Process Control Factors for Continuous Microbial Perchlorate Reduction in the Presence of Zero-Valent Iron

by

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Abstract

Water is a staple of civilization, and in the event of drinking water contamination the use of the contaminant's source should be discontinued. Alternatively, a technology that can remove the contaminant from the water must be developed. Perchlorate (ClO_4^-) is a byproduct of munitions, and pyrotechnics, and has been detected in water sources throughout the United States. It is unlikely that the use of perchlorate will be discontinued as it is linked to the integrity of national security. Due to the toxicity to human health, the United States Environmental Protection Agency (US EPA) announced in February 2011 that perchlorate will be federally regulated. It is expected that the maximum contaminant level (MCL) could be 1 ppb (µg/L). Therefore, it is necessary to develop a safe and inexpensive technology that is capable of completely removing the contaminant. Technologies for the perchlorate removal include: ion exchange, activated carbon adsorption, chemical reduction, and microbial reduction. Several studies demonstrated that zero-valent iron (ZVI) can be used as an electron donor for the microbial perchlorate reduction process. The core of our research approach is on the use of ZVI and mixed microbial culture.

Process control parameters influencing microbial perchlorate reduction by a flow-through ZVI column reactor were investigated in order to optimize perchlorate removal in water. Mixed perchlorate reducers were obtained from a wastewater treatment plant (aerobic activated sludge and anaerobic digester) and inoculated into

the reactor without further acclimation. Examined parameters include; hydraulic retention time (HRT), pH, nutrient requirement, and both chemical and microbial kinetics.

The minimum HRT required for our system that can completely reduce 10 mg/L of perchlorate was 8 hours. Perchlorate removal was reduced by 60% without pH control. As pH was determined to be an important parameter for microbial perchlorate reduction, a viable alternative of pH buffer is also discussed. Unlike other systems that used laboratory cultured microorganisms, our system needed no additional nutrients for the complete reduction of 10 mg/L of perchlorate in water. This is likely due to the plethora of nutrients available within activated sludge based seed cultures. The perchlorate reduction reaction follows the first order kinetics, with an average rate constant (K) of 0.761 hr^{-1}. The microbial growth in the column follows the Monod growth kinetics. The average maximum growth rate (μ_{max}) and the average half saturation constant (K_s) were determined to be 0.55 hr^{-1} and 15.4 mg/L, respectively. Also, a numerical model using Monod kinetics, transport, and attachment and detachment was used to verify the experimental result pertaining to the microbial growth kinetics in the ZVI supported perchlorate reducing column system.

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List of Abbreviations

EPA: Environmental Protection Agency
MCL: Maximum Contaminant Level
RfD: Reference Dose
CDHS: California Department of Health Services
USDA: United States Department of Agriculture
NIS: Sodium Iodine Symporter
Ppb: Parts per billion (µg/L)
NAS: National Academy of Sciences
CDC: Center for Disease Control
GAC: Granular Activated Carbon
CTAC: Cetyltrimethylammonium Chloride
HMX: High Melting Explosive
RDX: Cyclotrimethylene Trinitramine
IX: Ion Exchange
Ppm: Parts per million (mg/L)
PRB: Perchlorate Reducing Bacteria
EBCT: Empty Bed Contact Time
WWTP: Wastewater Treatment Plant
ZVI: Zero-Valent Iron
HRT: Hydraulic Retention Time
TE: Tris-EDTA Buffer
AGW: Artificial Groundwater
$\overline{\mu_{max}}$: Average Maximum Growth Rate
$\overline{Ks}$: Average Half Saturation Rate Constant
V: Velocity
D: Hydrodynamic Dispersion Coefficient
k_{att}: Biomass Attachment Factor
k_{det}: Biomass Detachment Factor

Chapter 1: Introduction

1.1. Introduction

The United States Environmental Protection Agency (US EPA) announced in 2011 that the policy for perchlorate will be changed, and that they are currently working towards federal regulation. The new regulation is expected to be as low as 1 µg/L [1]. The previous reference doses (RfD), were determined based on the potential adverse effect on human health and were 24.5 µg/L (2005, [2]) and 15 µg/L (2008, [3]). As compared to the previous reference doses, the new regulation level of nearly 1 µg/L will pose a significant undertaking on the perchlorate regulation and mitigation in near future [1, 4]. Perchlorate is an oxidized form of chlorine. Due to its high solubility and mobility in water ($217x10^3$ mg/L); it tends to be very difficult to remove from ground water [5]. Perchlorate in our drinking water primarily came from: munitions, and pyrotechnics. Other uses of perchlorate include: matches, refinement of aluminum, the manufacturing of rubber, it can also be found in the inflator of a vehicles airbag [6-8].

1.2. Contamination

Perchlorate contaminated groundwater flumes tend to originate or at least traverse areas where rocket fuels or other contaminant sources are either prepared or stored. The EPA states, at the two plants that manufacture ammonium perchlorate, which is

the major form perchlorate is used in, that the majority of the wells surrounding the plants contain some concentration of perchlorate [9]. Although production of ammonium perchlorate has declined by 76% since production peaked in the mid 1980's, contaminations still occur for multiple reasons: (1) because of the physical characteristics of perchlorate; the density of perchlorate is 1.95 g/cm^3 and the solubility ranges from 2,010-220 mg/L, which allows high concentrations of perchlorate to dissolve into groundwater and settle, and (2) the limited life of the compound; the perchlorate containing solid rocket fuel must be exchanged routinely and disposed of [6]. In 2007 scientist reported finding small amounts of naturally occurring perchlorate in America's southwest deserts, but there is only one known substantial natural perchlorate deposit, northern Chile's Atacama Desert [10]. This natural deposit lies in the desert's large nitrate deposit which is imported into this country as feedstock for fertilizer. This has caused many to suspect fertilizers as the prime culprit for perchlorate contamination. The EPA funded many studies to determine if fertilizers were contaminated with perchlorate. The finding was that fertilizers were not the main contributors to the nation's perchlorate laden ground water [11]. The majority of the samples contain no perchlorate while a few contained trace amounts of perchlorate. Some even hypothesized the perchlorate could be contributed to the use of oils used to keep the fertilizer dry ,as well as the brine used to control acidity during manufacturing and transport [7]. Based on this data as well as many more reports, it can be concluded that the perchlorate contamination of our drinking water is an anthropogenic problem. In a study performed by a host of interstate agencies it was reported that in 153 public water systems across 36 states

perchlorate was detected [12]. This range of contaminations is much more wide spread than thought previous to the late 1990's. In 1997 the California Department of Health Services (CDHS) developed a method to detect concentrations of perchlorate as low 4 parts per billion (µg/L). Soon after this technology was created many sites thought to be free of contamination were now proven to be contaminated. The contamination of perchlorate is thought to be widespread now ranging from coast to coast in 36 states. According to the United States Department of Agriculture (USDA) perchlorate can be found in other items such as food, and others state it can accumulate in human milk increasing the risk of health effects in infants [13-17]. Dasgupta reports that in a study done in 2005 that perchlorate was present in all 36 human milk samples analyzed [14]. In a later study done at the University of Texas at Arlington by Dasgupta et.al, 15 lactating women were chosen and breast milk samples were taken. From the 15 sample sets only 13 were usable and out of these 13 samples all contained some levels of perchlorate and 70% were above safe levels for infants [13]. With such a widespread contamination and with a large percentage of the population being affected, we must look at the health effects perchlorate ingestion causes.

1.3. Health Hazards and Regulation

In many studies it has been proven that the ingestion of perchlorate inhibits the thyroid gland's, a very important part in the development of fetus and young children's brains, ability to take up iodine into the Sodium Iodide Symporter (NIS) which is a key compound of brain development [7, 18]. Another study describes how

this inhibition takes place. Iodide passage through the NIS is severely inhibited by perchlorate because of the NIS's increased affinity towards perchlorate. As a result the production of thyroid hormones decrease, this can cause hypothyroidism in adults and severe birth defects and abnormal growth and development in infants and fetuses [2]. There is an ongoing debate on these health effects that perchlorate contaminations may have, but since it is thought to adversely affect such an important system in the development of young children, even unborn ones, it must be taken seriously. To measure the effect low levels of perchlorate has on humans, the EPA reviewed a study completed by Greer where a group of healthy men and women ingested variable concentrations of perchlorate and the effects were analyzed. This report states that no adverse effects were noted until greater than 7 μg/kg-day was ingested [18]. This level was then reduced by a factor of safety of 10 for pregnant women and their fetuses. Using this data as well as data relating perchlorate concentration in food, the EPA initially implemented a safe drinking water threshold of 24.5 μg/L (ppb) [2, 19]. Deborah Swackamer the EPA's Science Advisory Board disagreed with this level, stating "*The administration has just asked us for recommendations on how to strengthen the use of science, and here we are confronted by a case of the agency moving forward when not all of the science is in yet*". She also stated that using the EPA's own calculations and threshold, infants will receive 2-5 times the National Academy of Sciences reference dose (RfD) of 0.7 μg/kg-day [19]. Other studies have been done, including one by the Center for Disease Control (CDC) which states that the reference dose levels should be even an order of magnitude lower [19, 20]. As many as 7 states have set their own

regulations, proving that many agencies fill regulation of perchlorate is necessary. A table of the individual state regulations can be seen below.

Table 1: State Regulations

State	Perchlorate concentration (μg/L)	Year
California	6	2007
Massachusetts	2	2006
Texas	4	2002
Arizona	14	2003
Nevada	18	2005
New York	5	2008
New Mexico	1	2006

All of these regulations were set before the EPA recommended, even after a bill was passed by Congress allowing for National Regulation of perchlorate, that perchlorate not be regulated at a national level, citing that 99% of perchlorate contamination in water is of no health concern to the public. In 2009 the EPA published a Supplemental Request for comments Federal Register, and the date to submit reviews was extended until October of that same year. In February of 2011 the EPA announced it plans to regulate perchlorate, in the same press release it was stated that it might take 2 additional years to determine the necessary level of regulation. Which could be as low as 1 ppb (μg/L) [1, 4]. It is imperative a cost effective, efficient remediation technology be operational once this new regulation comes into effect.

