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Treatment of Perchlorate and Multiple Oxyanions: Chemical and Biological Investigations

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TREATMENT OF PERCHLORATE AND MULTIPLE OXYANIONS: CHEMICAL AND BIOLOGICAL INVESTIGATIONS

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ABSTRACT

Groundwater contamination with oxyanions is an issue that must be addressed for environmental, ecological and societal reasons. Chemical and biological treatment methods of reduction are already known and practiced, but the simultaneous presence of multiple oxyanions can add complicating effects to treatment sequence and efficacy. This research was concerned with the investigation of the chemical reductive treatment of Cr(VI) using both calcium polysulfide (CaS_x) and ferrous sulfate ($FeSO_4$), and the biological reductive treatment of chlorate, Cr(VI), nitrate and perchlorate using multiple organic substrates. The chemical treatment phase consisted of initial jar tests, using CaS_x and $FeSO_4$, followed by a laboratory column study using CaS_x. The biological treatment phase first implemented microcosm testing, using EOS-PRO, Industrial Sugar Wastewater (ISW) and Molasses as organic substrates, followed by laboratory column testing utilizing EOS-PRO and ISW.

The chemical jar tests showed that high doses (around 10 mg Cr(VI)/L) could be treated, to a great extent, by 2-3 times stoichiometric doses of CaS_x and 10-20 times stoichiometric doses of $FeSO_4$, (perhaps even lower for one groundwater/soil). Treatment was improved in the presence of solids, and the type of solids was also found to have importance. Treatment of low Cr(VI) concentrations (about 0.5 mg Cr(VI)/L) was seen to be less effective. It is thought that at lower Cr(VI) concentrations, dissolved oxygen competes with Cr(VI) for reduction and thereby reduces the efficacy of the reductant. The in-situ-simulating column tests resulted in very strong and reliable removal of Cr(VI) and total dissolved chromium from the column effluent water, regardless of initial Cr(VI) concentration (~1 mg Cr(VI)/L or ~10 mg Cr(VI)/L).

Biological treatment testing was not thoroughly isolated from the possible chemical effects of the organic substrate additions. The microcosm testing showed that reduction of all the oxyanions was possible, except for perchlorate which was only found to reduce minimally. However, different substrates were observed to greatly affect the treatment efficacy. For example, Cr(VI) was found to have low detection after just 7 days with ISW use (thought to be partially a chemical process) in one groundwater, while EOS-PRO resulted in higher Cr(VI) concentration after 36 days. On the other hand, EOS-PRO greatly reduced nitrate in one groundwater after 26 days, while nitrate concentrations were still higher after 99 days when ISW was used. Different contaminants were noted to have very different reactions to substrates. ISW treated Cr(VI) very quickly, but was very ineffective at reducing chlorate. Overall, Cr(VI) reduction kinetics were found to be first-order, but the reduction reaction orders of other oxyanions were thought to be affected by the presence of the co-occurring oxyanions. The groundwater/soil also had dramatic impacts on treatment. Biological column reduction testing indicated reduction of all oxyanions was possible, but treatment effectiveness varied noticeably between different groundwater/soils.

In general, the order of treatment from easiest to most difficult seemed to be Cr(VI), nitrate, chlorate and perchlorate, although the groundwater/soil and substrate had dramatic effects on the results.

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COD: Chemical Oxygen Demand

EBCT: Empty Bed Contact Time

EVO: Emulsified Vegetable Oil

FBR: Fluidized Bed Reactor

GAC: Granular Activated Carbon

ICP: Inductively Coupled Plasma

IX: Ion Exchange

LOEC: Lowest-Observed-Effect Concentration

MCL: Maximum Contaminant Level

MDL: Method Detection Limit

MIC: Minimum Inhibitory Concentration

NOEC: No-Observed-Effect Concentration

PHG: Public Health Goal

ppb: parts per billion

PRB: Perchlorate-Reducing Bacteria

QC: Quality Control

RO: Reverse-Osmosis

TDS: Total Dissolved Solids

US EPA: United States Environmental Protection Agency

ZVI: Zero-Valent Iron

CHAPTER 1

INTRODUCTION

1.1 Problem Statement

A former industrial site, the basis of the current investigation, is the location of extensive soil and groundwater contamination with multiple oxyanions, including chlorate (ClO_3^-), chromate (CrO_4^{-2}), nitrate (NO_3^-) and perchlorate (ClO_4^-). The contamination has migrated and reached vital water bodies; therefore, the remediation of the site is a priority. The geology of the site includes upper quaternary sediments (QS) (i.e. alluvial fan deposits) consisting of poorly sorted gravels, sands and cobbles (which are very permeable), with a small portion of clay and silt. It has no distinct or continuous units (Batista et al. 2003). In the area of the referred study, this layer varies from roughly 3 to 18 m in depth. The alluvial deposits have channel and inter-channel portions with generally different characterization of sediments. Underneath, is the Tertiary formation (TCF), which, in general, consists of silty clays, sand clays, clays, clayey sands, gypsiferous sandy clays, and conglomerates (Batista et al. 2003). The interface between the two layers is poorly defined. In one portion, there is also a caliche layer between the strata (Batista et al. 2003). Currently, the contaminated site is being characterized and potential clean-up technologies are being evaluated to find methods which will simplify and improve remediation.

