	4	2.4E+06	1.6E+06	1.2E+06		**1.7E+06**	#/gram
									1.3E+09	8.5E+08	6.6E+08		**9.4E+08**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	167	6	178	6	150	6	1.7E+08	1.8E+08	1.5E+08		**1.7E+08**	#/L
		solids	27	8	34	8	210	7	2.7E+09	3.4E+09	2.1E+09		**2.7E+09**	#/gram
									1.5E+12	1.9E+12	1.2E+12		**1.5E+12**	
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	32	5	39	5	45	5	3.2E+06	3.9E+06	4.5E+06		**3.9E+06**	#/L
		solids	143	6	172	6	154	6	1.4E+08	1.7E+08	1.5E+08		**1.6E+08**	#/gram
									7.9E+10	9.5E+10	8.5E+10		**8.6E+10**	
methanogens	methanogenic archaea	liquid	14	3	18	3	19	3	1.4E+04	1.8E+04	1.9E+04		**1.7E+04**	#/L
		solids	176	3	198	3	179	3	1.8E+05	2.0E+05	1.8E+05		**1.8E+05**	#/gram
									9.7E+07	1.1E+08	9.8E+07		**1.0E+08**	
Selanate Reducing Bacteria		liquid	78	3	72	3	86	3	7.8E+04	7.2E+04	8.6E+04		**7.9E+04**	#/L
		solids	121	5	177	5	148	5	1.2E+07	1.8E+07	1.5E+07		**1.5E+07**	#/gram
									6.7E+09	9.7E+09	8.1E+09		**8.2E+09**	
fungi	Czapek's Agar	liquid	8	4	9	4	6	4	8.0E+04	9.0E+04	6.0E+04		**7.7E+04**	#/L
		solids	37	4	36	4	43	4	3.7E+05	3.6E+05	4.3E+05		**3.9E+05**	#/gram
									2.0E+08	2.0E+08	2.4E+08		**2.1E+08**	
Yeast	Malt Extract Agar	liquid	3	3	4	3	2	3	3.0E+03	4.0E+03	2.0E+03		**3.0E+03**	#/L
		solids	12	4	15	4	7	4	1.2E+05	1.5E+05	7.0E+04		**1.1E+05**	#/gram
									6.6E+07	8.3E+07	3.9E+07		**6.2E+07**	
Aerobic Fastidious	Chocholate Agar	liquid	7	5	13	5	21	5	7.0E+05	1.3E+06	2.1E+06		**1.4E+06**	#/L
		solids	12	5	15	5	11	5	1.2E+06	1.5E+06	1.1E+06		**1.3E+06**	#/gram
									6.6E+08	8.3E+08	6.1E+08		**7.0E+08**	
Aerobic Non Fastidious	Nutrient Agar	liquid	16	4	24	4	10	4	1.6E+05	2.4E+05	1.0E+05		**1.7E+05**	#/L
		solids	15	5	9	5	8	5	1.5E+06	9.0E+05	8.0E+05		**1.1E+06**	#/gram
									8.3E+08	5.0E+08	4.4E+08		**5.9E+08**	

BIO 3 MID COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution				Average N	UNITS
Facultativ	Thioglycolate Media												
		liquid	24	4	32	4	21	4	2.4E+05	3.2E+05	2.1E+05	**2.6E+05**	#/L
		solids	4	5	72	4	35	4	4.0E+05	7.2E+05	3.5E+05	**4.9E+05**	#/gram
									2.2E+08	4.0E+08	1.9E+08	**2.7E+08**	
Anaerobic	Tryptocas	liquid	99	6	87	6	121	6	9.9E+07	8.7E+07	1.2E+08	**1.0E+08**	#/L
		solids	187	7	194	7	208	7	1.9E+09	1.9E+09	2.1E+09	**2.0E+09**	#/gram
									1.0E+12	1.1E+12	1.1E+12	**1.1E+12**	
Sulfate Re	sulfate re	liquid	87	5	98	5	132	5	8.7E+06	9.8E+06	1.3E+07	**1.1E+07**	#/L
		solids	113	6	158	6	185	6	1.1E+08	1.6E+08	1.9E+08	**1.5E+08**	#/gram
									6.2E+10	8.7E+10	1.0E+11	**8.4E+10**	
methanog	methanog	liquid	14	3	18	3	23	3	1.4E+04	1.8E+04	2.3E+04	**1.8E+04**	#/L
		solids	32	4	41	4	28	4	3.2E+05	4.1E+05	2.8E+05	**3.4E+05**	#/gram
									1.8E+08	2.3E+08	1.5E+08	**1.9E+08**	
Selanate Reducing Ba		liquid	87	3	95	3	121	3	8.7E+04	9.5E+04	1.2E+05	**1.0E+05**	#/L
		solids	188	4	29	5	28	5	1.9E+06	2.9E+06	2.8E+06	**2.5E+06**	#/gram
									1.0E+09	1.6E+09	1.5E+09	**1.4E+09**	
fungi	Czapek's A	liquid	5	4	5	4	2	4	5.0E+04	5.0E+04	2.0E+04	**4.0E+04**	#/L
		solids	18	4	26	4	21	4	1.8E+05	2.6E+05	2.1E+05	**2.2E+05**	#/gram
									9.9E+07	1.4E+08	1.2E+08	**1.2E+08**	
Yeast	Malt Extra	liquid	4	3	3	3	3	3	4.0E+03	3.0E+03	3.0E+03	**3.3E+03**	#/L
		solids	26	4	32	4	25	4	2.6E+05	3.2E+05	2.5E+05	**2.8E+05**	#/gram
									1.4E+08	1.8E+08	1.4E+08	**1.5E+08**	
Aerobic Fa	Chocholat	liquid	21	5	14	5	9	5	2.1E+06	1.4E+06	9.0E+05	**1.5E+06**	#/L
		solids	11	5	14	5	7	5	1.1E+06	1.4E+06	7.0E+05	**1.1E+06**	#/gram
									6.1E+08	7.7E+08	3.9E+08	**5.9E+08**	
Aerobic N	Nutrient A	liquid	14	4	18	4	9	4	1.4E+05	1.8E+05	9.0E+04	**1.4E+05**	#/L
		solids	21	5	15	5	13	5	2.1E+06	1.5E+06	1.3E+06	**1.6E+06**	#/gram
									1.2E+09	8.3E+08	7.2E+08	**9.0E+08**	

BIO 3 effluent			Count	Dilution	Count	Dilution	Count	Dilution				Average N	UNITS
Facultativ	Thioglycolate Media												
		liquid	31	4	23	4	14	4	3.1E+05	2.3E+05	1.4E+05	**2.3E+05**	#/L
		solids	24	4	67	4	51	4	2.4E+05	6.7E+05	5.1E+05	**4.7E+05**	#/gram
									1.3E+08	3.7E+08	2.8E+08	**2.6E+08**	
Anaerobic	Tryptocas	liquid	78	6	154	6	89	6	7.8E+07	1.5E+08	8.9E+07	**1.1E+08**	#/L
		solids	135	7	108	7	97	7	1.4E+09	1.1E+09	9.7E+08	**1.1E+09**	#/gram
									7.4E+11	5.9E+11	5.3E+11	**6.2E+11**	
Sulfate Re	sulfate re	liquid	76	5	84	5	89	5	7.6E+06	8.4E+06	8.9E+06	**8.3E+06**	#/L
		solids	180	6	176	6	184	6	1.8E+08	1.8E+08	1.8E+08	**1.8E+08**	#/gram
									9.9E+10	9.7E+10	1.0E+11	**9.9E+10**	
methanog	methanog	liquid	11	3	12	3	16	3	1.1E+04	1.2E+04	1.6E+04	**1.3E+04**	#/L
		solids	210	3	226	3	203	3	2.1E+05	2.3E+05	2.0E+05	**2.1E+05**	#/gram
									1.2E+08	1.2E+08	1.1E+08	**1.2E+08**	
Selanate Reducing Ba		liquid	88	3	101	3	87	3	8.8E+04	1.0E+05	8.7E+04	**9.2E+04**	#/L
		solids	123	4	87	4	79	4	1.2E+06	8.7E+05	7.9E+05	**9.6E+05**	#/gram
									6.8E+08	4.8E+08	4.3E+08	**5.3E+08**	
fungi	Czapek's A	liquid	5	4	8	4	4	4	5.0E+04	8.0E+04	4.0E+04	**5.7E+04**	#/L
		solids	25	4	28	4	32	4	2.5E+05	2.8E+05	3.2E+05	**2.8E+05**	#/gram
									1.4E+08	1.5E+08	1.8E+08	**1.6E+08**	
Yeast	Malt Extra	liquid	4	3	3	3	2	3	4.0E+03	3.0E+03	2.0E+03	**3.0E+03**	#/L
		solids	9	4	12	4	6	4	9.0E+04	1.2E+05	6.0E+04	**9.0E+04**	#/gram
									5.0E+07	6.6E+07	3.3E+07	**5.0E+07**	
Aerobic Fa	Chocholat	liquid	6	5	8	5	5	5	6.0E+05	8.0E+05	5.0E+05	**6.3E+05**	#/L
		solids	7	5	12	5	10	5	7.0E+05	1.2E+06	1.0E+06	**9.7E+05**	#/gram
									3.9E+08	6.6E+08	5.5E+08	**5.3E+08**	
Aerobic N	Nutrient A	liquid	14	4	15	4	12	4	1.4E+05	1.5E+05	1.2E+05	**1.4E+05**	#/L
		solids	23	5	12	5	11	5	2.3E+06	1.2E+06	1.1E+06	**1.5E+06**	#/gram
									1.3E+09	6.6E+08	6.1E+08	**8.4E+08**	