1.4. Treatment Options

Perchlorate's molecular structure, a single chlorine atom surrounded by four oxygen atoms, is very stable therefore common reducing agents are not able to efficiently reduce perchlorate to chloride in a timely manner. Therefore, much research has been done in the field of perchlorate remediation, and multiple technologies do exist. Many of the technologies are young, and not enough data is available in order to make an educated decision on which might be the best practice. Two different types of treatment exist in water treatment: Removal and Destruction and each have their advantages and disadvantages. With such stringent regulation forthcoming from the EPA an economical, yet effective treatment option for perchlorate laden water must be determined.

1.4.1. Activated Carbon

Activated Carbon has been used in water treatment for years, and research has been done to test its viability as a perchlorate removal technology. Granular Activated Carbon (GAC) is carbon heated to above 500°C in the absence of oxygen; which causes multiple fractures to the surface of the granule, which increases the surface area for particles to adsorb to. This increased surface area allows for more substrate to come in contact to and adsorb to said surface. Based on reports from Parette, virgin GAC is not an effective perchlorate removal technology [21]. The efficiency of this technology though is greatly increased when the GAC is tailored to remove perchlorate. In 2005 Chen and Cannon loaded an ammonium surfactant onto AC and witnessed efficiency 30 times greater than that of just AC alone. Therefore it was

reported that loading AC with cationic surfactants is an effective method of treating perchlorate laden water [21]. In experiments performed by Parette and Cannon where cetyltrimethylammonium chloride (CTAC) was loaded and not only the efficiency of removing perchlorate was analyzed, but also efficiency of removing nitro-organics such as HMX and RDX a different conclusion was reached. Altering the surface charges of the GAC proved to affect adsorption of HMX and RDX adversely. The breakthrough bed volumes decreased from 300,000 BV to 7800 BV [21]. It was concluded that using GAC tailored with CTAC should be used as "pre-treatment" and should always be followed with a virgin GAC treatment process [22]. Although this process would treat perchlorate as well as other water contaminants, because it is simply a removal technique the contaminants are only being concentrated onto the surface of the carbon. Increasing the amount of beds needed to be used in an effective system only magnifies this problem of proper and safe disposal of heavily concentrated granules or powder [22].

1.4.2. Ion Exchange

The most commonly used technology to treat perchlorate contaminations is ion exchange (IX) because it is thought to be the most effective type of treatment [7], or maybe because the industry is more comfortable with this technology because of its long track record [7]. However ion exchange technology is very expensive and requires disposal of the ion exchange resin or regenerate brine [23]. Because perchlorate concentrations in water tend to be low, a highly selective ion exchange resin must be used. It has been reported that if a less selective ion exchange resin is

used in perchlorate remediation 99.9% of the resin could be wasted. Even once these selective resins are used, regeneration becomes more difficult because of the selectivity. Gu and Gilbert developed an ion exchange system that treated the perchlorate with low HRT and also required small amounts of water to regenerate the resin [24]. In other studies they determined that the solution used to regenerate the resin could be used to completely reduce the concentrated perchlorate to chloride. This reduction would only take place at high temperatures and pressures. This phenomenon is useful when treating groundwater containing only perchlorate, which is not very probable [23, 25]. Therefore the highly concentrated brine still needs to be disposed of. This is not only environmentally unsafe, permits and fees go along with dumping hazardous materials. Most of the time regenerate solution is cycled thru a settling basin in order to reduce the amount of waste that needs to be disposed of. Although ion exchange is a very promising technology, because it is a removal technology it is still not the final solution, some concentrated medium must be disposed of, costing money and also a threat to leach and contaminate other sites [7]. Other removal treatment options such as membrane filtration and reverse osmosis are too expensive to be feasible.

1.4.3. Chemical Treatment

As mentioned previously, perchlorate is difficult to reduce chemically because of such a high energy barrier. Two methods to overcome this high activation energy are high pressures or temperatures, or both. To create an environment suitable for chemical reduction is costly and not normally accepted in wastewater treatment

practices. Increasing the temperature also increases the rate at which microorganisms grow, which increases the amount of microorganisms that need to be treated. Much research has been done in finding suitable catalysts which can overcome this energy barrier. Reduction of perchlorate was witnessed at reaction rates similar to those of other treatment processes, the reaction occurred at very high pressures. Others have also found catalysts that will reduce perchlorate, but due to the complexity of the system and the expense of the conditions needed, state that full scale utilization is improbable. Others use waste products from water treatment facilities to reduce perchlorate, although reduction rates were high, so were initial perchlorate concentrations. At 250 mg/L this concentration is much higher than found in contaminated water [5, 7, 26]. Some have been able to achieve complete reduction in conditions that are likely to be present in wastewater treatment; the major limiting factor is kinetics. One of these technologies is the reduction of perchlorate using zero-valent iron. Interestingly iron acts as a reducing agent and catalyst all at the same time. The kinetics of this reduction were too slow for practical purposes. Due to the slow reaction times, as well as environments not readily available during normal wastewater practices chemical reduction of perchlorate is not a viable option.

1.4.4. Microbial Reduction

Much research has been performed in the field of microbial reduction of perchlorate, and many have stated that this technology is the most promising technology for perchlorate reduction [6, 27]. A large list has been compiled of microorganisms capable of reducing perchlorate and two things each of these have in

common is they all contain two enzymes which allow them to reduce perchlorate to the non toxic chloride. These two enzymes responsible for this reduction are perchlorate Reductase and chlorite dismutase [28, 29]. A schematic can be seen below to illustrate the reduction pathway.

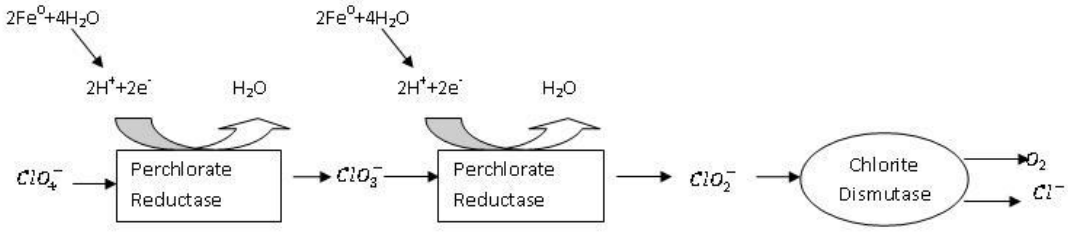


Figure 1: Perchlorate Reduction Pathway

The above illustration revised from Xu et. al [27] shows a three step process in which perchlorate and chlorate are reduced by Perchlorate Reductase, an enzyme which by character is able to overcome high energy barriers, and chlorite is then further reduced to chloride through the dismutase enzyme which simultaneously reduces chlorite and oxidizes oxygen. It should be noted here that oxygen, which has been reported to be inhibiting to this reaction is a by-product.

Research has been performed in many different areas of microbial reduction, and many different types of treatment have been examined. Treatment methods range from permeable reactive barriers to fluidized bed reactors. All these options have one thing in common; they all utilize microorganisms (PRB) to reduce perchlorate. The variables in these systems are the electron donor and the avenue in which the microorganism come into contact with the perchlorate. Permeable Reactive Barriers are in situ remediation techniques where barriers are installed into the ground, and

contaminated groundwater flows thru the barrier becoming less contaminated as it passes thru the barrier. The majorities of the other treatment methods are pump and treat techniques where the contaminated water is pumped to the surface and treated. These technologies include fixed bed reactors, and fluidized bed reactors. These reactors are packed with some consortium of microorganisms, whether pure or mixed cultures, and the contaminated water is pumped thru the system along with a nutrient solution containing vital elements necessary for perchlorate reduction. Although not much research has been done on this medium solution, one study cites many of the perchlorate reducing bacteria cannot grow without a "trace metal solution" which contains: Molybdenum, and Selenium [27]. The microorganisms used in the reduction of perchlorate are cited by many to be ubiquitous in throughout the environment. It was once believed that all chlorate respiring bacteria were also capable of reducing perchlorate, although the reverse is true, it has now been proven that there is one set of CRB that cannot reduce perchlorate [30]. Although many perchlorate reducing bacteria PRB exist; some exhibit different characteristics; some are purely heterotrophic while other are autotrophs. Some show growth only on acetate, while others will only grow on hydrogen, and still others can survive in multiple environments [6, 7, 27]. Wastewater processes are home to many microorganisms, and many PRB are able to live in these environments, and in systems tailored for these microorganisms they are able to thrive in the presence of other microorganisms. The use of these "mixed cultures" tends to be a less labor intensive process, allowing the microorganisms found in WWTP to be directly used in a reactor tailored for PRB, where pure cultures require some sort of laboratory

procedure to institute growth of a pure culture in the reactor. In order to tailor a reactor for reduction of perchlorate using PRBs the microbial kinetics need be understood. Nerenberg et al. isolated Dechloromonas sp. PC1 from a hydrogen-fed autotrophic reactor, and conducted batch experiments in order to understand the kinetics of this PRB. Using 1 liter bottles with 200 mL of growth medium, PC1, and the headspace was filled with either a 95/5 hydrogen, carbon dioxide mixture of a pure hydrogen gas. Using the results analyzed by a Dionex AS-16 column and by using Monod substrate-utilization and biomass accumulation equations per Rittman and McCarty were able to determine the reaction kinetics [31]. Others have studied another strain, Perchlorate Respiring isolate KJ with acetate as the electron donor. Mixed and pure cultures were both used in column experiments. It was determined that the pure culture KJ could reduce perchlorate when fed acetate at influent concentrations of ~25 mg/L to below the detection limit of the system. This occurred when empty bed contact times ranged from 2-65 minutes. The column containing mixed cultures also reduced perchlorate to below detection limits, but at slower flow rates, and minimum EBCT of 31 minutes compared to 2.1 minutes for the pure culture [32]. Others though reports mixed cultures have the ability to reduce perchlorate at higher rates than pure cultures. The ability to use a cohort of microorganisms directly from a WWTP is very beneficial to the viability of this technology.