Previous evaluation of remediation potential for the groundwater of the site included Pump and Treat, which was accomplished with biological reduction of nitrate, chlorate and perchlorate, and chemical reduction of hexavalent chromium. Biological treatment of all the

contaminant oxyanions of this site is feasible because all of them have been proven to degrade biologically under certain conditions. Coincidentally, all the contaminants can be used as electron acceptors by microorganisms, in the presence of an electron donor and a carbon source. Another potential treatment strategy for the site is in-situ treatment. Chemical reducing agents or biological reduction supplements can be injected into wells to promote in-situ treatment as opposed to the current ex-situ configuration where water must be pumped and treated and then discharged into a water body. There are three important issues associated with ex-situ treatment. The first is that pumping, transporting and treating water can have significant operating costs associated with the processes, compared to in-situ treatment. The second is that ex-situ treatment generates an effluent that must be discharged into a water body. Third, removing and treating the contaminated groundwater does not address any contamination of the overlaying soils of the vadose zone. An example of combined perchlorate treatment of both a contaminated groundwater and a contaminated vadose zone, (where contaminated water was cyclically pumped up, an electron donor was added to it and the water was applied to the shallow layers of soil), was found successful at reducing perchlorate, with some limiting factors (Levakov, Ronen, and Dahan 2019).

The goal of this research is to generate data and evidence, to guide future in-situ treatment at this site and at other sites where multiple oxyanion contaminants are found.

1.2 Objectives and Hypotheses

The current research aims at investigating chemical reduction of Cr(VI) and understanding the conditions that will foster biodegradation of the several co-occurring

oxyanions, namely perchlorate, chlorate, nitrate, and chromate. If successful, the information gained from this research will support the application of in-situ treatment for contaminated sites. The principal objectives of this research are twofold:

Objective 1 - Hexavalent Chromium (Cr(VI)) Reduction:

To investigate chemical reduction of hexavalent chromium, using two reducing agents and actual soil and groundwater samples from a site.

Ferrous sulfate is currently used in a small plant at the site to reduce hexavalent chromium, and treatment of larger flowrates will necessitate the evaluation of competing technologies. This research will investigate and compare both ferrous sulfate and calcium polysulfide to determine if there are improved treatment effects, in terms of treatment quality, chemical consumption and sludge production when calcium polysulfide is used.

Hypothesis 1:

It is proposed that smaller mass ratios of CaS_x to Cr(VI), (versus $FeSO_4$ to Cr(VI)) will result in similar or lower Cr(VI) concentrations in treated groundwater, since the theoretical stoichiometric mass demand is smaller.

In a previous study, a Cr(VI) contaminated site showed limited Cr(VI) to Cr(III) reduction success with traditional remediation methods for Cr(VI)-contaminated land (organic matter—a reductant—and ferrous sulfate use). The researchers discussed laboratory studies on calcium polysulfide that were considered successful at reducing Cr(VI), rapidly and

quantitatively to Cr(III) for the typical pH range seen at the sites (Graham et al. 2006). Calcium polysulfide is known to be economically feasible and highly effective for Cr(VI) removal (Dahlawi and Siddiqui 2017). Moreover, a recent study demonstrated that Cr(VI) contaminated soil was better stabilized by calcium polysulfide than ferrous sulfate. Cr(VI) stabilization and immobilization were enhanced significantly by calcium polysulfide and its toxicity and leachability were reduced (Zhang, Xue, and Wei 2018). Calcium polysulfide clearly would be expected to be effective at the current site.

The stoichiometric ratio of coagulant to chromium is a key consideration. The ratios of CaS_x to Cr(VI) and $FeSO_4$ to Cr(VI) are 1.5 and 3 respectively. Therefore, at pure stoichiometric ratios, a larger mass of ferrous sulfate is required. Research showed that when treating ion exchange brines for Cr(VI), using calcium polysulfide, a molar ratio of up to 3.7 was needed to obtain 0.1 mg/L chromium in the effluent. It was also found that coagulation sludge solids production, and added CaS_5 amount, were directly proportional (Pakzadeh and Batista 2011). In other research investigating Cr(VI) treatment of concentrated regenerant brine using $FeSO_4$, the authors found that close to stoichiometric ratio doses removed total chromium almost completely (Li et al. 2016). Interestingly, according to another reference, ferrous sulfate reduction of Cr(VI) is not attractive, (although it is simple) because of sludge formation which is excessive (Dutta et al. 2010).

Clearly, treatment behavior and sludge production may depend on site and groundwater matrix characteristics; therefore, investigating this technology for specific site conditions is useful. For the given in-situ treatment objective, sludge solids may cause soil clogging issues

and a laboratory column investigation should provide useful insight on this matter. It is important, however, to investigate its treatment effects with the site-specific soils and groundwaters, as each new environment provides novel chemical interactions.

The specific questions to be answered by this research are:

1. Is CaS_x effective at treating Cr(VI) in site groundwater? At what doses and to what treatment levels?
2. When compared to treatment using $FeSO_4$, is treatment better? How do doses compare? How does sludge production vary?
3. Would simulated in-situ conditions result in effective treatment? For example, would simulated in situ retention times be adequate for Cr(VI) reduction? Would reaction precipitates cause disruption in flow conditions?

Objective 2 - Biodegradation of Oxyanions:

To use various organic substrates as electron donors/carbon sources, to understand the conditions that support biological reduction; also, to investigate the reduction sequence of the multiple oxidized contaminants present at the site.

Currently, a part of the site's contaminated water is treated biologically for three of the four oxidized contaminants, but not for chromium. The key issues to investigate here are: treatment quality, speed, required doses of substrates, potential of treating multiple co-occurring oxyanions, reaction order, and interferences of oxyanion contaminants. Additionally, microbial

communities can vary from site to site and this will have influence on the degradation. Investigating the presence of oxyanion degrading microbes present will also be important. The investigation of these issues is integral to the second hypothesis.