BIO 4 COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution					Average Number		UNITS
Facultative Aerobes	Thioglycolate Media														
		liquid	17	6	12	6	7	6	1.7E+07	1.2E+07	7.0E+06	**1.2E+07**			#/L
		solids	8	5	9	5	15	5	8.0E+05	9.0E+05	1.5E+06	**1.1E+06**			#/gram
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	210	5	28	6	25	6	2.1E+07	2.8E+07	2.5E+07	**2.5E+07**			#/L
		solids	39	7	48	7	54	7	3.9E+08	4.8E+08	5.4E+08	**4.7E+08**			#/gram
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	89	5	93	5	121	5	8.9E+06	9.3E+06	1.2E+07	**1.0E+07**	#/L		#/L
		solids	195	6	224	6	213	6	2.0E+08	2.2E+08	2.1E+08	**2.1E+08**	#/gram		#/gram
methanogens	methanogenic archaea	liquid	7	3	9	3	6	3	7.0E+03	9.0E+03	6.0E+03	**7.3E+03**	#/L		#/L
		solids	200	3	187	3	145	3	2.0E+05	1.9E+05	1.5E+05	**1.8E+05**	#/gram		#/gram
Selanate Reducing Bacteria		liquid	168	3	151	3	173	3	1.7E+05	1.5E+05	1.7E+05	**1.6E+05**	#/L		#/L
		solids	145	4	132	4	98	4	1.5E+06	1.3E+06	9.8E+05	**1.3E+06**	#/gram		#/gram
fungi	Czapek's Agar	liquid	4	4	5	4	4	4	4.0E+04	5.0E+04	4.0E+04	**4.3E+04**	#/L		#/L
		solids	17	4	19	4	8	4	1.7E+05	1.9E+05	8.0E+04	**1.5E+05**	#/gram		#/gram
Yeast	Malt Extract Agar	liquid	12	3	9	3	14	3	1.2E+04	9.0E+03	1.4E+04	**1.2E+04**	#/L		#/L
		solids	16	4	21	4	9	4	1.6E+05	2.1E+05	9.0E+04	**1.5E+05**	#/gram		#/gram
Aerobic Fastidious	Chocholate Agar	liquid	5	5	5	5	2	5	5.0E+05	5.0E+05	2.0E+05	**4.0E+05**	#/L		#/L
		solids	5	5	9	5	7	5	5.0E+05	9.0E+05	7.0E+05	**7.0E+05**	#/gram		#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	10	4	11	4	8	4	1.0E+05	1.1E+05	8.0E+04	**9.7E+04**	#/L		#/L
		solids	12	5	8	5	7	5	1.2E+06	8.0E+05	7.0E+05	**9.0E+05**	#/gram		
									6.6E+08	4.4E+08	3.9E+08	**5.0E+08**			

BIO 4 Middle			Count	Dilution	Count	Dilution	Count	Dilution				Average Number		UNITS
Facultativ	Thioglycolate Media													
		liquid	28	6	24	6	27	6	2.8E+07	2.4E+07	2.7E+07	**2.6E+07**		#/L
		solids	18	5	21	5	27	5	1.8E+06	2.1E+06	2.7E+06	**2.2E+06**		#/gram
Anaerobic	Tryptocas	liquid	51	5	45	6	61	6	5.1E+06	4.5E+07	6.1E+07	**3.7E+07**		#/L
		solids	31	7	34	7	43	7	3.1E+08	3.4E+08	4.3E+08	**3.6E+08**		#/gram
Sulfate Re	sulfate re	liquid	76	5	68	5	63	5	7.6E+06	6.8E+06	6.3E+06	**6.9E+06**	#/L	#/L
		solids	150	6	151	6	134	6	1.5E+08	1.5E+08	1.3E+08	**1.5E+08**	#/gram	#/gram
methanog	methanog	liquid	5	3	3	3	8	3	5.0E+03	3.0E+03	8.0E+03	**5.3E+03**	#/L	#/L
		solids	141	3	132	3	78	3	1.4E+05	1.3E+05	7.8E+04	**1.2E+05**	#/gram	#/gram
Selanate Reducing Ba		liquid	114	3	102	3	89	3	1.1E+05	1.0E+05	8.9E+04	**1.0E+05**	#/L	#/L
		solids	89	4	98	4	76	4	8.9E+05	9.8E+05	7.6E+05	**8.8E+05**	#/gram	#/gram
fungi	Czapek's A	liquid	3	4	16	4	11	4	3.0E+04	1.6E+05	1.1E+05	**1.0E+05**	#/L	#/L
		solids	28	4	24	4	14	4	2.8E+05	2.4E+05	1.4E+05	**2.2E+05**	#/gram	#/gram
Yeast	Malt Extra	liquid	24	3	34	3	31	3	2.4E+04	3.4E+04	3.1E+04	**3.0E+04**	#/L	#/L
		solids	32	4	29	4	18	4	3.2E+05	2.9E+05	1.8E+05	**2.6E+05**	#/gram	#/gram
Aerobic Fa	Chocholat	liquid	13	5	9	5	21	5	1.3E+06	9.0E+05	2.1E+06	**1.4E+06**	#/L	#/L
		solids	16	5	21	5	19	5	1.6E+06	2.1E+06	1.9E+06	**1.9E+06**	#/gram	#/gram
Aerobic N	Nutrient A	liquid	18	4	17	4	23	4	1.8E+05	1.7E+05	2.3E+05	**1.9E+05**	#/L	#/L
		solids	21	5	18	5	15	5	2.1E+06	1.8E+06	1.5E+06	**1.8E+06**	#/gram	#/gram
									1.2E+09	9.9E+08	8.3E+08	**9.9E+08**		

BIO 4Effluent			Count	Dilution	Count	Dilution	Count	Dilution				Average Number		UNITS
Facultativ	Thioglycolate Media													
		liquid	56	6	42	6	35	6	5.6E+07	4.2E+07	3.5E+07	**4.4E+07**		#/L
		solids	58	5	44	5	42	5	5.8E+06	4.4E+06	4.2E+06	**4.8E+06**		#/gram
Anaerobic	Tryptocas	liquid	154	6	185	6	132	6	1.5E+08	1.9E+08	1.3E+08	**1.6E+08**		#/L
		solids	57	7	69	7	56	7	5.7E+08	6.9E+08	5.6E+08	**6.1E+08**		#/gram
Sulfate Re	sulfate re	liquid	154	5	131	5	128	5	1.5E+07	1.3E+07	1.3E+07	**1.4E+07**	#/L	#/L
		solids	226	6	198	6	214	6	2.3E+08	2.0E+08	2.1E+08	**2.1E+08**	#/gram	#/gram
methanog	methanog	liquid	3	3	2	3	3	3	3.0E+03	2.0E+03	3.0E+03	**2.7E+03**	#/L	#/L
		solids	43	3	56	3	43	3	4.3E+04	5.6E+04	4.3E+04	**4.7E+04**	#/gram	#/gram
Selanate	Reducing Ba	liquid	87	3	67	3	55	3	8.7E+04	6.7E+04	5.5E+04	**7.0E+04**	#/L	#/L
		solids	57	4	65	4	51	4	5.7E+05	6.5E+05	5.1E+05	**5.8E+05**	#/gram	#/gram
fungi	Czapek's A	liquid	8	4	21	4	23	4	8.0E+04	2.1E+05	2.3E+05	**1.7E+05**	#/L	#/L
		solids	36	4	34	4	28	4	3.6E+05	3.4E+05	2.8E+05	**3.3E+05**	#/gram	#/gram
Yeast	Malt Extra	liquid	31	3	38	3	32	3	3.1E+04	3.8E+04	3.2E+04	**3.4E+04**	#/L	#/L
		solids	26	4	21	4	24	4	2.6E+05	2.1E+05	2.4E+05	**2.4E+05**	#/gram	#/gram
Aerobic Fa	Chocholat	liquid	21	5	21	5	35	5	2.1E+06	2.1E+06	3.5E+06	**2.6E+06**	#/L	#/L
		solids	28	5	40	5	32	5	2.8E+06	4.0E+06	3.2E+06	**3.3E+06**	#/gram	#/gram
Aerobic N	Nutrient A	liquid	24	4	32	4	38	4	2.4E+05	3.2E+05	3.8E+05	**3.1E+05**	#/L	#/L
		solids	27	5	25	5	31	5	2.7E+06	2.5E+06	3.1E+06	**2.8E+06**	#/gram	#/gram
									1.5E+09	1.4E+09	1.7E+09	1.5E+09		