The choice of electron donor is also one that should be addressed, acetate a common electron donor for perchlorate reducing bacteria is expensive, and the addition of organic materials during water treatment process is hardly accepted.

Hydrogen, another common electron donor for PRBs cannot be ruled out economically, but the explosion risk that goes along with the use of hydrogen gas is a danger we need not take [7, 33]. The use of zero valent iron as a substitute for hydrogen as an electron donor for PRB has been studied [33, 34]. It has been stated that H_2 can be supplied to the PRB from the zero valent iron (ZVI) packed throughout the column. Yu et.al suggests that thru anaerobic corrosion of iron in the presence of water, hydrogen gas is released. This phenomenon can be seen illustrated in equation 1.

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2(g) \qquad \text{(eq.1)}$$

Using iron fillings from metal fabrication as a hydrogen supply is cost effective and safe. Yu et. al states that a column packed with iron fillings could treat perchlorate laden water at concentrations as high as 1000 ppb as long as 4000 pore volumes. Both a mineral solution and a synthetic ground water were pumped thru the column, and the system was able to remove perchlorate from an influent concentration of 600 ppm to below the detection limit of 4 µg/L [34]. Another study using ZVI as source of electrons was conducted and relatively high concentrations of 10 ppm were reduced to below the same detection level of 4 ppb. This system was a column packed with glass beads and ZVI and inoculated with activated sludge, and anaerobic digester from a local WWTP, a mineral solution with a perchlorate concentration around 10 mg/L was allowed to flow up into the column at a flow rate that produced a HRT of 12 hours. The effect of pH was also studied with the use of Hepes pH buffer. The buffer was added to the influent solution the assist in maintaining a neutral pH,

and after sometime the pH buffer was no longer added and the system was able still to treat the perchlorate laden water [33].

In our study we investigate four process controls: 1) hydraulic retention time (HRT), 2) pH, 3) nutrient requirement, and 4) kinetics for both perchlorate reduction and microbial growth. Particularly we will attempt to normalize kinetic parameters within a complex microbial population. Using this information as well as information from our previous studies [33, 35], a viable technology to treat perchlorate contaminated water to below regulated levels should be developed.

Chapter 2: Material and Methods

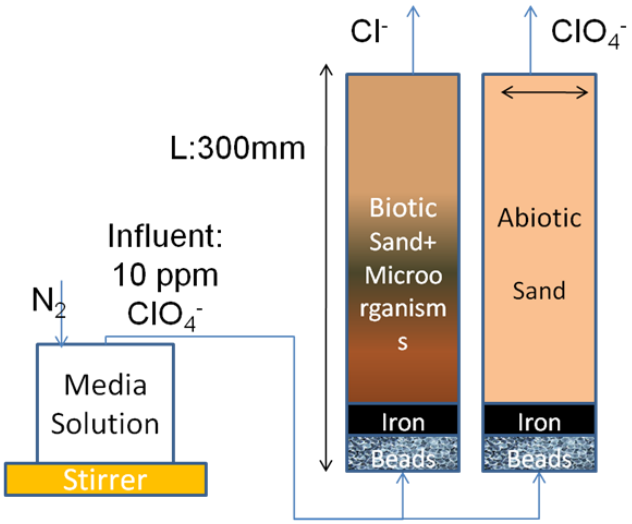
2.1. Microorganisms and flow-through column system

Mixed microbial communities were obtained from wastewater treatment processes and were used to inoculate the column. This sludge was collected from two separate facilities: anaerobic digester from the South Columbus Wastewater Treatment Facility (Columbus, GA) and activated sludge from the H.C. Morgan Pollution Center (Auburn, AL). The total suspended solids of each sample was measured and determined to be 15,945 mg/L for the anaerobic digester and 3,154 mg/L for the activated sludge. A medium solution was prepared with: $NaHCO_3$ (0.476 mM), $MgSO_4 \times 7H_2O$ (0.001 mM), $NH_4H_2PO_4$ (0.08 mM) and a trace metal solution of $NiCl_4 \times 6H_2O$ and $NaSeO_3 \times 5H_2O$ at concentrations of 0.04 mg/L each. It was then spiked with sodium perchlorate ($NaClO_4^-$) at a concentration of around 10 mg/L which is within the range of known perchlorate contamination levels [9]. Since our previous study has shown that the reduction could take place at lower concentrations, the test concentration used in this study was chosen to further demonstrate our system's ability to completely reduce perchlorate at high concentrations [33, 36]. HEPES buffer in both acid ($C_8H_{18}N_2O_4S$) and base ($C_8H_{17}N_2O_4NaS$) forms were added (70 mM and 38 mM, respectively) to maintain pH of 7.3 against the pH increase during anaerobic iron corrosion. The chemicals used in this research were obtained from VWR international (Bridgeport, NJ). A glass column (5 cm x 30 cm)

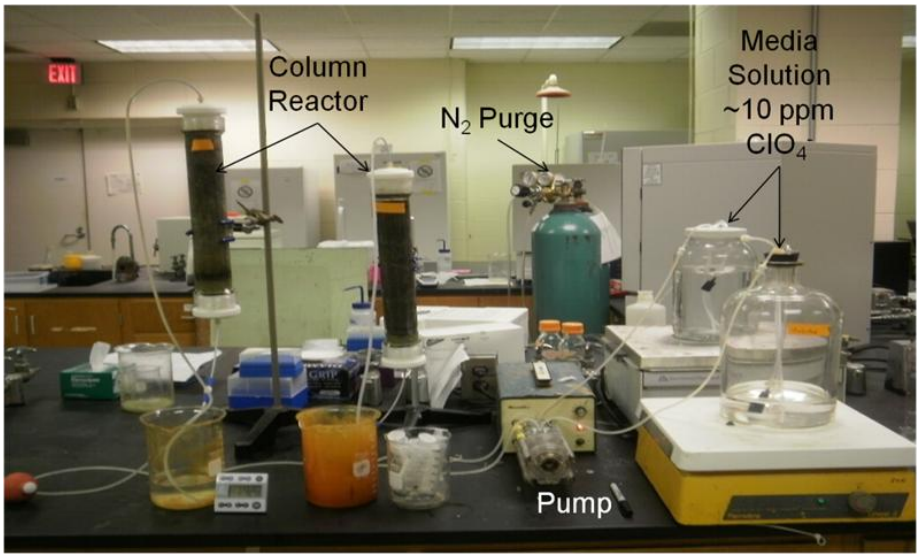
with Teflon® end caps (Ace Glass, Vineland, NJ) was filled in layers with 20 mL of glass beads, 17 g of Iron fillings (Fisher Scientific, Pittsburgh, PA), and then (2% v/v) a mixture of sand (Durham Geo, Stone Mountain, GA) and seed microbial culture were added. A control column was constructed in the same manner and was not inoculated with microorganisms. The column had a porosity and a pore volume of 0.27 and 160 mL, respectively. The column was purged with nitrogen during the packing process, between each layer, and also for five minutes after the column was packed to remove any oxygen from the column's pore volume. After this nitrogen purging, the column was subjected to the acclimation for perchlorate. During this acclimation, perchlorate spiked growth medium was pumped into the column for seven days to ensure at least ten pore volumes passed through before samples were collected. A schematic and picture of the described experimental setup can be seen in Figure 2.

2.2. Hydraulic Retention Time

Various HRTs (12, 8, 6 hours) were chosen and tested in order to determine the optimum HRT. Samples were collected every day for ten days for each HRT. The flow rate was subsequently increased after ten days to establish a new HRT. When the concentration of perchlorate exceeded the EPA's reference dose (RfD) of 15µg/L, the HRT was increased until complete perchlorate reduction was again resumed. The breakthrough HRT was revisited to ensure the correct HRT was concluded.



(a)



(b)

Figure 2: Experimental Setup

(a) Schematic of experimental setup. (b) Picture of experimental setup. Media Solution, purged with nitrogen to ensure anaerobic conditions, was pumped in an upward direction through a peristaltic pump (Masterflex) at varying flow rates into glass columns (5 cm x 30 cm; Ace Glass) filled in layers with glass beads, zero valent iron, and sand. Influent and Effluent samples were taken and analyzed.

2.3. pH effect

The flow was setup such that the column was subjected to an 8 hour HRT which was concluded to be the optimum HRT for this system. The reactor was subjected to ten days of influent media solution spiked to 10 mg/L of perchlorate and the pH was regulated using the same HEPEs buffer (EMD Millipore, MP Biomedical) solution (pH=7.3). After ten days the influent was switched an identical solution without pH buffer. The influent and effluent pH, as well as the perchlorate concentrations, was measured for twenty days. After this twenty day period, the HRT was increased to 12 hours, and the influent was switched to a solution with regulated pH. After the same ten day period, the influent was again switched out to an identical, but unbuffered solution and the same parameters were analyzed (influent and effluent pH and perchlorate concentration).

2.4. Alternate pH buffer

A single column was packed as aforementioned, and it was allowed to equilibrate. The system was subjected to the optimum HRT of 8 hours. The influent solution was spiked with 10 mg/L perchlorate, and was buffered with a Tris-EDTA (TE) buffer (EMD Millipore). Effluent samples were collected daily, and the pH and perchlorate concentrations were analyzed to determine whether this TE buffer is able to regulate the system's pH in a manner that allows for complete reduction of perchlorate.