Hypothesis 2:

i. **It is proposed that treatment using EOS-PRO will result in lower concentrations of chlorate, Cr(VI), nitrate and perchlorate in treated groundwater than treatment with molasses and industrial sugar wastewater, for equivalent initial substrate doses (measured as COD) and equivalent reaction times. Although all three electron donors are rich in organics, EOS-PRO includes rapidly biodegradable substrates and micro-nutrients that will further promote biological activity.**

The type of organic carbon added can have significant effects on treatment. For example, in research on a bacterial strain, different sources of carbon were found to have different effectiveness for Cr(VI) bacterial reduction stimulation (Batool, Yrjälä, and Hasnain 2012). Carbon sources can differ in a multitude of ways: they can vary drastically in their reducing capacity (concentration of organics), rapidity of treatment (availability to microorganisms), and so on. Additionally, a very important consideration is the source of organic substrates. The organics to be tested in this research come from varied sources. One is an industrial wastewater, the other a dedicated remediation substrate and the third a commercial food product. Required doses and substrate costs can be very different for each. Clearly, the current investigation on organic sources, with their wide variations will be warranted, particularly since the industrial

wastewater used in this work for bioremediation is not known to have been used previously for such purposes.

ii. **It is proposed that the current rare contaminant combination of chlorate, Cr(VI), nitrate and perchlorate in the groundwater may be concurrently degraded biologically, because all those compounds are electron acceptors and all have been found to be biodegradable alone or in other combinations.**

There has been previous research on the treatment of multiple oxidized contaminants: Cr(VI) and chlorate (Holger and Lagerkvist 1996), Cr(VI), nitrate, sulfate and more (Xia et al. 2013), but to the knowledge of the author, such concurrent large combinations of oxyanions are rare and the specific combination of chlorate, Cr(VI), nitrate and perchlorate has not been investigated. It is very likely that bacteria capable of reducing one of the oxyanions in the water will be capable of treating one, or more, of the others. For example, perchlorate and chlorate are utilized as terminal electron acceptors by dissimilatory (per)chlorate-reducing bacteria during anaerobic respiration (Youngblut et al. 2016). Furthermore, soils and groundwater can host a wide array of species of bacteria, with far more diversity and treatment potential than a single species.

iii. **It is proposed that the oxyanion reduction order may not necessarily follow the preferred order expected based on standard reduction potentials (chlorate > perchlorate > chromate > nitrate), since other factors such as structural stability may also affect the reduction series. In the case of**

perchlorate, for example, the presence and orientation of its oxygen atoms sterically block the attack of reductant molecules (Ye et al. 2012).

Treatment order does not necessarily proceed according to standard reduction potentials. The order of standard or possible standard reduction potentials, from highest to lowest, is: chlorate > perchlorate > chromate > nitrate (MWH 2005; Vanýsek n.d.). For example, although perchlorate has a higher standard or possible standard reduction potential than nitrate (MWH 2005; Vanýsek n.d.), in nitrate presence, perchlorate reduction lags were noted by Zhu et al. (2016). The prediction of reduction order using standard reduction potentials fails to consider certain factors. For example, perchlorate is thermodynamically unstable, but it is very inert, resulting from the energetically stable oxygen tetrahedral structure (Ye et al. 2012). Another example of the lack of predictability based on standard reduction potentials is that (per)chlorate respiring organisms do not experience standard reduction potentials since an intermediates-bypassing dismutation reaction is not accounted for (Youngblut et al. 2016). The reaction order is interesting for predicting the timeframe for the reduction of various compounds, but it is also of interest because it may affect the required carbon substrate doses. For example, while nitrate might not be the treatment target, it may be necessary to completely reduce nitrate, prior to the initiation of reduction of a target constituent.

Key research questions to be investigated:

1. Of the organic substrates tested, which ones are effective at reducing pollutant oxyanions in the water?

2. Which substrates are more/less effective in terms of reduction time and treatment effectiveness?
3. What will be the reduction sequence of oxyanions and will they all be reduced?
4. Will retention times for simulated in situ treatment be adequate for the degradation of all oxyanions? If not, which ones?
5. Will simulated in situ treatment result in flow inhibition?

CHAPTER 2

STATE OF THE KNOWLEDGE

The following chapter provides some relevant background information on topics such as the investigated contaminants, current treatment technologies, etc., that are related to this research project.

2.1 Oxyanion Occurrence, Sources, Regulations, Health Effects and Typical Concentrations

2.1.1 Chromate

2.1.1.1 Sources and Occurrence

Leather tanning, chrome plating, textile, pigment, and wood preservation, are among the areas of use of chromium (Rai et al. 2016). Barrera-Diaz et al. (2012) provide a valuable overview on hexavalent chromium and its reductive treatment. Cr(VI) is carcinogenic, mutagenic, and a strong oxidizing agent. Its diffusion through aquatic environments and soil is rapid. It is not possible to separate Cr(VI) by precipitation, since in aqueous solutions no insoluble compounds are formed. Cr(III) cations, as opposed to Cr(VI) oxyanions, are neither environmentally toxic nor highly mobile. Insoluble precipitates are formed by Cr(III). Therefore, toxicity, mobility and difficulty of removal from effluent are all diminished by reducing Cr(VI) to Cr(III). The relative proportions of $Cr_2O_7^{2-}$, CrO_4^{2-}, H_2CrO_4, and $HCrO_4^-$, the species of Cr(VI) most likely found in aqueous solution, depend on the pH of the solution, the redox potential and the concentration of hexavalent chromium (Barrera-Díaz et al., 2012). A method of direct precipitation for separation is not feasible, since insoluble precipitates are

formed by none of those species (Barrera-Díaz et al., 2012). The following diagrams and captions (Figures 1 and 2) are taken from Barrera-Diaz et al., 2012.