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BIO 2 INFLUENT														
			Count	Dilution	Count	Dilution	Count	Dilution					Average Number	UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	43	6	41	6	48	6	4.3E+07	4.1E+07	4.8E+07		**4.4E+07**	#/L
		solids	38	6	41	6	50	6	3.8E+07	4.1E+07	5.0E+07		**4.3E+07**	#/gram
									2.1E+10	2.3E+10	2.8E+10		**2.4E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar		65	6	55	6	52	6	6.5E+07	5.5E+07	5.2E+07		**5.7E+07**	#/L
			59	6	67	6	58	6	5.9E+07	6.7E+07	5.8E+07		**6.1E+07**	#/gram
									3.2E+10	3.7E+10	3.2E+10		**3.4E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar plates		8	5	11	5	6	5	8.0E+05	1.1E+06	6.0E+05		**8.3E+05**	#/L
			30	5	32	5	34	5	3.0E+06	3.2E+06	3.4E+06		**3.2E+06**	#/gram
									1.7E+09	1.8E+09	1.9E+09		**1.8E+09**	
methanogens	methanogenic archaea		0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00		**0.0E+00**	#/L
			8	3	15	3	11	3	8.0E+03	1.5E+04	1.1E+04		**1.1E+04**	#/gram
									4.4E+06	8.3E+06	6.1E+06		**6.2E+06**	
Selanate Reducing Bacteria			9	3	21	3	23	3	9.0E+03	2.1E+04	2.3E+04		**1.8E+04**	#/L
			48	4	56	4	53	4	4.8E+05	5.6E+05	5.3E+05		**5.2E+05**	#/gram
									2.6E+08	3.1E+08	2.9E+08		**2.9E+08**	
fungi	Czapek's Agar		53	4	46	4	44	4	5.3E+05	4.6E+05	4.4E+05		**4.8E+05**	#/L
			21	5	17	5	24	5	2.1E+06	1.7E+06	2.4E+06		**2.1E+06**	#/gram
									1.2E+09	9.4E+08	1.3E+09		**1.1E+09**	
Yeast	Malt Extract Agar		97	2	89	2	58	2	9.7E+03	8.9E+03	5.8E+03		**8.1E+03**	#/L
			65	4	61	4	79	4	6.5E+05	6.1E+05	7.9E+05		**6.8E+05**	#/gram
									3.6E+08	3.4E+08	4.3E+08		**3.8E+08**	
Aerobic Fastidious	Chocholate Agar		74	6	66	6	78	6	7.4E+07	6.6E+07	7.8E+07		**7.3E+07**	#/L
			35	5	52	5	66	5	3.5E+06	5.2E+06	6.6E+06		**5.1E+06**	#/gram
									1.9E+09	2.9E+09	3.6E+09		**2.8E+09**	
Aerobic Non Fastidious	Nutrient Agar		78	6	73	6	89	6	7.8E+07	7.3E+07	8.9E+07		**8.0E+07**	#/L
			75	6	89	6	92	6	7.5E+07	8.9E+07	9.2E+07		**8.5E+07**	#/gram
									4.1E+10	4.9E+10	5.1E+10		**4.7E+10**	

BIO 2 middle													
		Count	Dilution	Count	Dilution	Count	Dilution					Average N	UNITS
Facultative Aerobes	Thioglycolate Media												
	liquid	26	6	45	6	58	6	2.6E+07	4.5E+07	5.8E+07		**4.3E+07**	#/L
	solids	35	6	53	6	61	6	3.5E+07	5.3E+07	6.1E+07		**5.0E+07**	#/gram
								1.9E+10	2.9E+10	3.4E+10		**2.7E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	18	6	23	6	27	6	1.8E+07	2.3E+07	2.7E+07		**2.3E+07**	#/L
		89	6	78	6	94	6	8.9E+07	7.8E+07	9.4E+07		**8.7E+07**	#/gram
								4.9E+10	4.3E+10	5.2E+10		**4.8E+10**	
Sulfate Rducing Bacteria	sulfate reducing aga	26	5	43	5	47	5	2.6E+06	4.3E+06	4.7E+06		**3.9E+06**	#/L
		68	5	89	5	82	5	6.8E+06	8.9E+06	8.2E+06		**8.0E+06**	#/gram
								3.7E+09	4.9E+09	4.5E+09		**4.4E+09**	
methanogens	methanogenic archa	0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00		**0.0E+00**	#/L
		78	3	87	3	93	3	7.8E+04	8.7E+04	9.3E+04		**8.6E+04**	#/gram
								4.3E+07	4.8E+07	5.1E+07		**4.7E+07**	
Selanate Reducing Bacteria		13	4	15	4	11	4	1.3E+05	1.5E+05	1.1E+05		**1.3E+05**	#/L
		64	5	52	5	45	5	6.4E+06	5.2E+06	4.5E+06		**5.4E+06**	#/gram
								3.5E+09	2.9E+09	2.5E+09		**3.0E+09**	
fungi	Czapek's Agar	52	4	43	4	33	4	5.2E+05	4.3E+05	3.3E+05		**4.3E+05**	#/L
		32	5	28	5	16	5	3.2E+06	2.8E+06	1.6E+06		**2.5E+06**	#/gram
								1.8E+09	1.5E+09	8.8E+08		**1.4E+09**	
Yeast	Malt Extract Agar	32	3	24	3	22	3	3.2E+04	2.4E+04	2.2E+04		**2.6E+04**	#/L
		49	4	46	4	62	4	4.9E+05	4.6E+05	6.2E+05		**5.2E+05**	#/gram
								2.7E+08	2.5E+08	3.4E+08		**2.9E+08**	
Aerobic Fastidious	Chocholate Agar	16	7	18	7	21	7	1.6E+08	1.8E+08	2.1E+08		**1.8E+08**	#/L
		56	5	55	5	62	5	5.6E+06	5.5E+06	6.2E+06		**5.8E+06**	#/gram
								3.1E+09	3.0E+09	3.4E+09		**3.2E+09**	
Aerobic Non Fastidious	Nutrient Agar	75	6	66	6	52	6	7.5E+07	6.6E+07	5.2E+07		**6.4E+07**	#/L
		41	6	45	6	28	6	4.1E+07	4.5E+07	2.8E+07		**3.8E+07**	#/gram
								2.3E+10	2.5E+10	1.5E+10		**2.1E+10**	

BIO 2 EFFLUENT			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	56	6	62	6	51	6	5.6E+07	6.2E+07	5.1E+07	**5.6E+07**	#/L
		solids	78	5	82	5	68	6	7.8E+06	8.2E+06	6.8E+07	**2.8E+07**	#/gram
									4.3E+09	4.5E+09	3.7E+10	**1.5E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	31	6	52	6	62	6	3.1E+07	5.2E+07	6.2E+07	**4.8E+07**	#/L
		solids	17	7	18	7	29	7	1.7E+08	1.8E+08	2.9E+08	**2.1E+08**	#/gram
									9.4E+10	9.9E+10	1.6E+11	**1.2E+11**	
Sulfate Rducing Bacteria	sulfate reducing agar plates	liquid	46	5	61	5	52	5	4.6E+06	6.1E+06	5.2E+06	**5.3E+06**	#/L
		solids	98	6	74	6	82	6	9.8E+07	7.4E+07	8.2E+07	**8.5E+07**	#/gram
									5.4E+10	4.1E+10	4.5E+10	**4.7E+10**	
methanogens	methanogenic archaea	liquid	0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
		solids	57	3	85	3	92	3	5.7E+04	8.5E+04	9.2E+04	**7.8E+04**	#/gram
									3.1E+07	4.7E+07	5.1E+07	**4.3E+07**	
Selanate Reducing Bacteria	senanate reducing bacteria	liquid	91	4	89	4	108	4	9.1E+05	8.9E+05	1.1E+06	**9.6E+05**	#/L
		solids	22	6	16	6	24	6	2.2E+07	1.6E+07	2.4E+07	**2.1E+07**	#/gram
									1.2E+10	8.8E+09	1.3E+10	**1.1E+10**	
fungi	Czapek's Agar	liquid	78	4	72	4	68	4	7.8E+05	7.2E+05	6.8E+05	**7.3E+05**	#/L
		solids	95	4	82	4	91	4	9.5E+05	8.2E+05	9.1E+05	**8.9E+05**	#/gram
									5.2E+08	4.5E+08	5.0E+08	**4.9E+08**	
Yeast	Malt Extract Agar	liquid	67	4	96	4	89	4	6.7E+05	9.6E+05	8.9E+05	**8.4E+05**	#/L
		solids	27	4	32	4	30	4	2.7E+05	3.2E+05	3.0E+05	**3.0E+05**	#/gram
									1.5E+08	1.8E+08	1.7E+08	**1.6E+08**	
Aerobic Fastidious	Chocholate Agar	liquid	27	6	31	6	24	6	2.7E+07	3.1E+07	2.4E+07	**2.7E+07**	#/L
		solids	67	4	61	4	58	4	6.7E+05	6.1E+05	5.8E+05	**6.2E+05**	#/gram
									3.7E+08	3.4E+08	3.2E+08	**3.4E+08**	
Aerobic Non Fastidious	Nutrient Agar	liquid	98	6	78	6	71	6	9.8E+07	7.8E+07	7.1E+07	**8.2E+07**	#/L
		solids	40	5	34	5	32	5	4.0E+06	3.4E+06	3.2E+06	**3.5E+06**	#/gram
									2.2E+09	1.9E+09	1.8E+09	**1.9E+09**	

Bio 3 Influent			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	38	4	55	4	34	4	3.8E+05	5.5E+05	3.4E+05	**4.2E+05**	#/L
		solids	45	5	38	5	34	5	4.5E+06	3.8E+06	3.4E+06	**3.9E+06**	#/gram
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	28	7	24	7	21	7	2.8E+08	2.4E+08	2.1E+08	**2.4E+08**	#/L
		solids	48	8	45	8	40	8	4.8E+09	4.5E+09	4.0E+09	**4.4E+09**	#/gram
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	41	5	47	5	49	5	4.1E+06	4.7E+06	4.9E+06	**4.6E+06**	#/L
		solids	26	7	23	7	31	7	2.6E+08	2.3E+08	3.1E+08	**2.7E+08**	#/gram
methanogens	methanogenic archaea	liquid	28	3	23	3	25	3	2.8E+04	2.3E+04	2.5E+04	**2.5E+04**	#/L
		solids	22	4	33	4	27	4	2.2E+05	3.3E+05	2.7E+05	**2.7E+05**	#/gram
Selanate Reducing Bacteria		liquid	95	3	83	3	97	3	9.5E+04	8.3E+04	9.7E+04	**9.2E+04**	#/L
		solids	20	6	26	6	32	6	2.0E+07	2.6E+07	3.2E+07	**2.6E+07**	#/gram
fungi	Czapek's Agar	liquid	18	4	11	4	15	4	1.8E+05	1.1E+05	1.5E+05	**1.5E+05**	#/L
		solids	48	4	52	4	56	4	4.8E+05	5.2E+05	5.6E+05	**5.2E+05**	#/gram
Yeast	Malt Extract Agar	liquid	7	3	15	3	6	3	7.0E+03	1.5E+04	6.0E+03	**9.3E+03**	#/L
		solids	19	4	28	4	23	4	1.9E+05	2.8E+05	2.3E+05	**2.3E+05**	#/gram
Aerobic Fastidious	Chocholate Agar	liquid	15	5	19	5	28	5	1.5E+06	1.9E+06	2.8E+06	**2.1E+06**	#/L
		solids	19	5	28	5	17	5	1.9E+06	2.8E+06	1.7E+06	**2.1E+06**	#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	25	4	31	4	25	4	2.5E+05	3.1E+05	2.5E+05	**2.7E+05**	#/L
		solids	21	5	17	5	19	5	2.1E+06	1.7E+06	1.9E+06	**1.9E+06**	#/gram