2.5. Required Nutrients

A single column was packed as previously described and was subjected to an optimum HRT of 8 hours. In order to investigate and identify the required nutrients for continuous microbial perchlorate reduction, the influent solution was alternated between: a full growth media solution (see section 2.1), a solution containing only nutrients ($NaHCO_3$ (0.476 mM), $MgSO_4 \times 7H_2O$ (0.001 mM), $NH_4H_2PO_4$ (0.08 mM)), a trace metal solution (0.04 mg/L of $NiCl_4 \times 6H_2O$ and $NaSeO_3 \times 5H_2O$) as well as an artificial groundwater (AGW) solution containing only 48 ppm $CaCO_3$ to simulate that found in natural groundwater [32]. The effluent perchlorate concentration was measured and plotted in order to determine the necessary nutrients and trace metals, if any, are required for complete microbial perchlorate reduction.

2.7. Kinetics

2.7.1. Perchlorate Reduction Rate

A single column was packed as previously described, and it was allowed to run for a ten day start up period. The column was subjected to different influent perchlorate concentrations: 0.1, 1, 10, 20, and 100 (mg/L). The influent solution contained the same growth nutrients, trace metal solution, and pH buffer as described above. Effluent samples were collected after two pore volumes had passed through the columns. The column's ZVI was replaced after all five prescribed influent concentrations were examined. A total of four different volumes of iron were examined: 0.5, 1, 2, and 4 % of the total reactor volume. The results were studied

using an approach described by Logan, and the degradation rate (R) was plotted against the perchlorate concentration [37].

2.7.2. Microbial Growth Kinetics Experiment

A column was packed as mentioned above. This column was subjected to an influent perchlorate concentration above the limit of complete reduction in order to accurately determine the necessary microbial growth kinetics previously described. In order to determine the microbial kinetics the system must be subjected to varying hydraulic residence times; these HRTs were: 4, 6, 8, 10, and 12 (hours). Samples were collected after 2 pore volumes had been allowed to pass through the system, and effluent perchlorate concentrations were measured. A plot of HRT ($1/\mu$) versus the inverse of the effluent substrate concentration (1/S) was generated and Excel's linear regression employed to create a trend line. This trend line was used to determine the maximum growth rate (μ_{max}) and the half saturation substrate concentration (K_s).

2.7.3. Numerical Modeling

A numerical model was constructed within Visual Basic for Microsoft Excel using Monod equations to describe the microbial growth kinetics of the perchlorate reducing bacteria in the column system. In this study, we used the operator split strategy to solve the equations (16-18) numerically. Operator split strategy is one of the numerical strategies used in solving multi-species reactive transport problems [38]. The kinetic parameters (K_s and μ_{max}) obtained from the growth kinetic

experiment as well as the theoretical yield coefficients (Y) that were calculated for both autotrophic and heterotrophic bacteria were used as the inputs for the model.

2.8. Analytical Analysis

The samples were collected in a 15 mL vial, and then they were filtered using 0.45 µl filters. They were stored in the refrigerator for no more than 28 days as described in EPA method 314.0. A Dionex (Sunnyvale, CA) DX-120 Ion Chromatograph with a 4mm IonPac AS-16 column and an AG-16 guard column was used to analyze for perchlorate. Analysis of the samples was based on the EPA's suggested method for analyzing perchlorate in drinking water (EPA Method 314.0). A Thermo Scientific Orion 3Star pH meter was used to determine each sample's pH.

Chapter 3: Results and Discussion

3.1. Hydraulic Retention Time

In order to optimize process controls factors for continuous microbial perchlorate reduction, an optimum hydraulic retention time needs to be examined. With an optimized (minimum HRT we can ensure that the proper flow rate is used during experiments. This will also allow us to calculate the chemical reaction rate. Various HRTs (12, 8, 6 hours) were applied to our column in order to determine the optimum HRT, and the result is presented in Figure 3. The optimum HRT was determined to be 8 hours because of the repeated breakthrough of perchlorate at a 6 hour HRT. It should also be noted that the Abiotic control column was only able to achieve 35% reduction of perchlorate. The results from the abiotic reactor illustrate that iron itself cannot efficiently reduce perchlorate, and furthermore, that perchlorate does not significantly adsorb to column materials. It can be concluded that abiotic perchlorate reduction in a flow through reactor is not an efficient remediation method. These results can be found below in Figure 3.

After the initial start up period the flow rate was set at 12 hours based on previous studies performed by our group [36] The breakthrough is concluded to be 6 hours because of the repeated breakthrough of perchlorate above the EPA's RfD and suspected MCL of 15 µg/L and 1 µg/L respectively [1, 7].

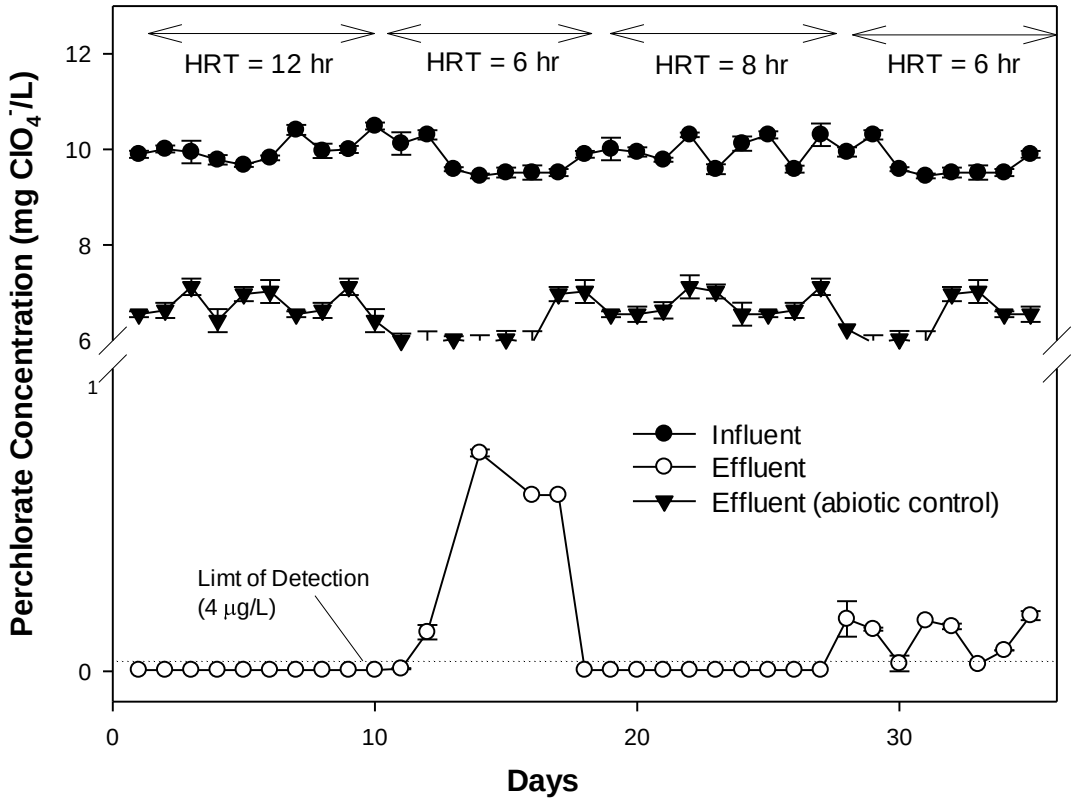


Figure 3: Hydraulic Retention Time

Perchlorate reductions in the ZVI-supported microbial column reactor under various HRTs. The breakthrough of perchlorate was repeatedly observed at a 6 hours of HRT. The optimum (minimum) HRT for the complete removal of perchlorate (below the limit of detection: 4 μg/L, depicted by the dotted line) was determined to be 8 hours. The abiotic control column (without microbial culture) was unable to significantly reduce perchlorate. The concentration and error bar represent mean and standard deviation based on triplicate samples from the reactor.

To determine if the values of perchlorate concentration were indeed above 15 ppb a one tailed *t*-test was performed. A p value was computed to be 0.008; because this value is less than 0.05 we can conclude that at 6 hours our column can no longer reduce perchlorate below the EPA's reference dose. After the initial sustained breakthrough the HRT was adjusted to 8 hours, and complete reduction resumed. The 6 hour HRT was revisited to ensure the sustained breakthrough indeed does take place, and once again perchlorate broke through the Rfd. This reoccurrence as well as the results from the statistical analysis allows one to conclude that the optimum HRT is 8 hours.

An 8 hour HRT in our system (300 mm x 50 mm) describes a water velocity of 0.2 feet per day, which is within the range of common groundwater velocities [39] . The average perchlorate concentration in the first 6 hour HRT trial was 560 µg/L compared to the second trial average of 160 µg/L. This can be attributed to the microorganism's ability to adjust to the environment and therefore become more efficient for the perchlorate respiration. The 35 % reduction of perchlorate by the abiotic reactor can be attributed to both adsorption to the iron fillings as well as reduction, a phenomenon discussed in the literature [40]. The pH in this system was maintained to 7.2-7.5 using a HEPEs buffer solution. To ensure the iron corrosion process did not raise the pH to a level that would hinder reduction as discussed in the literature [29, 41] . From the figure above as well as the results from the *t*-test ($P = 0.008 < 0.05$) we conclude that the optimum hydraulic retention time for our system and systems similar to ours is 8 hours.

3.2. pH effect

The effect that pH had on the reactor was studied to determine the necessity of pH control within the system. This was investigated by alternating influent solutions with and without controlled pH (7.3). Okeke et.al states that the two major enzymes involved in the reduction pathway are perchlorate reductase and chlorite dismutase, and that perchlorate reductase has an optimum pH range of 7.0-8.0 [29]. Due to this optimum range, it is possible that perchlorate reduction could be inhibited when no pH buffer is used in our system, because of the hydroxide ions released during the iron corrosion process.