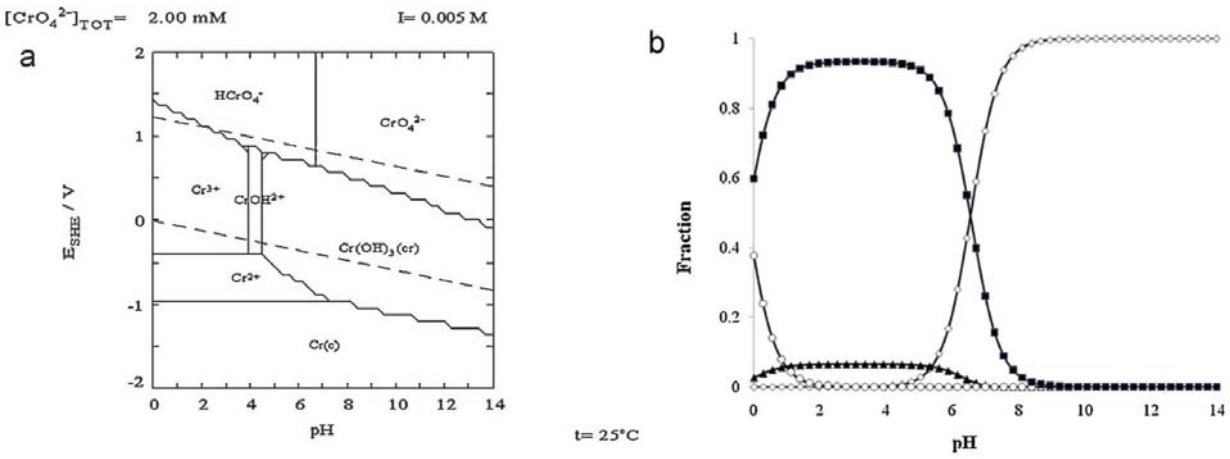


Figure 1: (a) Pourbaix Diagram for Cr Chemical Species in Aqueous Solution. $[CrO_4^{2-}]$ = 2.00 mM, I = 0.005 M and T = 25 °C. (b) Predominance Zone Diagram for Cr(VI) Chemical Species in Aqueous Solution. (◊) CrO_4^{2-}, (▲) $Cr_2O_7^{2-}$ (○) H_2CrO_4 and (■) $HCrO_4^-$. Figures and Subtitle Taken from (Barrera-Díaz et al. 2012).

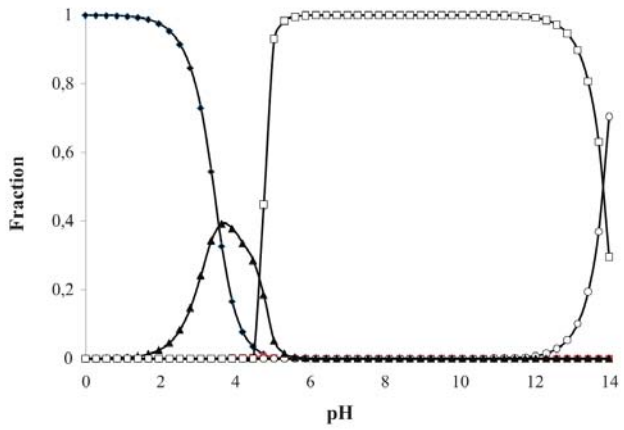


Figure 2: Predominance Zone Diagram for Cr(III) Chemical Species in Aqueous Solution. (♦) Cr^{3+}, (□) $Cr(OH)_{3(S)}$ (▲) $Cr(OH)_2^+$, (○) $Cr(OH)_4^-$. Figures and Subtitle Taken from (Barrera-Díaz et al. 2012).

The authors stated that the diagrams (Figure 1) show that pH variations do not result in insoluble species of Cr(VI). For pH values shown in Figure 2, however, insoluble species are formed by Cr(III) (Barrera-Díaz et al. 2012).

2.1.1.2 Regulations

The US EPA has a 100 ppb drinking water standard for total chromium (all chromium forms included). Testing for total chromium is required by water systems. Long term, potential negative dermatological effects inspired the current standard. Starting in 2008, due to new chromium-6 science, its health effects are under a comprehensive and rigorous review (US EPA 2017a). By comparison, California has a stricter MCL of just 50 ppb for total chromium, and as of July 1, 2014, a 10 ppb Cr(VI) MCL. The California MCL should approach, as nearly as

possible, considering costs and technical feasibility, the public health goal (PHG). A PHG of 0.02 ppb for Cr(VI) was completed in 2011 (California Water Boards 2015).

2.1.1.3 Health Effects

Chromium exposure can be through contaminated air inhalation or through ingestion of contaminated drinking water or food. High levels of Cr(VI) can cause nose damage and cancer. Chromium (VI) ingested at high levels can cause stomach and intestinal damage or anemia. Chromium (III) is a nutrient which is essential. Breathing Cr(III), as well as Cr(VI), can cause nose and breathing problems but at much higher concentrations. Both Cr(III) and Cr(VI) can cause skin allergies and some compounds of Cr(VI) can lead to ulcers of the skin (ATSDR 2012).

2.1.1.4 Concentrations

The literature provides levels of chromium that have been previously reported. Polluted groundwater in India was reported to have 0.295 mg/L of chromium (Ramachandran et al. 2017). Unfiltered well samples from the Tucson International Airport Area Superfund site had reported ranges from 0.2 to 10.8 µg/L (hexavalent chromium) and 0.2 to 15.7 µg/L (total chromium), (Tillman, McCleskey, and Hermosillo 2016), while wells near the contamination source of a contaminated industrial site in Mexico were reported to have hexavalent chromium levels ranging from 3.93 to 116 mg/L (Castro-Rodríguez et al. 2015). As shown by this snapshot of the literature, the ranges of chromium contamination are wide, and the levels can be quite significant.