BIO 3 MID COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution				Average N	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	36	4	43	4	44	4	3.6E+05	4.3E+05	4.4E+05	**4.1E+05**	#/L
		solids	4	5	72	4	35	4	4.0E+05	7.2E+05	3.5E+05	**4.9E+05**	#/gram
Anaerobic Heterotrc	Tryptocas	liquid	13	7	18	7	19	7	1.3E+08	1.8E+08	1.9E+08	**1.7E+08**	#/L
		solids	21	8	25	8	22	8	2.1E+09	2.5E+09	2.2E+09	**2.3E+09**	#/gram
Sulfate Reducing Ba	sulfate re	liquid	109	5	135	5	18	6	1.1E+07	1.4E+07	1.8E+07	**1.4E+07**	#/L
		solids	23	7	21	7	19	7	2.3E+08	2.1E+08	1.9E+08	**2.1E+08**	#/gram
methanogens	methanog	liquid	25	3	25	3	29	3	2.5E+04	2.5E+04	2.9E+04	**2.6E+04**	#/L
		solids	48	4	67	4	38	4	4.8E+05	6.7E+05	3.8E+05	**5.1E+05**	#/gram
Selanate Reducing Bacteria		liquid	14	4	18	4	21	4	1.4E+05	1.8E+05	2.1E+05	**1.8E+05**	#/L
		solids	38	5	39	5	27	5	3.8E+06	3.9E+06	2.7E+06	**3.5E+06**	#/gram
fungi	Czapek's A	liquid	9	4	8	4	6	4	9.0E+04	8.0E+04	6.0E+04	**7.7E+04**	#/L
		solids	29	4	39	4	26	4	2.9E+05	3.9E+05	2.6E+05	**3.1E+05**	#/gram
Yeast	Malt Extra	liquid	11	3	9	3	7	3	1.1E+04	9.0E+03	7.0E+03	**9.0E+03**	#/L
		solids	43	4	37	4	41	4	4.3E+05	3.7E+05	4.1E+05	**4.0E+05**	#/gram
Aerobic Fastidious	Chocholat	liquid	45	5	23	5	19	5	4.5E+06	2.3E+06	1.9E+06	**2.9E+06**	#/L
		solids	19	5	24	5	21	5	1.9E+06	2.4E+06	2.1E+06	**2.1E+06**	#/gram
Aerobic Non Fastidic	Nutrient A	liquid	23	4	31	4	18	4	2.3E+05	3.1E+05	1.8E+05	**2.4E+05**	#/L
		solids	34	5	31	5	26	5	3.4E+06	3.1E+06	2.6E+06	**3.0E+06**	#/gram

BIO 3 effluent			Count	Dilution	Count	Dilution	Count	Dilution				Average N	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	46	4	42	4	38	4	4.6E+05	4.2E+05	3.8E+05	**4.2E+05**	#/L
		solids	79	4	74	4	84	4	7.9E+05	7.4E+05	8.4E+05	**7.9E+05**	#/gram
Anaerobic Heterotrop	Tryptocas	liquid	12	7	19	7	21	7	1.2E+08	1.9E+08	2.1E+08	**1.7E+08**	#/L
		solids	22	8	19	8	26	8	2.2E+09	1.9E+09	2.6E+09	**2.2E+09**	#/gram
Sulfate Reducing Bact	sulfate re	liquid	95	5	108	5	112	5	9.5E+06	1.1E+07	1.1E+07	**1.1E+07**	#/L
		solids	29	7	23	7	28	7	2.9E+08	2.3E+08	2.8E+08	**2.7E+08**	#/gram
methanogens	methanog	liquid	19	3	26	3	29	3	1.9E+04	2.6E+04	2.9E+04	**2.5E+04**	#/L
		solids	41	4	37	4	35	4	4.1E+05	3.7E+05	3.5E+05	**3.8E+05**	#/gram
Selanate Reducing Bacteria		liquid	16	4	21	4	20	4	1.6E+05	2.1E+05	2.0E+05	**1.9E+05**	#/L
		solids	18	5	21	5	26	5	1.8E+06	2.1E+06	2.6E+06	**2.2E+06**	#/gram
fungi	Czapek's A	liquid	8	4	14	4	11	4	8.0E+04	1.4E+05	1.1E+05	**1.1E+05**	#/L
		solids	42	4	39	4	51	4	4.2E+05	3.9E+05	5.1E+05	**4.4E+05**	#/gram
Yeast	Malt Extra	liquid	9	3	8	3	5	3	9.0E+03	8.0E+03	5.0E+03	**7.3E+03**	#/L
		solids	18	4	23	4	15	4	1.8E+05	2.3E+05	1.5E+05	**1.9E+05**	#/gram
Aerobic Fastidious	Chocholat	liquid	9	5	14	5	21	5	9.0E+05	1.4E+06	2.1E+06	**1.5E+06**	#/L
		solids	16	5	21	5	15	5	1.6E+06	2.1E+06	1.5E+06	**1.7E+06**	#/gram
Aerobic Non Fastidiou	Nutrient A	liquid	25	4	29	4	25	4	2.5E+05	2.9E+05	2.5E+05	**2.6E+05**	#/L
		solids	45	5	38	5	32	5	4.5E+06	3.8E+06	3.2E+06	**3.8E+06**	#/gram

BIO 4 COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution				Average Number		UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	28	6	14	6	15	6	2.8E+07	1.4E+07	1.5E+07	**1.9E+07**		#/L
		solids	21	5	11	5	24	5	2.1E+06	1.1E+06	2.4E+06	**1.9E+06**		#/gram
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	17	6	19	6	19	6	1.7E+07	1.9E+07	1.9E+07	**1.8E+07**		#/L
		solids	49	7	55	7	54	7	4.9E+08	5.5E+08	5.4E+08	**5.3E+08**		#/gram
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	78	5	85	5	93	5	7.8E+06	8.5E+06	9.3E+06	**8.5E+06**	#/L	#/L
		solids	23	7	39	7	34	7	2.3E+08	3.9E+08	3.4E+08	**3.2E+08**	#/gram	#/gram
methanogens	methanogenic archaea	liquid	15	3	11	3	11	3	1.5E+04	1.1E+04	1.1E+04	**1.2E+04**	#/L	#/L
		solids	27	4	29	4	32	4	2.7E+05	2.9E+05	3.2E+05	**2.9E+05**	#/gram	#/gram
Selanate Reducing Bacteria		liquid	25	4	21	3	17	4	2.5E+05	2.1E+04	1.7E+05	**1.5E+05**	#/L	#/L
		solids	20	5	19	5	26	5	2.0E+06	1.9E+06	2.6E+06	**2.2E+06**	#/gram	#/gram
fungi	Czapek's Agar	liquid	8	4	6	4	8	4	8.0E+04	6.0E+04	8.0E+04	**7.3E+04**	#/L	#/L
		solids	24	4	26	4	21	4	2.4E+05	2.6E+05	2.1E+05	**2.4E+05**	#/gram	#/gram
Yeast	Malt Extract Agar	liquid	19	3	17	3	19	3	1.9E+04	1.7E+04	1.9E+04	**1.8E+04**	#/L	#/L
		solids	25	4	22	4	15	4	2.5E+05	2.2E+05	1.5E+05	**2.1E+05**	#/gram	#/gram
Aerobic Fastidious	Chocholate Agar	liquid	9	5	8	5	6	5	9.0E+05	8.0E+05	6.0E+05	**7.7E+05**	#/L	#/L
		solids	11	5	13	5	8	5	1.1E+06	1.3E+06	8.0E+05	**1.1E+06**	#/gram	#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	15	4	19	4	21	4	1.5E+05	1.9E+05	2.1E+05	**1.8E+05**	#/L	#/L
		solids	18	5	15	5	15	5	1.8E+06	1.5E+06	1.5E+06	**1.6E+06**	#/gram	