From Figure 4 it can be concluded that when the pH raises above 8.0 the microbial ability to reduce perchlorate becomes inhibited in the reactor. When two separate HRTs are examined we notice that reduction did not completely cease, but only slowed down when the pH rose above the optimum range. We can then conclude that the longer residence time enables the system to further reduce perchlorate though not completely. The first cycle of unbuffered solution resulted in higher influent pH readings than the second, but both were as high as expected, and higher than the prescribed "optimum" range. Although not shown in the figure, one point to note is the influent pH. The influent pH was not always above the optimum range, but because the effluent lacks the buffering capacity it always is. This can be attributed to the iron corrosion process, a schematic of can be seen in the previous pages [34].

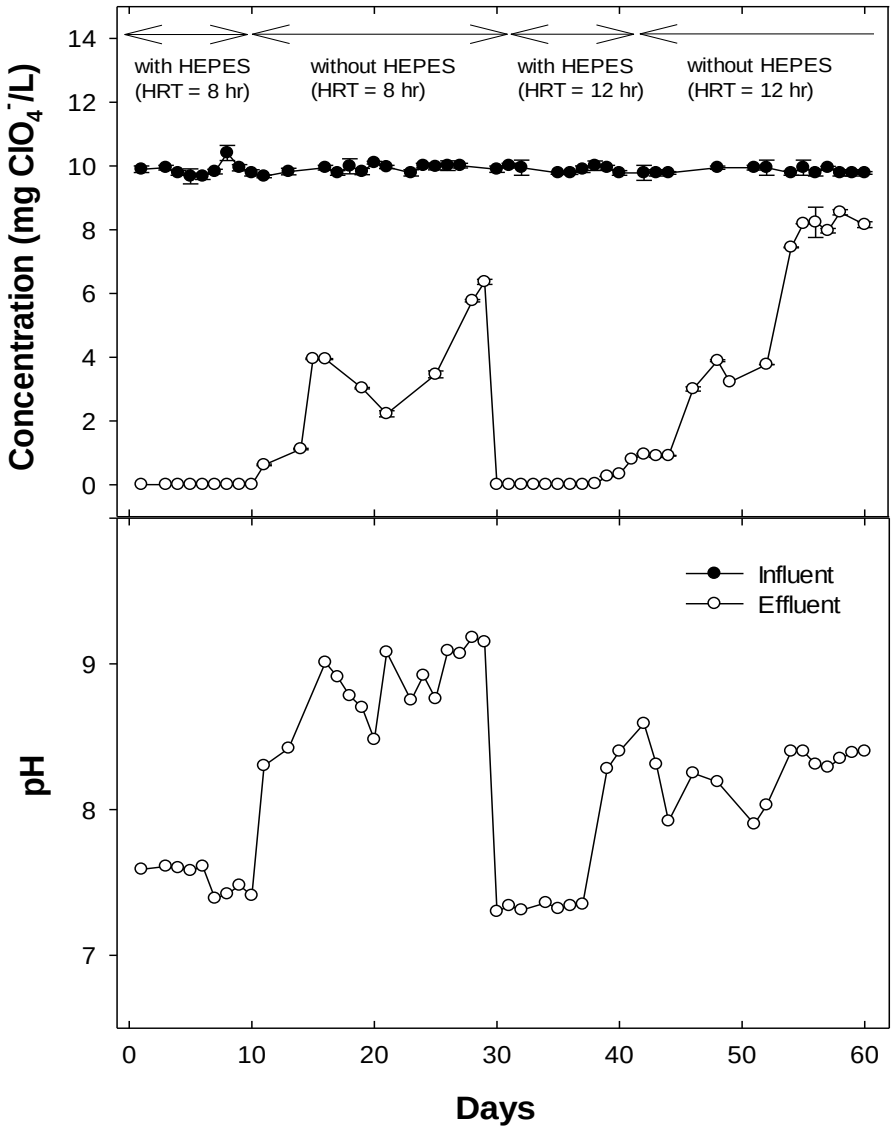


Figure 4: pH Effect on Perchlorate Reduction

Perchlorate reduction in the ZVI-supported microbial column reactor with and without pH buffer. While the pH effect was investigated by alternating a buffered and unbuffered influent solution, the HRT targeted for this experiment also varied from 8 hours to 12 hours. The concentration (or pH) and error bar represent mean and standard deviation based on triplicate samples from the reactor.

During this corrosion as proposed in eq. 1, two moles of hydroxide are released for every one of hydrogen, resulting in an increased pH.

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2(g) \qquad \text{(eq.1)}$$

Another possible reason for inhibition is that when the pH rises above certain levels, iron precipitates encapsulate the microorganisms, thus reducing the amount of microorganisms able to reduce perchlorate. This conclusion is disputed because the study manually injected large concentrations of Fe^2 into their reactors to study the effect the precipitates had on reduction [41]. A number of studies indicated that higher pH results in slower reduction rates [29, 42, 43].

The results of this experiment allow a conclusion that pH has a significant effect on microbial perchlorate reduction. When the HRT was increased it seemed that the microorganisms were able to reduce perchlorate to lower levels than in the 8 hour cycle. It does seem that when pH reaches a certain maximum, 8.5, the microorganism's ability to reduce is severely affected. Therefore we conclude that pH at certain ranges slows microbial perchlorate reduction, and at higher levels will severely affect perchlorate reduction.

3.3. Alternate Buffer

As pH control is shown to be an important parameter for the perchlorate reduction a cost effective pH buffer should be determined. HEPEs buffer has been used throughout the experiments, but due to its cost it is an unlikely option in full scale systems. A cost effective pH buffer is required that can maintain the system pH

within the optimum range mentioned earlier. The three major pH buffers considered were: (1) carbonate, (2) phosphate, and (3) a Tris-EDTA (TE) buffer.

Carbonate Buffer systems cannot buffer pH within the optimum range of our system, and phosphate buffers tend to inhibit iron corrosion, which is an important supply of electrons in our system. Therefore it was determined that the buffering ability of the TE buffer should be analyzed.

Figure 5 shows that the pH of the system buffered by 50 mM TE buffer was near pH 8. It was observed that only partial reduction takes place. Complete reduction was attained for two days immediately following the introduction of the TE buffer, but it appeared that 50 mM was not sufficient to maintain complete reduction for longer periods of time. When the concentration of the buffer was doubled (100 mM) the pH of the system decreased to pH 7.5, and complete reduction was sustained. Therefore we can conclude that 100 mM TE buffer is a viable alternative to the expensive HEPEs buffer because of its ability to buffer pH within the optimum range (pH 7-8). The TRIS-EDTA buffer system is a viable alternative to the HEPEs buffer because of its ability to regulate the pH within the optimum range; it does not interfere with the iron corrosion process, and is significantly more cost-effective than its counterpart, HEPEs. The HEPEs buffer system is comprised of a HEPEs base, and HEPEs acid, at concentrations of 70mM and 38mM respectively. The cost of this system is $20 per liter of contaminated water treated. The TE buffer system contains TRIS base and EDTA the concentration of each are; 100 mM and 10 mM. The TE buffer system costs a little more than $2.25 per liter of contaminated water treated. This is almost 90% less than the HEPEs buffer system.

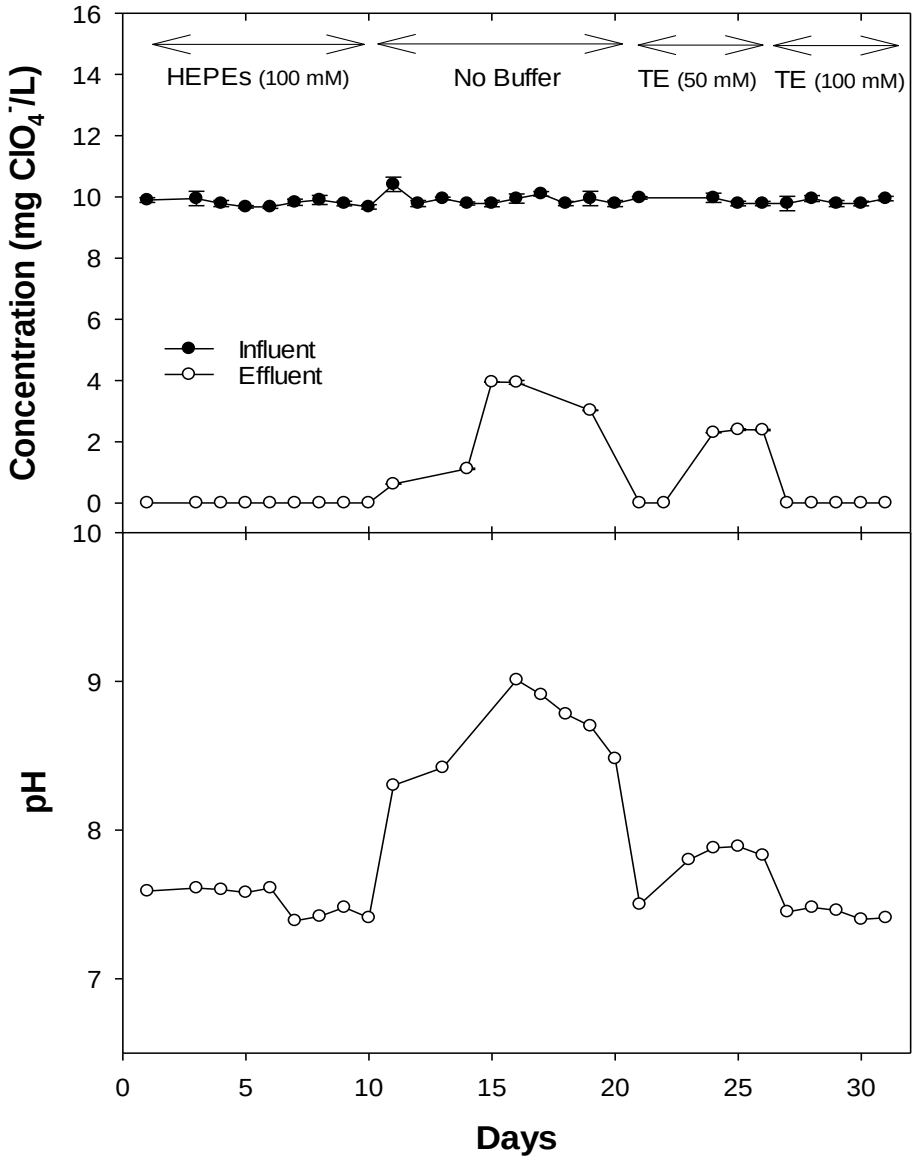


Figure 5: Alternate pH Buffer

A TE pH buffer was tested to the system in order to determine its ability to maintain a neutral pH. When a 50 mM TE solution was used, the pH rose above the prescribed range for PRBs, but when the concentration was increased to 100 mM the system remained within the optimum range for reduction. Perchlorate reduction was sensitively affected by the pH changes.