Groundwater samples in the current work were found to have Cr(VI) at concentrations between about 23 and 21,000 µg/L.

2.1.2 Nitrate, Chlorate, Perchlorate

2.1.2.1 Sources and Occurrence

Groundwater nitrate pollution can have various pathways of origin, including, for example, dairy lagoons, septic systems intensive livestock farming, and wastewater effluents (point) and for example, pesticides, fertilizers, heavy metals, manure application, and atmospheric deposition, etc. (non-point) sources (Zhai et al. 2017; Almasri, 2007; Arauzo and Martínez- Bastida, 2015). Chlorate has applications as a soil sterilant, an herbicide, a post-harvest desiccant, a pulp-bleaching agent and in chlorine dioxide manufacture (Hunter 2002). Perchlorate has applications in rocket propellants, fireworks, explosives, and in certain fertilizer components it may be a contaminant that is present (Hunter 2002).

2.1.2.2 Regulations and Health Effects

Table 1 presents various state and federal contaminant standards and some of the health impacts associated with each of the oxidized contaminants of interest. The information is derived from, (US EPA 2017b), (US EPA 2017d), (AWWA 2014), (US EPA 2017c).

Table 1: Standards and Health Impacts Associated with Various Oxidizes Compounds

Compound	Standard	Health Impacts	Reference
Chlorate (ClO_3^-)	210 ug/L[a]	May decrease thyroid function. Can decrease uptake of iodide. Impairs oxygen carrying ability of blood, etc.[b]	AWWA, 2014 (citing: NAS 1987)
Nitrate- N (NO_3^--N)	10 mg/L[c]	Serious illness and possibly death in infants[d]	US EPA, 2017b
Perchlorate (ClO_4^-)	15 ug/L[e], 6 ug/L[f], 2 ug/L[g]	Iodide uptake interference and effects on thyroid. Hyperthyroidism historically treated with potassium perchlorate. Exposure to certain perchlorate salts may cause irritation, diarrhea, etc. Corrosive to eyes, etc. is perchloric acid.[b]	US EPA, 2017d (citing: EPA 2008, 2012; Cal/EPA 2016c; Massachusetts DEP 2016; ATSDR 2008; Cal/EPA 2015; National Research Council 2005; NIOSH 2014)
Sulfate (SO_4^{2-})	250 mg/L[h]	Aesthetic Effect: salty taste	US EPA, 2017c
Total Chromium	0.1 mg/L[c]	Allergic dermatitis[d]	US EPA, 2017b

[a] US EPA calculated health reference level

[b] Not an exhaustive list

[c] EPA MCL

[d] Possible long term above MCL exposure effects on health

[e] Non-legally enforceable Interim Drinking Water Health Advisory by EPA

[f] Enforceable drinking water standard in California

[g] Enforceable drinking water standard in Massachusetts

[h] EPA secondary MCL

2.1.2.3 Concentrations

The following map (Figure 3), taken from WellWaterGuide.net, shows concentrations of groundwater and well nitrates across the United States.

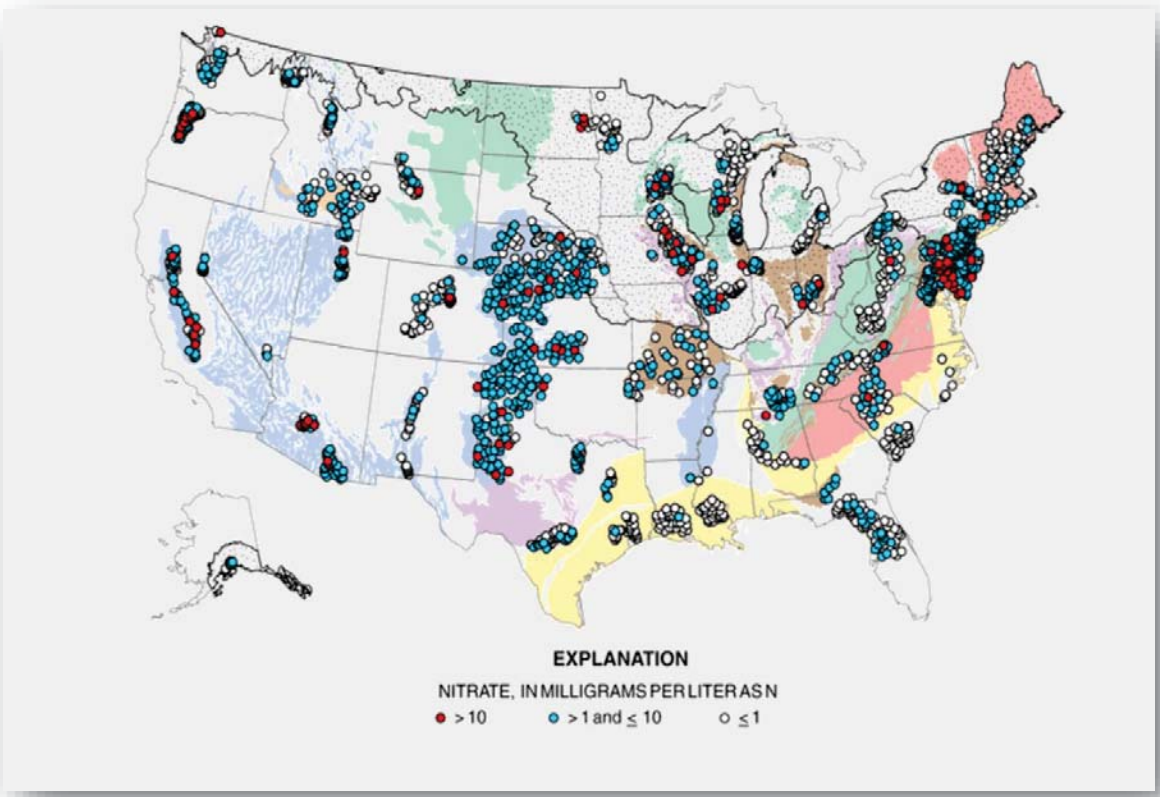


Figure 3: Well and Groundwater Nitrates (Anon 2017)