BIO 4 Middle			Count	Dilution	Count	Dilution	Count	Dilution				Average Number		UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	39	6	33	6	17	6	3.9E+07	3.3E+07	1.7E+07	**3.0E+07**		#/L
		solids	15	5	17	5	21	5	1.5E+06	1.7E+06	2.1E+06	**1.8E+06**		#/gram
Anaerobic Heterotrophs	Tryptocase Soy A	liquid	58	5	51	6	49	6	5.8E+06	5.1E+07	4.9E+07	**3.5E+07**		#/L
		solids	27	7	25	7	32	7	2.7E+08	2.5E+08	3.2E+08	**2.8E+08**		#/gram
Sulfate Reducing Bacteria	sulfate reducing a	liquid	68	5	75	5	81	5	6.8E+06	7.5E+06	8.1E+06	**7.5E+06**	#/L	#/L
		solids	18	7	23	7	19	7	1.8E+08	2.3E+08	1.9E+08	**2.0E+08**	#/gram	#/gram
methanogens	methanogenic ar	liquid	9	3	5	3	9	3	9.0E+03	5.0E+03	9.0E+03	**7.7E+03**	#/L	#/L
		solids	21	4	18	4	19	4	2.1E+05	1.8E+05	1.9E+05	**1.9E+05**	#/gram	#/gram
Selanate Reducing Bacteria		liquid	14	4	18	4	11	4	1.4E+05	1.8E+05	1.1E+05	**1.4E+05**	#/L	#/L
		solids	9	5	11	5	12	5	9.0E+05	1.1E+06	1.2E+06	**1.1E+06**	#/gram	#/gram
fungi	Czapek's Agar	liquid	7	4	9	4	3	4	7.0E+04	9.0E+04	3.0E+04	**6.3E+04**	#/L	#/L
		solids	23	4	25	4	19	4	2.3E+05	2.5E+05	1.9E+05	**2.2E+05**	#/gram	#/gram
Yeast	Malt Extract Agar	liquid	22	3	25	3	24	3	2.2E+04	2.5E+04	2.4E+04	**2.4E+04**	#/L	#/L
		solids	38	4	34	4	27	4	3.8E+05	3.4E+05	2.7E+05	**3.3E+05**	#/gram	#/gram
Aerobic Fastidious	Chocholate Agar	liquid	21	5	15	5	16	5	2.1E+06	1.5E+06	1.6E+06	**1.7E+06**	#/L	#/L
		solids	26	5	29	5	25	5	2.6E+06	2.9E+06	2.5E+06	**2.7E+06**	#/gram	#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	23	4	28	4	27	4	2.3E+05	2.8E+05	2.7E+05	**2.6E+05**	#/L	#/L
		solids	26	5	24	5	27	5	2.6E+06	2.4E+06	2.7E+06	**2.6E+06**	#/gram	#/gram

BIO 4Effluent			Count	Dilution	Count	Dilution	Count	Dilution					Average Number		UNITS
Facultative Aerobes	Thioglycolate Media														
		liquid	51	6	58	6	47	6	5.1E+07	5.8E+07	4.7E+07	**5.2E+07**			#/L
		solids	51	5	46	5	38	5	5.1E+06	4.6E+06	3.8E+06	**4.5E+06**			#/gram
Anaerobic Heterotrop	Tryptocase Soy Agar	liquid	108	6	135	6	113	6	1.1E+08	1.4E+08	1.1E+08	**1.2E+08**			#/L
		solids	52	7	54	7	49	7	5.2E+08	5.4E+08	4.9E+08	**5.2E+08**			#/gram
Sulfate Reducing Bact	sulfate reducing agar plates	liquid	11	6	12	6	10	6	1.1E+07	1.2E+07	1.0E+07	**1.1E+07**	#/L		#/L
		solids	18	7	17	7	20	7	1.8E+08	1.7E+08	2.0E+08	**1.8E+08**	#/gram		#/gram
methanogens	methanogenic archaea	liquid	5	3	6	3	9	3	5.0E+03	6.0E+03	9.0E+03	**6.7E+03**	#/L		#/L
		solids	67	3	89	3	72	3	6.7E+04	8.9E+04	7.2E+04	**7.6E+04**	#/gram		#/gram
Selanate Reducing Bacteria		liquid	92	3	78	3	69	3	9.2E+04	7.8E+04	6.9E+04	**8.0E+04**	#/L		#/L
		solids	62	4	71	4	67	4	6.2E+05	7.1E+05	6.7E+05	**6.7E+05**	#/gram		#/gram
fungi	Czapek's Agar	liquid	9	4	14	4	16	4	9.0E+04	1.4E+05	1.6E+05	**1.3E+05**	#/L		#/L
		solids	31	4	26	4	22	4	3.1E+05	2.6E+05	2.2E+05	**2.6E+05**	#/gram		#/gram
Yeast	Malt Extract Agar	liquid	24	3	21	3	18	3	2.4E+04	2.1E+04	1.8E+04	**2.1E+04**	#/L		#/L
		solids	21	4	15	4	18	4	2.1E+05	1.5E+05	1.8E+05	**1.8E+05**	#/gram		#/gram
Aerobic Fastidious	Chocholate Agar	liquid	32	5	26	5	41	5	3.2E+06	2.6E+06	4.1E+06	**3.3E+06**	#/L		#/L
		solids	38	5	45	5	42	5	3.8E+06	4.5E+06	4.2E+06	**4.2E+06**	#/gram		#/gram
Aerobic Non Fastidio	Nutrient Agar	liquid	31	4	39	4	37	4	3.1E+05	3.9E+05	3.7E+05	**3.6E+05**	#/L		#/L
		solids	36	5	44	5	38	5	3.6E+06	4.4E+06	3.8E+06	**3.9E+06**	#/gram		#/gram

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BIO 2 INFLUENT														
			Count	Dilution	Count	Dilution	Count	Dilution					Average Number	UNITS
Facultative Aerobes	Thioglycolate Media													
		liquid	34	6	22	6	21	6		3.4E+07	2.2E+07	2.1E+07	**2.6E+07**	#/L
		solids	24	6	32	6	41	6		2.4E+07	3.2E+07	4.1E+07	**3.2E+07**	#/gram
										1.3E+10	1.8E+10	2.3E+10	**1.8E+10**	
Anaerobic Heterotrophs	Tryptocase Soy Agar		24	6	32	6	34	6		2.4E+07	3.2E+07	3.4E+07	**3.0E+07**	#/L
			23	6	44	6	23	6		2.3E+07	4.4E+07	2.3E+07	**3.0E+07**	#/gram
										1.3E+10	2.4E+10	1.3E+10	**1.7E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar plates		1	5	2	5	1	5		1.0E+05	2.0E+05	1.0E+05	**1.3E+05**	#/L
			23	5	19	5	12	5		2.3E+06	1.9E+06	1.2E+06	**1.8E+06**	#/gram
										1.3E+09	1.0E+09	6.6E+08	**9.9E+08**	
methanogens	methanogenic archaea		0	0	0	0	0	0		0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
			6	3	13	3	8	3		6.0E+03	1.3E+04	8.0E+03	**9.0E+03**	#/gram
										3.3E+06	7.2E+06	4.4E+06	**5.0E+06**	
Selanate Reducing Bacteria			6	3	8	3	15	3		6.0E+03	8.0E+03	1.5E+04	**9.7E+03**	#/L
			25	4	42	4	42	4		2.5E+05	4.2E+05	4.2E+05	**3.6E+05**	#/gram
										1.4E+08	2.3E+08	2.3E+08	**2.0E+08**	
fungi	Czapek's Agar		22	4	14	4	18	4		2.2E+05	1.4E+05	1.8E+05	**1.8E+05**	#/L
			8	5	7	5	14	5		8.0E+05	7.0E+05	1.4E+06	**9.7E+05**	#/gram
										4.4E+08	3.9E+08	7.7E+08	**5.3E+08**	
Yeast	Malt Extract Agar		33	2	21	2	45	2		3.3E+03	2.1E+03	4.5E+03	**3.3E+03**	#/L
			38	4	35	4	43	4		3.8E+05	3.5E+05	4.3E+05	**3.9E+05**	#/gram
										2.1E+08	1.9E+08	2.4E+08	**2.1E+08**	
Aerobic Fastidious	Chocholate Agar		43	6	21	6	32	6		4.3E+07	2.1E+07	3.2E+07	**3.2E+07**	#/L
			16	5	20	5	25	5		1.6E+06	2.0E+06	2.5E+06	**2.0E+06**	#/gram
										8.8E+08	1.1E+09	1.4E+09	**1.1E+09**	
Aerobic Non Fastidious	Nutrient Agar		45	6	36	6	32	6		4.5E+07	3.6E+07	3.2E+07	**3.8E+07**	#/L
			27	6	31	6	28	6	750000	2.7E+07	3.1E+07	2.8E+07	**2.9E+07**	#/gram
										1.5E+10	1.7E+10	1.5E+10	**1.6E+10**	