3.4. Nutrient Requirement

In order to examine the requirement of nutrients (macro-nutrients and trace metals) as indicated in the literature [27, 31, 34, 42], a variety of influent solutions were tested and presented in Figure 4. The first phase of this experiment used an influent containing additional nutrients, trace metals, and pH buffer. Complete reduction was achieved as expected. The next phase the influent solution contained only additional nutrients, but no trace metals. As a number of studies indicated the need for the trace metal solution, particularly Ni and Se, it was hypothesized that only partial reduction would occur. However complete reduction was achieved in the second phase and it is likely that trace metals in the column was derived from the activated sludge that was used as the seed culture [44]. The third influent solution which contained no macro-nutrients only trace metals; enabled the complete reduction, because of the nutrients available in the wastewater sludge. The buffered AGW solution (no nutrient or trace metals) assisted in concluding that complete perchlorate reduction can be accomplished without the addition of nutrients or trace metals, when a wastewater sludge is used to inoculate the reactor. This is because as we previously discussed, the amount of nutrients and trace metals in the sludge itself. The last phase was an unbuffered AGW; breakthrough was expected because of the importance of optimum pH, which we have already discussed.

In order for the enzymes to completely reduce perchlorate to chloride, it is necessary for a carbon source as well as a nitrogen source to be made available and utilized for the synthesis of microbial cells. The energy sources for microbial activity were derived from the biomass consortium in our system. In comparison to the

majority of existing systems where each column was inoculated with laboratory enriched pure cultures [29, 34, 42, 43] . Our system used a mixture of anaerobic digester sludge as well as aerobic activated sludge from waste water treatment facilities as the microbial consortium for the column system. Wastewater sludge has been used as an agricultural fertilizer for decades because of its nutrient concentration. This consortium of microorganisms plus the groundwater minerals could contribute carbon and nitrogen sources from which the PRB could utilize. An activated sludge solution is a very diverse solution, and can conceivably be able to provide even the trace metals needed for complete perchlorate reduction [44, 45].

In some cases when influent solutions are alternated, the effluent concentration could have a slight time lag. In our experiment we ensured a long enough time to prove our conclusions. The fact that breakthrough was only experienced when the HEPEs buffer was removed proves that pH, not nutrient addition is the governing parameter in microbial perchlorate reduction.

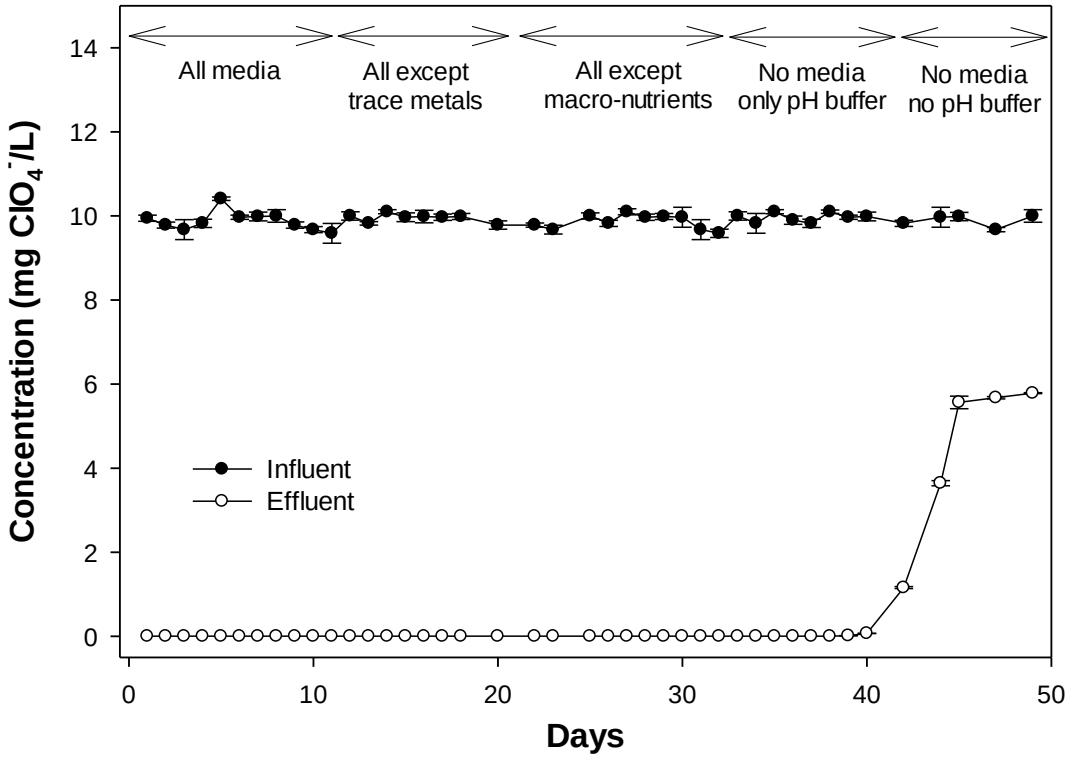


Figure 6: Required Nutrients

Effect of macro-nutrients and trace elements on the perchlorate reduction. All required media, macro-nutrient only, trace elements only, no nutrient and pH buffer only, and no nutrient and no pH were applied for perchlorate reduction in the ZVI supported microbial column reactor. The system was able to completely reduce perchlorate without the addition of any nutrients as long as the pH was buffered to neutral (7-8).

3.5. Kinetics

It is important to investigate the controlling kinetics of the system because, in biological systems, the active biomass concentration controls the rate the pollutant is reduced, and the biomass is grown through the utilization of available energy (electron donor, electron acceptor, nitrogen source, carbon source). Therefore, the rate at which pollutants are reduced is proportional to the rate at which biomass is synthesized. This is imperative because the knowledge of kinetic parameters allows users to efficiently utilize the reduction technology. In this paper we investigate both microbial and chemical kinetic parameters in our flow through column reactor. When both systems are analyzed it ensures complete understanding of the controlling kinetics.

Many microbial kinetic models are available, and four were investigated in order to determine which best represents the system. The governing equations of each can be seen below.

$$\frac{dS}{dT} = \frac{\frac{\mu}{Y} * S * X}{Ks + S} \qquad \text{(eq.2a)}$$

$$\frac{dX}{dT} = \frac{\mu * S * X}{Ks + S} \qquad \text{(eq.2b)}$$

$$\frac{dS}{dT} = \frac{q * S * X}{BX + S} \qquad \text{(eq.3)}$$

$$\frac{dS}{dT} = \frac{q * S * X}{K + S^{-\gamma}} \quad \text{(eq.4)}$$

$$\frac{dS}{dT} = -q(1 - e^{S/K})X \quad \text{(eq.5)}$$

Where;

μ (eq.2; *Monod*): growth rate (T^{-1})

Y: yield coefficient (M-biomass/M-perchlorate)

S: substrate concentration (mg/L) * In this research S: perchlorate concentration

X: Biomass concentration, VSS (mg/L)

Ks: Half saturation constant (mg/L)

B (eq.3; *Contois*): fitting parameter

q (eq.5; *Tessier*): substrate utilization rate (M-perchlorate/M-biomass/T)

γ (eq.4; *Moser*): fitting parameter

Much debate exists between these models, and the only valid arguments between them is goodness of fit, mathematical utility, and acceptance [46, 47]. The Monod kinetic model was developed for single bacterial cultures feeding on single organic substrates. Therefore, when modeling mixed/heterogeneous cultures questions arise to whether the model can accurately predict the growth and reduction of substrate. Much research has been done on this debate and the general consensus is that Monod kinetics can accurately depict mixed systems. Hence, one must realize that the kinetic parameters deduced are not necessarily parameters for one single microorganism, but an average for the complete system [46]. Other kinetic models such as Contois and Moser are more complex versions of the original Monod model. The addition of the "ill defined" coefficients only add skepticism to the models

results [47]. Due to the fact that the Monod model is widely used and accepted as an accurate model for perchlorate reducing system, and also because of the accurate fit to our results, Monod expressions were used to formulate the kinetic parameters. From these kinetic parameters, as well as the other process controls investigated throughout this report, an efficient flow through reactor can be developed.