Of the wells, approximately 4% had nitrate concentrations above the 10 mg/L EPA MCL (Anon 2017). Levels such as: up to 300 mg NO_3/L (in a southern Portugal nitrate vulnerable zone), (Stigter, Carvalho Dill, and Ribeiro 2011), 29.4 mg/L NO_3^- (in natural groundwater in a Chinese study), (Tingliang et al. 2011) and 0.1 to 157.4 mg NO_3^-/L (in groundwater samples from a study in Syria), (Abou Zakhem and Hafez 2015), have been reported. Industrial area aquifer

groundwater samples in India were found to have nitrate-nitrogen levels ranging from 0.10 to 64.1 mg/L NO_3-N (Singh et al. 2006).

In terms of groundwater contamination, Sunset Well groundwater in Pasadena California was reported to have 0.091 – 0.10 mg/L of chlorate (M C Ziv-El and Rittmann 2009), while a shallow unconfined aquifer in Italy was once reported to have concentrations ranging between 0.01 to 38 (average 2.9) mg of chlorates per L (Mastrocicco et al. 2017).

In terms of groundwater perchlorate concentrations ranges, Ye, et al., 2012 summarize the following literature information from different study locations: 0.02 – 0.74 µg/L, three samples greater than 1 µg/L (India), up to 54.4 µg/L (China), up to 280 µg/L (California), 0.12 – 1.8 µg/L (Middle Rio Grande Basin), (Ye et al. 2012). Out of 326 samples tested for "pristine" sites, 137 could be quantified (120 ng/L MRL): 109 had less than 1000 ng/L and 28 had 1000 – 10400 ng/L, with greater than 10000 ng/L thought to be anomalous (across the coterminous United States), (Parker, Seyfferth, and Reese 2008). Other perchlorate levels have also been reported in the literature. For example groundwater in Las Vegas, Nevada was reported to have 0.18 – 3.7 g perchlorate/L (Sarria Cortes 2016), while contaminated groundwater from Edwards Air Force Base, California, was reported to contain 460 µg/L of perchlorate (Gu, Ku, and Brown 2003).

In the current work, groundwater samples were found to have nitrate levels which ranged from about 20 mg/L to over 1100 mg/L as NO_3^-, perchlorate from about 225 to over 1300 mg/L, while chlorate was measured at 3500/3600 mg/L.

2.2 In-Situ vs. Ex-Situ Treatment

A key decision that must be made when treating groundwater contamination, in addition to the treatment process to be used, is whether that water will be treated in place (in-situ treatment), or treated elsewhere (ex-situ treatment, typically pumping the water from the ground). Fruchter (2002) discusses in-situ treatment of groundwater contaminated with chromium. With in-situ treatment of an aquifer, the goal is the appropriate reagent delivery to aquifer contamination. Fruchter (2002) discusses abiotic approaches typically involving electron donors such as reduced sulfur and/or iron compounds. The methods to implement in-situ treatment include: permeable reactive barriers, iron particle barriers and other permeable reactive barriers—i.e. combining biological and chemical treatment (Fruchter 2002). Additional methods are: in-situ redox manipulation (a permeable zone for subsurface treatment), and chemically enhanced pump and treat—adding chemical reducing agent in reinjected treated groundwater for in situ treatment of residual hexavalent chromium (Fruchter, 2002). Calcium polysulfide is one usual choice of reagent for chemically enhanced pump and treat of Cr(VI) contamination. Other possibilities reported are electrochemical methods, also called electrokinetic remediation, which places direct current, low-voltage electrodes in the zone of contamination. Biological methods of in situ treatment include, microbial reduction and phytoremediation—plant remediation via uptake, accumulation/sequestration, or biochemical degradation (Fruchter 2002).

Ex-situ treatment of contaminated groundwater involves its removal by pumping and transportation to a different location for treatment (Kuppusamy et al. 2016). The treatment could then presumably include any known water contaminant treatment method.

2.3 Occurrence/Treatment of Multiple Oxyanions

The co-occurrence of perchlorate and nitrate in groundwater contamination is documented (Nam et al. 2016). Ammonium perchlorate wastes can contaminate groundwater as a result of rocket propellant manufacturing (Parkinson 2000). With time, ammonium can be oxidized to nitrate. Also reported, was the study of treatment of industrial filter sludge containing chlorate and chromium VI (Holger and Lagerkvist 1996). However, the co-occurrence of chlorate, chromate (Cr(VI)), nitrate, and perchlorate at the same site appears to be unique. The investigation of this dissertation deals with potential treatment of groundwater containing all four contaminants. Such co-occurrence can have interfering or delaying effects on the degradation of a given contaminant. It is also possible that the removal of more than one such oxyanion contaminant is desired or required by regulatory agencies. As mentioned earlier, all the oxyanion contaminants can be used as electron acceptors by bacteria and are therefore potentially biodegradable. The biodegradation of individual contaminants has been studied; however, co-biodegradation has evidently not been fully investigated to date. The following studies provide some insight on this topic.