BIO 2 middle													
			Count	Dilution	Count	Dilution	Count	Dilution					Average N	UNITS
Facultativ	Thioglycolate Media												
		liquid	23	6	24	6	32	6		2.3E+07	2.4E+07	3.2E+07	**2.6E+07**	#/L
		solids	16	6	18	6	21	6		1.6E+07	1.8E+07	2.1E+07	**1.8E+07**	#/gram
										8.8E+09	9.9E+09	1.2E+10	**1.0E+10**	
Anaerobic	Tryptocase Soy Agar		8	6	9	6	11	6		8.0E+06	9.0E+06	1.1E+07	**9.3E+06**	#/L
			41	6	24	6	38	6		4.1E+07	2.4E+07	3.8E+07	**3.4E+07**	#/gram
										2.3E+10	1.3E+10	2.1E+10	**1.9E+10**	
Sulfate Rd	sulfate reducing aga		11	5	13	5	16	5		1.1E+06	1.3E+06	1.6E+06	**1.3E+06**	#/L
			24	5	26	5	31	5		2.4E+06	2.6E+06	3.1E+06	**2.7E+06**	#/gram
										1.3E+09	1.4E+09	1.7E+09	**1.5E+09**	
methanog	methanogenic archa		0	0	0	0	0	0		0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
			32	3	36	3	24	3		3.2E+04	3.6E+04	2.4E+04	**3.1E+04**	#/gram
										1.8E+07	2.0E+07	1.3E+07	**1.7E+07**	
Selanate	Reducing Bacteria		17	3	21	3	33	3		1.7E+04	2.1E+04	3.3E+04	**2.4E+04**	#/L
			24	5	17	5	12	5		2.4E+06	1.7E+06	1.2E+06	**1.8E+06**	#/gram
										1.3E+09	9.4E+08	6.6E+08	**9.7E+08**	
fungi	Czapek's Agar		45	4	31	4	26	4		4.5E+05	3.1E+05	2.6E+05	**3.4E+05**	#/L
			55	4	35	4	56	4		5.5E+05	3.5E+05	5.6E+05	**4.9E+05**	#/gram
										3.0E+08	1.9E+08	3.1E+08	**2.7E+08**	
Yeast	Malt Extract Agar		29	3	23	3	27	3		2.9E+04	2.3E+04	2.7E+04	**2.6E+04**	#/L
			21	4	26	4	22	4		2.1E+05	2.6E+05	2.2E+05	**2.3E+05**	#/gram
										1.2E+08	1.4E+08	1.2E+08	**1.3E+08**	
Aerobic Fa	Chocholate Agar		55	6	65	6	59	6		5.5E+07	6.5E+07	5.9E+07	**6.0E+07**	#/L
			24	5	18	5	32	5		2.4E+06	1.8E+06	3.2E+06	**2.5E+06**	#/gram
										1.3E+09	9.9E+08	1.8E+09	**1.4E+09**	
Aerobic N	Nutrient Agar		32	6	20	6	21	6		3.2E+07	2.0E+07	2.1E+07	**2.4E+07**	#/L
			15	6	22	6	11	6		1.5E+07	2.2E+07	1.1E+07	**1.6E+07**	#/gram
										8.3E+09	1.2E+10	6.1E+09	**8.8E+09**	

BIO 2 EFFLUENT			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	23	6	16	6	21	6	2.3E+07	1.6E+07	2.1E+07	**2.0E+07**	#/L
		solids	21	5	23	5	32	6	2.1E+06	2.3E+06	3.2E+07	**1.2E+07**	#/gram
									1.2E+09	1.3E+09	1.8E+10	**6.7E+09**	
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	11	6	8	6	24	6	1.1E+07	8.0E+06	2.4E+07	**1.4E+07**	#/L
		solids	12	7	9	7	12	7	1.2E+08	9.0E+07	1.2E+08	**1.1E+08**	#/gram
									6.6E+10	5.0E+10	6.6E+10	**6.1E+10**	
Sulfate Rducing Bacteria	sulfate reducing agar plates	liquid	13	5	15	5	10	5	1.3E+06	1.5E+06	1.0E+06	**1.3E+06**	#/L
		solids	14	6	21	6	32	6	1.4E+07	2.1E+07	3.2E+07	**2.2E+07**	#/gram
									7.7E+09	1.2E+10	1.8E+10	**1.2E+10**	
methanogens	methanogenic archaea	liquid	0	0	0	0	0	0	0.0E+00	0.0E+00	0.0E+00	**0.0E+00**	#/L
		solids	11	3	7	3	4	3	1.1E+04	7.0E+03	4.0E+03	**7.3E+03**	#/gram
									6.1E+06	3.9E+06	2.2E+06	**4.0E+06**	
Selanate Reducing Bacteria	senanate reducing bacteria	liquid	22	4	15	4	18	4	2.2E+05	1.5E+05	1.8E+05	**1.8E+05**	#/L
		solids	27	5	36	5	45	5	2.7E+06	3.6E+06	4.5E+06	**3.6E+06**	#/gram
									1.5E+09	2.0E+09	2.5E+09	**2.0E+09**	
fungi	Czapek's Agar	liquid	21	4	15	4	16	4	2.1E+05	1.5E+05	1.6E+05	**1.7E+05**	#/L
		solids	34	4	26	4	22	4	3.4E+05	2.6E+05	2.2E+05	**2.7E+05**	#/gram
									1.9E+08	1.4E+08	1.2E+08	**1.5E+08**	
Yeast	Malt Extract Agar	liquid	12	4	8	4	14	4	1.2E+05	8.0E+04	1.4E+05	**1.1E+05**	#/L
		solids	7	4	78	3	67	3	7.0E+04	7.8E+04	6.7E+04	**7.2E+04**	#/gram
									3.9E+07	4.3E+07	3.7E+07	**3.9E+07**	
Aerobic Fastidious	Chocholate Agar	liquid	56	5	74	5	39	5	5.6E+06	7.4E+06	3.9E+06	**5.6E+06**	#/L
		solids	19	4	16	4	12	4	1.9E+05	1.6E+05	1.2E+05	**1.6E+05**	#/gram
									1.0E+08	8.8E+07	6.6E+07	**8.6E+07**	
Aerobic Non Fastidious	Nutrient Agar	liquid	22	6	21	6	17	6	2.2E+07	2.1E+07	1.7E+07	**2.0E+07**	#/L
		solids	9	5	13	5	5	5	9.0E+05	1.3E+06	5.0E+05	**9.0E+05**	#/gram
									5.0E+08	7.2E+08	2.8E+08	**5.0E+08**	

Bio 3 Influent			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultative Aerobes	Thioglycolate Media												
		liquid	5	4	6	4	5	4	5.0E+04	6.0E+04	5.0E+04	**5.3E+04**	#/L
		solids	29	5	36	4	56	4	2.9E+06	3.6E+05	5.6E+05	**1.3E+06**	#/gram
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	55	6	67	6	72	6	5.5E+07	6.7E+07	7.2E+07	**6.5E+07**	#/L
		solids	13	8	11	8	102	7	1.3E+09	1.1E+09	1.0E+09	**1.1E+09**	#/gram
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	13	5	16	5	21	5	1.3E+06	1.6E+06	2.1E+06	**1.7E+06**	#/L
		solids	69	6	54	6	68	6	6.9E+07	5.4E+07	6.8E+07	**6.4E+07**	#/gram
methanogens	methanogenic archaea	liquid	56	2	15	2	11	2	5.6E+03	1.5E+03	1.1E+03	**2.7E+03**	#/L
		solids	55	2	76	2	43	2	5.5E+03	7.6E+03	4.3E+03	**5.8E+03**	#/gram
Selanate Reducing Bacteria		liquid	28	3	24	3	32	3	2.8E+04	2.4E+04	3.2E+04	**2.8E+04**	#/L
		solids	81	5	54	5	43	5	8.1E+06	5.4E+06	4.3E+06	**5.9E+06**	#/gram
fungi	Czapek's Agar	liquid	17	3	21	3	13	3	1.7E+04	2.1E+04	1.3E+04	**1.7E+04**	#/L
		solids	11	4	10	4	5	4	1.1E+05	1.0E+05	5.0E+04	**8.7E+04**	#/gram
Yeast	Malt Extract Agar	liquid	13	2	8	2	11	2	1.3E+03	8.0E+02	1.1E+03	**1.1E+03**	#/L
		solids	43	3	23	3	18	3	4.3E+04	2.3E+04	1.8E+04	**2.8E+04**	#/gram
Aerobic Fastidious	Chocholate Agar	liquid	4	5	7	5	6	5	4.0E+05	7.0E+05	6.0E+05	**5.7E+05**	#/L
		solids	3	5	5	5	3	5	3.0E+05	5.0E+05	3.0E+05	**3.7E+05**	#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	3	4	5	4	4	4	3.0E+04	5.0E+04	4.0E+04	**4.0E+04**	#/L
		solids	32	4	4	5	17	4	3.2E+05	4.0E+05	1.7E+05	**3.0E+05**	#/gram

BIO 3 MID COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution					Average N	UNITS
Facultativ	Thioglycolate Media													
		liquid	13	4	9	4	13	4	1.3E+05	9.0E+04	1.3E+05		**1.2E+05**	#/L
		solids	19	4	34	4	11	4	1.9E+05	3.4E+05	1.1E+05		**2.1E+05**	#/gram
Anaerobic	Tryptocas	liquid	43	6	35	6	61	6	4.3E+07	3.5E+07	6.1E+07		**4.6E+07**	#/L
		solids	46	7	21	7	78	7	4.6E+08	2.1E+08	7.8E+08		**4.8E+08**	#/gram
Sulfate Re	sulfate re	liquid	32	5	24	5	21	5	3.2E+06	2.4E+06	2.1E+06		**2.6E+06**	#/L
		solids	61	6	89	6	75	6	6.1E+07	8.9E+07	7.5E+07		**7.5E+07**	#/gram
methanog	methanog	liquid	5	3	8	3	10	3	5.0E+03	8.0E+03	1.0E+04		**7.7E+03**	#/L
		solids	16	4	22	4	12	4	1.6E+05	2.2E+05	1.2E+05		**1.7E+05**	#/gram
Selanate Reducing B		liquid	21	3	14	3	16	3	2.1E+04	1.4E+04	1.6E+04		**1.7E+04**	#/L
		solids	43	4	33	4	39	4	4.3E+05	3.3E+05	3.9E+05		**3.8E+05**	#/gram
fungi	Czapek's A	liquid	23	3	11	3	13	3	2.3E+04	1.1E+04	1.3E+04		**1.6E+04**	#/L
		solids	11	4	13	4	12	4	1.1E+05	1.3E+05	1.2E+05		**1.2E+05**	#/gram
Yeast	Malt Extra	liquid	8	2	16	2	11	2	8.0E+02	1.6E+03	1.1E+03		**1.2E+03**	#/L
		solids	12	4	11	4	5	4	1.2E+05	1.1E+05	5.0E+04		**9.3E+04**	#/gram
Aerobic Fa	Chocholat	liquid	6	5	7	5	3	5	6.0E+05	7.0E+05	3.0E+05		**5.3E+05**	#/L
		solids	12	4	14	4	23	4	1.2E+05	1.4E+05	2.3E+05		**1.6E+05**	#/gram
Aerobic N	Nutrient A	liquid	4	4	8	4	34	3	4.0E+04	8.0E+04	3.4E+04		**5.1E+04**	#/L
		solids	28	4	36	4	54	4	2.8E+05	3.6E+05	5.4E+05		**3.9E+05**	#/gram