3.5.1. Perchlorate Reduction Rate in Column Reactor

Chemical reduction rates are important parameters in any remediation, (redox) system. In order to determine the reaction rates for our system we took a similar approach to one found in the literature [37]. Logan states in his report that $R=KC^n$, so the removal rate, R, was plotted against the substrate concentration and when this produces a straight line, the slope is equal to the reaction rate. Excel was used to plot the data, and R^2 values were analyzed to ensure a 1st order approach was correct. According the other reports, when analyzing the reaction rates in flow through columns of our sort, one should use the log mean concentration due to the fact that the influent and effluent concentrations vary so much [48].

$$R = k \times C_{lm}{}^{n} \qquad \text{(eq. 6)}$$

Where;

R: Perchlorate removal rate= $\frac{C_{in} - C_{out}}{\theta}$

$C_{in/out}$: Influent and effluent concentrations (mg/L)

n: Reaction order

θ: Hydraulic residence time (hr)

k: 1st order reaction rate (hr^{-1})

C_{lm}: log mean coefficient= $C_{lm} = \frac{C_{in} - C_{out}}{\ln \frac{C_{in}}{C_{out}}}$ (eq.7)

These above equations were used in the approach to determine the chemical degradation rate. In our experiment we changed the influent flow rate, taking samples after at least 2 pore volumes passed. The influent and effluent concentrations as well as an HRT of 8 hours were used to create Figure 7.

ZVI is the electron source in our system. In order to investigate the effect it has on the reaction its amount was varied. It can be concluded from Table 2, that when the percent of zero valent iron increases the chemical reaction rate increases. There is only a slight increase in the reaction rates as ZVI was changed from 2% (v/v) to 4% this is because the enzyme responsible for perchlorate reduction were saturated with electrons, or possibly the water flowing through the system was saturated with H_2. In either scenario, no more electrons were available to be utilized, or transported to the enzyme perchlorate reductase. It was also observed that there is a significant reduction in the reaction rate when the system is limited to 0.5% ZVI. From Figure7 as well as Table 2 it can be concluded that the chemical reaction rate for our system is approximately 0.777 hr^{-1} when the system is saturated with electrons.

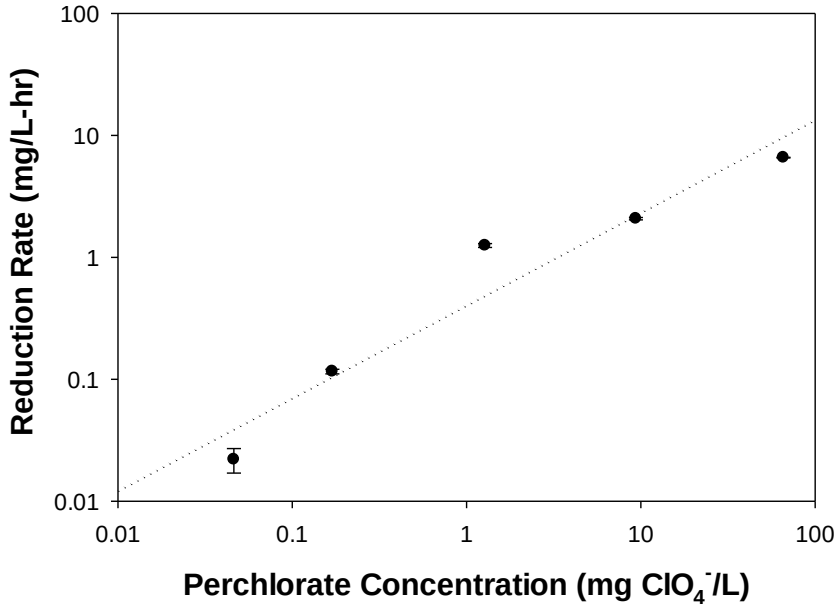


Figure 7: Chemical Reduction Rate

The perchlorate reduction rate increases slightly as the concentration of the electron donor increases. The increase in the rate decreases as more iron is introduced; this is because the microorganisms are saturated with the electron donor. The reduction rate was calculated using 1st order kinetics and linear regression.

Table 2: Effect of zero-valent iron on reduction rate

Zero Valent Iron (% v/v)	K (hr^{-1})
4%	0.777
2%	0.761
1%	0.735
0.50%	0.68

In the table it can be seen that when the percent of ZVI increases (increase in H_2) the chemical reduction rate increases. The rate at which the reaction rate increases is decreased as the percent of ZVI reaches four, because of the H_2 saturation.

3.5.2. Microbial Growth Kinetics (Column)

Experimental results for the growth kinetics of perchlorate reducing culture in the systems were presented in Figure 9 (data points). Experimentally determined microbial kinetic parameters were based on the Monod growth kinetics (eq. 8):

$$\mu = \frac{\mu_{\max} S}{S + K_S} \qquad \text{(eq. 8)}$$

Where μ is the growth rate (hr^{-1}), S is the concentration of perchlorate (mg/L), Ks is the half saturation perchlorate concentration (mg/L), and μ_{max} is the maximum growth rate (day^{-1}). The constants K_s and μ_{max} were evaluated from the linearized form represented by the eq. 10:

$$\frac{1}{\mu} = \frac{K_S}{\mu_{\max}} \frac{1}{S} + \frac{1}{\mu_{\max}} \qquad \text{(eq. 9)}$$

A plot of 1/μ against 1/S gives a linear line with a slope of (K_s / μ_{max}) and an intercept of $1/\mu_{max}$. The correlation coefficient (R^2) describing the goodness of fit to the linearized Monod curve was 0.91. The K_s and μ_{max} were 15.4 mg/L and 0.55 hr^{-1}. The results of this analysis can be seen in below in Figure 8.

The reactor system uses the mixed cultures for perchlorate reduction, K_s and μ_{max} represent the average value of all the perchlorate reducing culture in the column system: $\overline{\mu_{\max}}$ and $\overline{K_s}$. Note that these parameters are considered as the overall average of each individual strain's actual characteristics [46, 47].

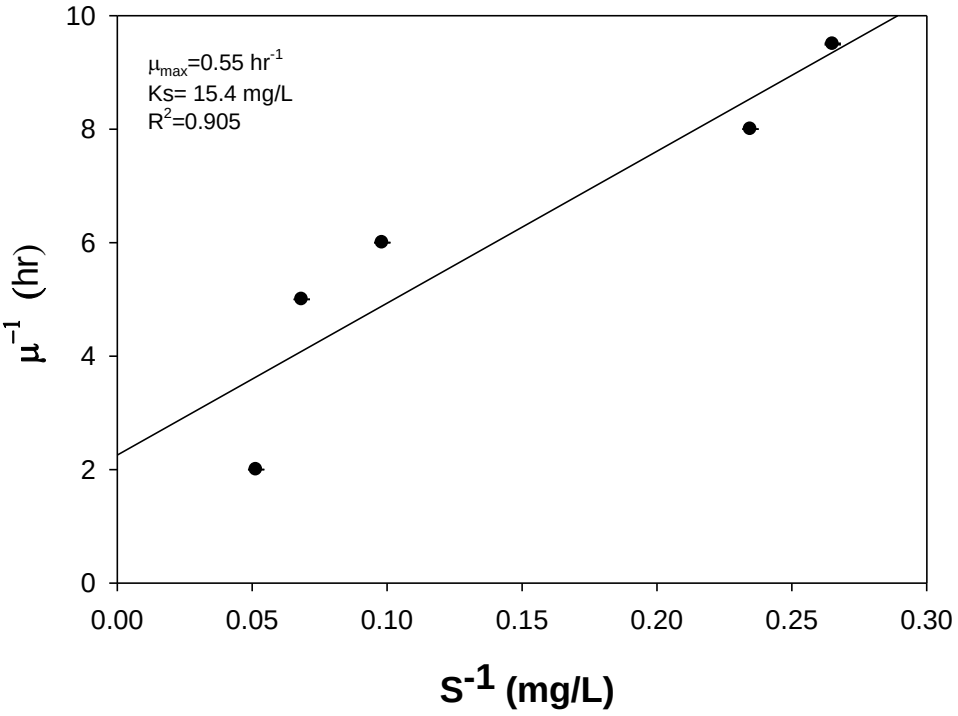


Figure 8: Microbial Kinetics (column)

Figure 8 plots the inverse of the average growth rate (μ) for our system with the inverse of the substrate concentration (perchlorate). With an R^2 value of 0.91 we can conclude that the maximum growth rate for our system (μ_{max}) is 0.55 hr $^{-1}$ and the half saturation constant (K_s) is 15.4 mg/L. The growth rate and half saturation constant are expressed as averages, because our system is a mixed culture and the parameters are averages of each individual strain present.

The yield coefficients were calculated as described by Rittmann and McCarty [47] using a perchlorate half reaction modified from Urbansky [49]. Yield coefficients for both autotrophic and heterotrophic bacteria were calculated, because our system is a mixed system and it has been previously reported that both heterotrophic and autotrophic perchlorate reduction takes place in similar systems [35]. The calculations for each yield coefficient can be found in the appendix, but a table summarizing the input parameters as well as calculated values can be seen below. These calculated values should be understood to be representative ranges of the true values, because theoretical yield coefficients can hardly predict actual mixed cultures. Therefore some range of these values was used to better reflect the true yield coefficient of our system.

Table 3: Theoretical yield calculation

	Autotrophic	**Heterotrophic**
Electron Acceptor	Perchlorate	Perchlorate
Electron Donor	Hydrogen	Domestic Wastewater
N-source	Ammonia	Ammonia
C-source	CO_2	Domestic Wastewater
Y (g cell /g E.D.)	0.074	0.227

A numerical model was constructed using equations of advection, dispersion, Monod growth, and attachment and detachment of biomass to describe the reduction of the perchlorate in the column system; the governing equations for all the species in this model are as follows:

$$\frac{\partial S}{\partial t} = -V\frac{\partial S}{\partial x} + D\frac{\partial^2 S}{\partial x^2} - \left[\frac{q_{max} S X}{S + K_s}\right] \qquad \text{(eq.11)}$$

$$\frac{\partial X_M}{\partial t} = -V\frac{\partial X_M}{\partial x} + D\frac{\partial^2 X_M}{\partial x^2} + \left[\frac{Y q_{max} S X_M}{S + K_s}\right] - k_{att} X_M + k_d \qquad \text{(eq.12)}$$

$$\frac{\partial X_{IM}}{\partial t} = \left[\frac{Y q_{max} S X_{IM}}{S + K_s}\right] + k_{att} X_M - k_{det} X_{IM} \qquad \text{(eq.13)}$$

Where, V is the velocity (cm/hr), D is hydrodynamic dispersion coefficient (cm^2/hr), S is perchlorate concentration (mg/L), X is the total biomass concentration (mg/L, $X_{IM}+X_M$), X_{IM} is the immobile biomass in column (mg/L), X_M is the mobile biomass concentration (mg/L), Y is the yield coefficient (mg of biomass/mg of substrate), q_{max} is maximum substrate-utilization rate (hr^{-1}), k_{att} is the rate of attachment of the mobile phase bacteria (hr^{-1}), k_{det} is the rate of detachment of the immobile phase bacteria (hr^{-1}).