Xia et al. (2013) implemented a lab-scale membrane biofilm reactor (hydrogen-based and continuously stirred), to reduce simultaneously BrO_3^-, Cr(VI), NO_3^--N, *para*-chloronitrobenzene (*p*-CNB), and SO_4^{2-}. Within one day, the reductions started. After a continuous operation of 112 days, there was more than 95% removal for all contaminants, with the exception of sulfate (sulfate was 37% removed, with high surface loading). The authors reported complete reductions of: BrO_3^- to Br^-, Cr(VI) to Cr(III), NO_3^--N via NO_2^--N to N_2, and *p*-CNB via *p*-CAN

to aniline. They reported that competition between the contaminants was indicated. With a limited H_2 supply, the authors noted that reductions of NO_3^--N and SO_4^{2-} controlled the electron consumption. Most of the total electron flux, over 99%, resulted from the combined reductions of NO_3^--N and SO_4^{2-}. The compounds accepted electrons in the following order: NO_3^--N, followed by SO_4^{2-} (Xia et al. 2013). The order of the remaining compounds was not apparent.

Table 2 presents relevant standard reduction potentials or possible standard reduction potentials from the literature for contaminants of interest in the present work.

Table 2: Standard Reduction Potentials, or Possible Standard Reduction Potentials for Relevant Oxidized Compounds (MWH 2005; Vanýsek n.d.).

Reaction/Reduction Half Reaction	E°/V
$ClO_3^- + 6\ H^+ + 6\ e \leftrightarrow Cl^- + 3\ H_2O$	1.451[a]
$ClO_4^- + 8\ H^+ + 8\ e \leftrightarrow Cl^- + 4\ H_2O$	1.389[a]
$Cr_2O_7^{2-} + 14\ H^+ + 6\ e \leftrightarrow 2\ Cr^{3+} + 7\ H_2O$	1.36[a]
$HCrO_4^- + 7H^+ + 3\ e \leftrightarrow Cr^{3+} + 4H_2O$	1.350[a]
$CrO_4^{2-} + 5H^+ + 3e \leftrightarrow Cr(OH)_3 + H_2O$	1.25
$2\ NO_3^- + 12\ H^+\ 10\ e^- \leftrightarrow N_2(g) + 6H_2O$	1.240[b]
$O_2(g) + 4H^+ + 4e^- \leftrightarrow 2H_2O$	1.230[b]
$ClO_4^- + 2H^+ + 2e^- \leftrightarrow ClO_3^- + H_2O$	1.190[b]

[a] Vanýsek, n.d.

[b] MWH, 2005

The standard reduction potentials shown in Table 2 suggest that the order of reduction, from least to most favorable (Brown et al. 2003), is expected to be nitrate, chromate, perchlorate and

chlorate, when all other variables are constant. The actual bio-reduction sequence will be investigated.

Chung et al. (2007) investigated whether the use of H_2 as an electron donor in the setting of membrane biofilm reactor (MBfR), permits concurrent reduction of oxidized contaminants in many different combinations. The MBfR simultaneously reduced varying combinations of arsenate, DBCP, nitrate, nitrite, and perchlorate plus chlorate, in contaminated groundwater. There was complete reduction to N_2 of nitrate for all groundwaters. Chlorate and perchlorate were reduced to ppb level. The authors also reported that switching the influent contaminant from chromate to selenite, or vice versa, in MBfRs not previously exposed to this contaminant, resulted in reductions in the new contaminant that were immediate and significant. The authors stated that these results support the notion that multiple oxidized contaminants can be simultaneously treated by the H_2-based MBfR (Chung et al. 2007).

To study the impact of nitrate and ammonium co-contaminants and different electron donors on perchlorate degradation, Guan et al. (2015) investigated several perchlorate degradation systems. In the ClO_4^- degradation, hydrogen was less easily used as an electron donor than acetate. Nitrate decreased acetate's inhibition and improved the degradation of perchlorate through Perchlorate-reducing bacteria (PRB) growth acceleration. PRB may have been able to use ammonia as a source of assimilable nitrogen for growth promotion. Compared to the heterotrophic systems (i.e. acetate as electron donor), the autotrophic system (i.e. hydrogen as electron donor) bacterial community was clearly different. Within systems studied, the most

dominant bacteria became *Azospira*. The most important factor influencing ClO_4^- degradation rate was the relative abundance of the bacteria (Guan et al. 2015).

As they sought mixed perchlorate reducing bacteria removal of high-strength perchlorate, Zhu et al. (2016) investigated kinetics of perchlorate reduction and the impacts of different environmental conditions on synthetic water perchlorate removal. They found that under optimal conditions, 50 – 1500 mg/L perchlorate could be rapidly degraded within 28 hours. The q_{max}, maximum specific perchlorate reduction rate, was 0.92 mg-perchlorate (mg-dry weight)$^{-1}$ h^{-1} and K_s (half saturation constant) was 157.7 mg/L. Perchlorate experienced reduction lags in the presence of nitrate, but the lags were recoverable. There was an increase in the lag time with increased nitrate to perchlorate ratios varying from 0.5 – 3. In systems containing perchlorate and sulfate, ratios for sulfate to perchlorate above 10 were needed for inhibition to occur. A temperature of 35° C and a pH of 6.85 were optimum. A ratio of roughly 2 for acetate-to-perchlorate was optimal to allow all acetate and perchlorate to be consumed simultaneously. Prominent among perchlorate-reducing bacteria, *Dechloromonas,* was the dominant bacterium present in the described culture (Zhu et al. 2016a).