BIO 3 effluent			Count	Dilution	Count	Dilution	Count	Dilution				Average Number	UNITS
Facultativ	Thioglycolate Media												
		liquid	11	4	6	4	9	4	1.1E+05	6.0E+04	9.0E+04	**8.7E+04**	#/L
		solids	11	4	16	4	13	4	1.1E+05	1.6E+05	1.3E+05	**1.3E+05**	#/gram
Anaerobic	Tryptocas	liquid	24	6	43	6	25	6	2.4E+07	4.3E+07	2.5E+07	**3.1E+07**	#/L
		solids	19	7	28	7	45	7	1.9E+08	2.8E+08	4.5E+08	**3.1E+08**	#/gram
Sulfate Re	sulfate re	liquid	21	5	15	5	19	5	2.1E+06	1.5E+06	1.9E+06	**1.8E+06**	#/L
		solids	29	6	28	6	34	6	2.9E+07	2.8E+07	3.4E+07	**3.0E+07**	#/gram
methanog	methanog	liquid	23	2	15	2	15	2	2.3E+03	1.5E+03	1.5E+03	**1.8E+03**	#/L
		solids	51	3	65	3	45	3	5.1E+04	6.5E+04	4.5E+04	**5.4E+04**	#/gram
Selanate Reducing B		liquid	11	3	23	3	14	3	1.1E+04	2.3E+04	1.4E+04	**1.6E+04**	#/L
		solids	16	4	15	4	21	4	1.6E+05	1.5E+05	2.1E+05	**1.7E+05**	#/gram
fungi	Czapek's A	liquid	11	3	9	3	21	3	1.1E+04	9.0E+03	2.1E+04	**1.4E+04**	#/L
		solids	7	4	14	4	11	4	7.0E+04	1.4E+05	1.1E+05	**1.1E+05**	#/gram
Yeast	Malt Extra	liquid	10	2	15	2	7	2	1.0E+03	1.5E+03	7.0E+02	**1.1E+03**	#/L
		solids	19	3	26	3	13	3	1.9E+04	2.6E+04	1.3E+04	**1.9E+04**	#/gram
Aerobic Fa	Chocholat	liquid	21	4	13	4	7	4	2.1E+05	1.3E+05	7.0E+04	**1.4E+05**	#/L
		solids	18	4	36	4	45	4	1.8E+05	3.6E+05	4.5E+05	**3.3E+05**	#/gram
Aerobic N	Nutrient A	liquid	12	3	15	3	23	3	1.2E+04	1.5E+04	2.3E+04	**1.7E+04**	#/L
		solids	7	5	4	5	45	4	7.0E+05	4.0E+05	4.5E+05	**5.2E+05**	#/gram

BIO 4 COMPOSITE			Count	Dilution	Count	Dilution	Count	Dilution					Average Number		UNITS
Facultative Aerobes	Thioglycolate Media														
		liquid	37	5	45	5	21	5		3.7E+06	4.5E+06	2.1E+06	**3.4E+06**		#/L
		solids	27	4	34	4	65	4		2.7E+05	3.4E+05	6.5E+05	**4.2E+05**		#/gram
Anaerobic Heterotrophs	Tryptocase Soy Agar	liquid	35	5	42	5	31	5		3.5E+06	4.2E+06	3.1E+06	**3.6E+06**		#/L
		solids	73	7	87	6	34	6		7.3E+08	8.7E+07	3.4E+07	**2.8E+08**		#/gram
Sulfate Reducing Bacteria	sulfate reducing agar plates	liquid	21	5	14	5	9	5		2.1E+06	1.4E+06	9.0E+05	**1.5E+06**	#/L	#/L
		solids	39	6	58	6	76	6		3.9E+07	5.8E+07	7.6E+07	**5.8E+07**	#/gram	#/gram
methanogens	methanogenic archaea	liquid	21	2	5	2	13	2		2.1E+03	5.0E+02	1.3E+03	**1.3E+03**	#/L	#/L
		solids	54	3	25	3	29	3		5.4E+04	2.5E+04	2.9E+04	**3.6E+04**	#/gram	#/gram
Selanate Reducing Bacteria		liquid	25	3	53	3	43	3		2.5E+04	5.3E+04	4.3E+04	**4.0E+04**	#/L	#/L
		solids	21	4	17	4	16	4		2.1E+05	1.7E+05	1.6E+05	**1.8E+05**	#/gram	#/gram
fungi	Czapek's Agar	liquid	94	2	67	2	11	3		9.4E+03	6.7E+03	1.1E+04	**9.0E+03**	#/L	#/L
		solids	43	3	23	3	38	3		4.3E+04	2.3E+04	3.8E+04	**3.5E+04**	#/gram	#/gram
Yeast	Malt Extract Agar	liquid	45	2	32	2	56	2		4.5E+03	3.2E+03	5.6E+03	**4.4E+03**	#/L	#/L
		solids	6	4	7	4	4	4		6.0E+04	7.0E+04	4.0E+04	**5.7E+04**	#/gram	#/gram
Aerobic Fastidious	Chocholate Agar	liquid	89	3	12	4	11	4		8.9E+04	1.2E+05	1.1E+05	**1.1E+05**	#/L	#/L
		solids	67	3	98	3	65	3		6.7E+04	9.8E+04	6.5E+04	**7.7E+04**	#/gram	#/gram
Aerobic Non Fastidious	Nutrient Agar	liquid	11	3	43	3	21	3		1.1E+04	4.3E+04	2.1E+04	**2.5E+04**	#/L	#/L
		solids	34	4	21	4	31	5		3.4E+05	2.1E+05	3.1E+06	**1.2E+06**	#/gram	

BIO 4 MID			Count	Dilution	Count	Dilution	Count	Dilution					Average Number		UNITS
Facultativ	Thioglycolate Media														
		liquid	13	5	78	4	19	4		1.3E+06	7.8E+05	1.9E+05	**7.6E+05**		#/L
		solids	32	4	15	4	22	4		3.2E+05	1.5E+05	2.2E+05	**2.3E+05**		#/gram
Anaerobic	Tryptocas	liquid	45	5	32	5	76	4		4.5E+06	3.2E+06	7.6E+05	**2.8E+06**		#/L
		solids	58	6	28	6	43	6		5.8E+07	2.8E+07	4.3E+07	**4.3E+07**		#/gram
Sulfate Re	sulfate re	liquid	38	4	24	4	43	4		3.8E+05	2.4E+05	4.3E+05	**3.5E+05**	#/L	#/L
		solids	13	6	21	6	9	6		1.3E+07	2.1E+07	9.0E+06	**1.4E+07**	#/gram	#/gram
methanog	methanog	liquid	2	2	1	2	2	2		2.0E+02	1.0E+02	2.0E+02	**1.7E+02**	#/L	#/L
		solids	7	3	8	3	4	3		7.0E+03	8.0E+03	4.0E+03	**6.3E+03**	#/gram	#/gram
Selanate Reducing B		liquid	11	3	7	3	6	3		1.1E+04	7.0E+03	6.0E+03	**8.0E+03**	#/L	#/L
		solids	54	3	7	4	5	4		5.4E+04	7.0E+04	5.0E+04	**5.8E+04**	#/gram	#/gram
fungi	Czapek's A	liquid	6	3	3	3	4	3		6.0E+03	3.0E+03	4.0E+03	**4.3E+03**	#/L	#/L
		solids	14	3	22	3	13	3		1.4E+04	2.2E+04	1.3E+04	**1.6E+04**	#/gram	#/gram
Yeast	Malt Extra	liquid	23	2	32	2	45	2		2.3E+03	3.2E+03	4.5E+03	**3.3E+03**	#/L	#/L
		solids	52	3	39	3	32	3		5.2E+04	3.9E+04	3.2E+04	**4.1E+04**	#/gram	#/gram
Aerobic F	Chocholat	liquid	21	4	34	4	24	4		2.1E+05	3.4E+05	2.4E+05	**2.6E+05**	#/L	#/L
		solids	33	4	36	4	43	4		3.3E+05	3.6E+05	4.3E+05	**3.7E+05**	#/gram	#/gram
Aerobic N	Nutrient A	liquid	18	3	23	3	13	3		1.8E+04	2.3E+04	1.3E+04	**1.8E+04**	#/L	#/L
		solids	61	4	54	4	24	4		6.1E+05	5.4E+05	2.4E+05	**4.6E+05**	#/gram	#/gram