Equation 11 describes the fate and transport of the perchlorate (S) in the column and its microbial reduction due to the presence of the biomass (X). Equations 12 and 13 describe the growth of mobile and immobile phase biomass and their attachment and detachment processes. It was assumed that some of the biomass detaches to form a mobile phase biomass which is eluted along the length of the column. Some of this detached mobile phase biomass reattaches to the porous media forming immobile phase biomass. This is a common way to model similar systems [50]. The model simulated the identical parameters of the column and the results were plotted

alongside our experimental data in Figure 14. The table below shows the parameters for the model simulations.

Table 4: Model parameters

Parameter	Value	Unit
Y	0.227	g-X/g-E.A.
$\overline{\mu_{max}}$	0.55	hr^{-1}
$\overline{Ks}$	15.4	mg/L
$\mathbf{K_{att}}$	0.09	hr^{-1}
$\mathbf{K_{det}}$	0.45	hr^{-1}

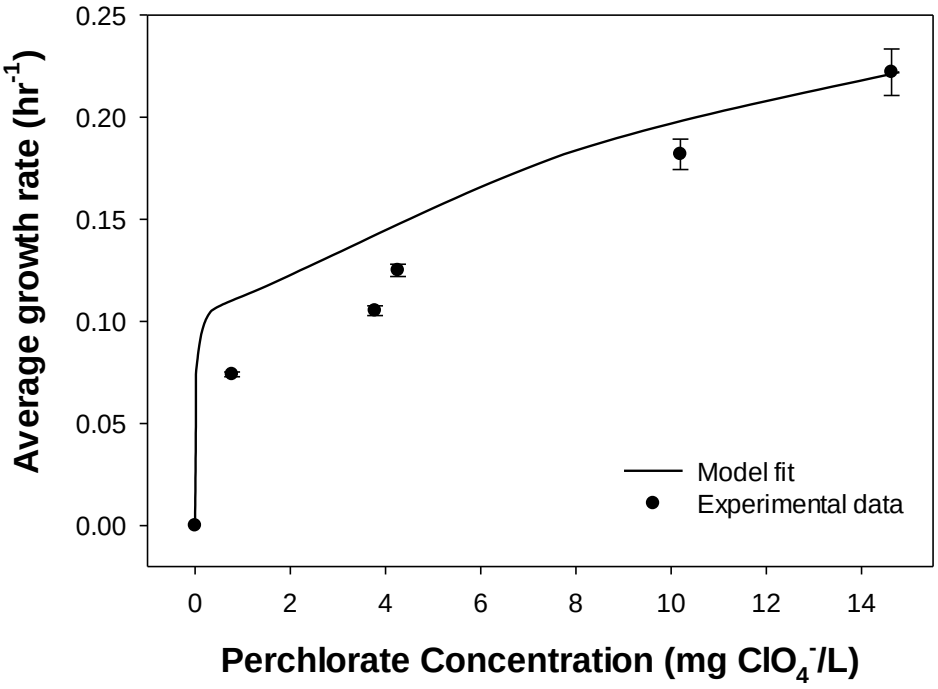


Figure 9: Monod Growth Curve

Microbial growth kinetics of perchlorate reducing bacteria in the ZVI-supported column reactor were determined by experiments (points) and numerical models (line). The growth rate (μ) similarly follows the Monod growth kinetics and model fits very well with the experimental data.

Chapter 4: Conclusions

In this study it has been concluded once again that unacclimated mixed microbial consortium feeding on H_2 from zero valent iron corrosion can completely reduce perchlorate in a continuous flow through column. Four process controls parameters are: 1) HRT, 2) pH 3) required nutrients, 4) kinetics. The parameters were analyzed in order to optimize a technology to safely and efficiently treat perchlorate laden water to levels below the forthcoming federal regulation of 1 µg/L. The optimum HRT was determined to be 8 hours for our flow through reactor, and it was determined that the hydroxide released during the essential iron corrosion causes the pH to rise above the enzyme's "optimum" range for perchlorate reduction. Both HEPEs and TE pH buffers proved to be viable to control the pH of the reactor within this "optimum" range (7.0-8.0). This research proved that additional nutrients might not be necessary when wastewater sludge is used to inoculate the reactor, because of the plethora of nutrients available within the sludge itself. Kinetics, both chemical and microbial, of this mixed microbial system were analyzed. The chemical degradation rate was concluded to be 0.777 hr^{-1} in a system saturated with H_2. It was also proven that the amount of ZVI has an effect on the degradation rate until some saturation limit is reached. While trying to determine if the microbial growth kinetics of a similar batch system could be compared to that of the flow through reactor it was determined that when H_2 concentration is low and dissolved oxygen is allowed to accumulate within

the reactors the reaction rate is severely inhibited. The microbial growth kinetics of the flow through reactor were studied and values for both the maximum growth rate as well as the half saturation constant were estimated to be 0.55 hr^{-1} and 15.4 mg/L, respectively. A novel concept presented in this research is that these values are simple averages of the entire system, not actual values for specific strains of PRBs. These values as well as others discussed throughout this paper should be treated as average values within some reasonable range for each system. We were also able to recreate our data using a mathematical model with simple Monod expressions. Using these process control factors as well as important parameters described elsewhere a safe and effective treatment option can now be implemented.

To summarize the conclusion:

(1) The minimum HRT for our system to completely reduce 10 mg/L of perchlorate is 8 hours.

(2) The pH in iron supported microbial perchlorate reducing systems is a governing process control and should be controlled to at neutral pH value.

(3) Additional nutrients are not needed when wastewater sludge is used to inoculate the system.

(4) Both perchlorate reduction kinetics and microbial growth kinetics were elucidated for the perchlorate reducing column system.

(5) The numerical model successfully simulated microbial growth kinetics within a continuous flow-through microbial column system.

These conclusions can be further used to optimize full scale perchlorate remediation systems, as well as assist in the understanding of other microbial remediation technologies which might follow similar kinetic characteristics.

Chapter 5: Future Work

Much progress has been made in the field of microbial reduction of perchlorate in the presence of zero-valent iron, but more studies are needed. The major process controls have been analyzed, and it has been determined that hydrogen gas from iron corrosion can be utilized. However a more in depth analysis of the active microbial communities could be extremely beneficial. As well as a more in depth study of the controlling kinetics in order to elucidate the system. A long term investigation into the use of microorganisms from WWTP including the options available to introduce additional sludge when needed would be an interesting research opportunity. Also a detailed pilot scale investigation with an in-depth cost analysis would be of great benefit to the viability of this technology.

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Appendix

Autotrophic Yield calculation

$1/8\ ClO_4^- + H^+ + e^- = 1/8Cl^- + ¼\ H2O \quad -7.66\ kCal*mol^{-1}$ (eq.14)

1. ΔG_r= E.D.-E.A. (eq.15)

   ΔG_r=-17.33

2. ΔG_p = C.S. – pyruvate (eq.16)

   ΔG_p =27.220

3. ΔG_N =0 (Ammonia is the N-source)

   Є = 0.4-0.8 (efficiency of system to create biomass)

   ΔG_c=7.5 (equivalent to create from ammonia and pyruvate)

4. $A=\dfrac{\frac{-\Delta G_P}{\varepsilon^m}-\frac{\Delta G_N}{\varepsilon}-\Delta G_c}{\varepsilon * \Delta G_R}$ =5.084 ⟶ $Ae=\dfrac{1}{1+A}$ = 0.164 (eq.17)

5. $Y=Ae^* \dfrac{\frac{g-cell}{e^- eq}}{\frac{g-EA}{e^- eq}}$ (eq.18)

   $\dfrac{g-cell}{e^- eq}$ =5.66 $\qquad$ $\dfrac{g-EA}{e^- eq}$ = $1/8ClO_4^-$: 1 e^- = 12.5

6. Y_{auto}=0.074 g cell/g E.A.

Heterotrophic Yield calculation

$1/8ClO_4^- + H^+ + e^- = 1/8Cl^- + ¼\ H_2O$ $\quad -7.66\ kCal*mol^{-1}$ (eq.14)

1. ΔG_r= E.D.-E.A. (eq.15)

   ΔG_r = -15.26

2. ΔG_p = C.S. – pyruvate (eq.16)

   ΔG_p =0.945

3. ΔG_N =0 (Ammonia is the N-source)

   Є= 0.4-0.8 (efficiency of system to create biomass)

   ΔG_c=7.5 (equivalent to create from ammonia and pyruvate)

4. $A=\dfrac{\frac{-\Delta G_P}{\varepsilon^m}-\frac{\Delta G_N}{\varepsilon}-\Delta G_c}{\varepsilon * \Delta G_R}$ =0.991 $\longrightarrow$ Ae= $\dfrac{1}{1+A}$ = 0.502 (eq.17)

5. $Y=Ae*\dfrac{\frac{g-cell}{e^- eq}}{\frac{g-EA}{e^- eq}}$ (eq.18)

   $\dfrac{g-cell}{e^- eq}$=5.66 $\quad \dfrac{g-EA}{e^- eq}$ = $\quad 1/8ClO_4^-$: 1 e^- $\quad$ =12.5

6. Y=0.227 g cell/g E.A.