BIO 4Effluent			Count	Dilution	Count	Dilution	Count	Dilution				Average Number		UNITS
Facultativ	Thioglycolate Media													
		liquid	56	6	42	6	35	6	5.6E+07	4.2E+07	3.5E+07	**4.4E+07**		#/L
		solids	58	5	44	5	42	5	5.8E+06	4.4E+06	4.2E+06	**4.8E+06**		#/gram
Anaerobic	Tryptocas	liquid	154	6	185	6	132	6	1.5E+08	1.9E+08	1.3E+08	**1.6E+08**		#/L
		solids	57	7	69	7	56	7	5.7E+08	6.9E+08	5.6E+08	**6.1E+08**		#/gram
Sulfate Re	sulfate re	liquid	154	5	131	5	128	5	1.5E+07	1.3E+07	1.3E+07	**1.4E+07**	#/L	#/L
		solids	226	6	198	6	214	6	2.3E+08	2.0E+08	2.1E+08	**2.1E+08**	#/gram	#/gram
methanog	methanog	liquid	3	3	2	3	3	3	3.0E+03	2.0E+03	3.0E+03	**2.7E+03**	#/L	#/L
		solids	43	3	56	3	43	3	4.3E+04	5.6E+04	4.3E+04	**4.7E+04**	#/gram	#/gram
Selanate	Reducing B	liquid	87	3	67	3	55	3	8.7E+04	6.7E+04	5.5E+04	**7.0E+04**	#/L	#/L
		solids	57	4	65	4	51	4	5.7E+05	6.5E+05	5.1E+05	**5.8E+05**	#/gram	#/gram
fungi	Czapek's A	liquid	8	4	21	4	23	4	8.0E+04	2.1E+05	2.3E+05	**1.7E+05**	#/L	#/L
		solids	36	4	34	4	28	4	3.6E+05	3.4E+05	2.8E+05	**3.3E+05**	#/gram	#/gram
Yeast	Malt Extra	liquid	31	3	38	3	32	3	3.1E+04	3.8E+04	3.2E+04	**3.4E+04**	#/L	#/L
		solids	26	4	21	4	24	4	2.6E+05	2.1E+05	2.4E+05	**2.4E+05**	#/gram	#/gram
Aerobic Fa	Chocholat	liquid	21	5	21	5	35	5	2.1E+06	2.1E+06	3.5E+06	**2.6E+06**	#/L	#/L
		solids	28	5	40	5	32	5	2.8E+06	4.0E+06	3.2E+06	**3.3E+06**	#/gram	#/gram
Aerobic N	Nutrient A	liquid	24	4	32	4	38	4	2.4E+05	3.2E+05	3.8E+05	**3.1E+05**	#/L	#/L
		solids	27	5	25	5	31	5	2.7E+06	2.5E+06	3.1E+06	**2.8E+06**	#/gram	#/gram

Appendix B Species Identification Data

August 2014

	Bio 2 Inlet	Bio 2 Outlet
Bacillus subtillus	5	7
Microbacterium arborescense	2	1
Enterobacter sp.	4	1
Psuedomonas stutzari	8	2
Desulfomusa spp.	0	3
Desulfomicrobium (norvegicum?)	0	4
Desulfovibrio desulfricans	3	8
Desulfobacterium spp.	4	12
geovibrio spp.	0	2
Shewanella putrifaciens	3	5
unidentified Se Reducers	18	5
Total Selenium Reducers Tested	47	50

November 2014

	Bio 2 Inlet	Bio 2 Middle	Bio 2 Outlet	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	9	8	5	2	2	3	2	6	7
Microbacterium arborescense	5	4	1	0	0	0	0	0	1
Enterobacter sp.	7	3	3	0	0	0	0	0	0
Psuedomonas stutzari	9	6	4	1	2	1	1	2	3
Desulfomusa spp.	0	2	3	6	8	5	2	3	1
Desulfomicrobium (norvegicum?)	0	1	4	7	9	7	3	1	1
Desulfovibrio desulfricans	2	3	7	8	11	14	8	6	3
Desulfobacterium spp.	3	4	11	14	16	17	11	8	5
geovibrio spp.	0	3	2	2	1	0	6	7	9
Shewanella putrifaciens	3	5	3	4	0	0	6	8	7
unidentified Se Reducers	12	11	7	6	1	3	11	9	13
Total Selenium Reducers Tested	50	50	50	50	50	50	50	50	50

May 2015

	Bio 2 Inlet	Bio 2 Middle	Bio 2 Outlet	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	11	9	4	1	2	2	1	4	6
Microbacterium arborescense	9	5	1	0	0	0	0	0	1
Enterobacter sp.	5	2	3	0	0	0	0	0	1
Psuedomonas stutzari	7	5	4	1	2	0	3	3	2
Desulfomusa spp.	0	2	4	5	8	4	1	2	1
Desulfomicrobium (norvegicum?)	0	0	1	8	9	8	2	3	1
Desulfovibrio desulfricans	1	6	9	15	11	16	9	5	2
Desulfobacterium spp.	2	3	14	14	16	19	16	9	4
geovibrio spp.	0	1	1	1	1	0	4	6	8
Shewanella putrifaciens	6	4	4	2	0	0	5	9	9
unidentified Se Reducers	9	13	5	3	1	2	9	9	15
Total Selenium Reducers Tested	50	50	50	50	50	51	50	50	50

November 2015

	Bio 2 Inlet	Bio 2 Middle	Bio 2 Outlet	Bio 3 Inlet	Bio 3 Middle	Bio 3 Outlet	Bio 4 Inlet	Bio 4 Middle	Bio 4 Outlet
Bacillus subtillus	5	6	6	1	1	2	1	4	6
Microbacterium arborescense	4	2	1	0	0	0	0	0	1
Enterobacter sp.	6	2	2	0	0	0	0	0	0
Psuedomonas stutzari	4	4	1	0	0	1	0	1	3
Desulfomusa spp.	0	1	1	4	3	4	1	2	0
Desulfomicrobium (norvegicum?)	0	0	2	3	7	5	3	1	0
Desulfovibrio desulfricans	3	1	4	4	8	8	5	4	2
Desulfobacterium spp.	2	2	6	8	9	10	8	3	3
geovibrio spp.	0	2	1	2	1	0	3	4	6
Shewanella putrifaciens	1	3	2	3	0	0	2	5	4
unidentified Se Reducers	5	7	4	5	1	0	7	6	6
Total Selenium Reducers Tested	30	30	30	30	30	30	30	30	31

Appendix C Optimization Data

Nitrogen Added	Selenium Reduction Rate
	mg/L/hr
0	1.48
0	1.56
0	1.53
0.5	1.59
0.5	1.63
0.5	1.61
1	1.78
1	1.89
1	1.75
2.5	2.11
2.5	2.34
2.5	2.41
5	2.27
5	2.43
5	2.26
7.5	2.37
7.5	2.39
7.5	2.45
10	2.41
10	2.45
10	2.38
10	2.49
15	2.41
15	2.44
15	2.5
20	2.45
20	2.52
20	2.41

Phosphorous Added	Selenium Reduction Rate
mg/L	mg/L/hr
0	1.51
0	1.48
0	1.53
0.5	1.58
0.5	1.67
0.5	1.64
1	1.65
1	1.69
1	1.78
2	1.56
2	1.67
2	1.68
3	1.77
3	1.65
3	1.73
5	1.53
5	1.62
5	1.48
7.5	1.59
7.5	1.67
7.5	1.71
10	1.77
10	1.78
10	1.65

Micronutrients	Selenium Reduction Rate
mL/L	mg/L/hr
0	1.58
0	1.49
0	1.54
0.5	1.98
0.5	1.92
0.5	2.05
1	2.24
1	2.56
1	2.34
2	2.45
2	2.53
2	2.45
3	2.49
3	2.56
3	2.52
5	2.49
5	2.65
5	2.54
7.5	2.65
7.5	2.67
7.5	2.52
10	2.66
10	2.73
10	2.59

Molybdenum added	Selenium Reduction Rate
ug/L	mg/L/hr
0	1.6
0	1.62
0	1.56
5	1.89
5	2.01
5	1.78
10	2.14
10	2.26
10	2.11
15	2.36
15	2.54
15	2.62
20	2.4
20	2.69
20	2.82
25	2.77
25	2.59
25	2.86
30	2.72
30	2.77
30	2.58
40	2.89
40	2.67
40	2.57

Zinc Added	Selenium Reduction Rate
ug/L	mg/L/hr
0	1.6
0	1.86
0	1.76
5	1.67
5	1.53
5	1.56
10	1.75
10	1.84
10	1.83
15	1.88
15	1.94
15	2.01
20	1.78
20	1.654
20	1.58
25	1.78
25	1.73
25	1.89
30	1.932
30	1.99
30	1.67
40	1.78
40	1.84
40	1.83

Cobalt Added	Se Reduction Rate
ug/L	mg/L/hr
0	1.6
0	1.62
0	1.56
5	1.89
5	1.64
5	1.56
10	1.68
10	1.78
10	1.83
15	1.67
15	1.78
15	1.82
20	1.93
20	1.83
20	1.78
25	1.68
25	1.87
25	1.88
30	1.93
30	1.76
30	1.58
40	1.87
40	1.94
40	1.99

Temp	Selenium Reduction Rate
oC	mg/L/hr
5	0.85
5	0.92
5	0.76
10	0.99
10	1.07
10	1.13
15	1.46
15	1.42
15	1.56
20	1.78
20	1.83
20	1.93
25	2.06
25	2.14
25	2.09
30	2.54
30	2.34
30	2.45
35	2.08
35	1.99
35	2.02
40	1.52
40	1.35
40	1.41
45	1.08
45	1.13
45	0.98

Appendix D Sugars Data

			hemicellu	cellulose		xylose	glucose
0	32	38	22	38	0	32	38
5	27	41	22	39	5	59	79
10	25	45	21	38	10	84	124
15	34	38	21	40	15	118	162
20	38	37	22	39	20	156	199
25	23	34	20	39	25	179	233
30	32	40	20	36	30	211	273
40	25	42	19	35	40	236	315
50	28	36	20	37	50	264	351
60	29	32	19	35	60	293	383
75	33	36	18	34	75	326	419
100	43	45	18	35	100	369	464
125	41	52	18	36	125	410	516
150	39	49	19	35	150	449	565