Using granular sludge biofilms in a sequencing batch reactor (SBR) and batch experiments, Reddy and Nancharaiah (2018) investigated Cr(VI) biological removal with or without nitrate present. With an electron donor present, activated-sludge-cultivated denitrifying granular sludge could directly reduce Cr(VI). The concentrations of granular sludge and initial Cr(VI) affected the bioreduction. Cr(VI) bioreduction preceded precipitation or sludge entrapment of Cr(III). In batch experiments, the denitrification of high-strength nitrate (3000

mg/L) was not greatly influenced by Cr(VI) addition, but that of nitrite was slowed. Nevertheless, because of the enrichment of denitrifying bacteria that were tolerant to Cr(VI), successful Cr(VI) removal and denitrification were found with the SBR experiment. During an operation of two months, 3000 mg/L of denitrification and up to 0.75 mM of Cr(VI) removal were demonstrated to be stable. The main removal mechanism for chromate was found to be bioreduction preceding Cr(III) precipitation or entrapment (Reddy and Nancharaiah 2018).

In batch studies, Farhan and Hatzinger (2009) studied *Azospira suillum* JPLRND, a groundwater isolated perchlorate-reducing strain. It can use, as terminal electron acceptor, nitrate, oxygen or perchlorate. For both perchlorate and nitrate utilization, the maximum specific growth rate was 0.16 per hour, lower than the 0.22 per hour, when oxygen was utilized. Nitrate was found to biodegrade prior to perchlorate, even when initial strain culturing was with oxygen or perchlorate. The bacterium's active perchlorate reduction was found to be inhibited by as little as 0.5 mM of nitrate (Farhan and Hatzinger 2009).

2.4 Abiotic Treatment Technologies

2.4.1 Abiotic Treatment Technologies for Cr(VI)

Chromium reduction can take place using various processes, such as, traditional reduction treatments (sulfur compounds and iron salts), electrochemical methods, photocatalytic reduction, and reducing bacteria—aerobic, anaerobic and fungi (Barrera-Díaz et al. 2012). The following paragraphs further develop some of the relevant methods.

Qin et. al., 2005 considered ferrous sulfate Cr(VI) to Cr(III) reduction as part of a pilot scale system for contaminated groundwater. Even when significant levels of dissolved oxygen were present, Fe(II) dose to Cr(VI) concentration ratios of 10-50, resulted in complete Cr(VI) to Cr(III) reduction (Qin et al. 2005). Chen et al, 2015 investigated the reduction of Cr(VI) to Cr(III) using sugarcane molasses. In the absence of bioreduction, the phenolic hydroxyl group is oxidized to quinone and Cr(VI) accepts electrons and is reduced to Cr(III). The reduction was found to occur in the pH range of 2.0 to 6.1. The rate constants for the reaction were found to decrease with increasing pH in that range (Chen et al. 2015). Similarly, Hansen et al. (2017) mention that between Cr(VI) and a molasses constituent, there was a rapid and direct reaction suggested, since almost immediate chromium reduction took place (prior to the appearance of biomass) in molasses amended microcosms. The authors mentioned that there was agreement with Chen et al. (2015) results, contradicting the so called classic conception that molasses is only a bio-stimulant (Hansen et al. 2017).

According to Barrera-Díaz et al. (2012), the industrial reducing agents that are most common are sulfur compounds, sulfur dioxide gas or an acidic solution of sodium bisulfite, which both form sulfurous acid, the active reducing agent. After the Cr(VI) reduction step, calcium hydroxide slurry/sodium hydroxide solution or sodium hydroxide, respectively, can be used for chromium precipitation. Acidic conditions are usual for Cr(VI) reductions using iron. Iron salts, such as $FeCl_2$ and $FeSO_4$, are often used to reduce Cr(VI) to Cr(III) followed by precipitation (Barrera-Díaz et al., 2012). Sodium sulfite and ferrous sulfate are used under acidic conditions to reduce Cr(VI) to Cr(III) followed by alkali precipitation. Shortcomings include, sulfur dioxide production with sodium sulfite (in acidic conditions) and ferric hydroxide

waste disposal requirements, with ferrous sulfate. Additionally, both compounds are not appropriate for use in dilute Cr(VI) solutions because of excessive chemical requirements (Barrera-Díaz et al., 2012). The basis for $FeSO_4$ remediation of Cr(VI) water contamination is Fe(II) reduction of Cr(VI) with the following precipitation of a Fe(III)/Cr(III) hydroxide mixture—reaction below also from reference (Geelhoed et al. 2003):

$$3Fe^{2+} + CrO_4^{2-} + 8H_2O \rightarrow 4Fe_{0.75}Cr_{0.25}(OH)_3 + 4H^+$$

The following reaction is also proposed as the reduction reaction mechanism of the ferrous sulfate reduction of hexavalent chromium to trivalent chromium (Kim, Park, and Gu 2002):

$$6Fe^{2+} + 2CrO_4^{2-} + 13H_2O \rightarrow 6Fe(OH)_3 + Cr_2O_3 + 8H^+$$

According to other research, chemical reduction with ferrous sulfate can employ the equation:

$$3Fe^{2+} + HCrO_4^- + 7H^+ \leftrightarrow 3Fe^{3+} + Cr^{3+} + 4H_2O$$

Precipitation as $Cr(OH)_3$ follows (Zhang, Xue, and Wei 2018). A few interesting properties of ferrous sulfate are presented in Table 3.

Table 3: Relevant Properties of $FeSO_4$

Parameter	Units	Value	Notes
Solubility[a]	g/100 g H_2O	29.5	at 25° C
Mol. Weight[a]		151.908	
Solubility Product Constant[b]		3.771	

[a] From (Physical Constants of Inorganic Compounds n.d.)
[b] Calculated according to (Anon n.d.), assuming 1L = 1000g

The ferrous sulfate solution used (Brenntag, Las Vegas, NV) had a relative density of 1.203 and contained 6% Fe by weight.

Calcium polysulfide treatment will be addressed later in the review.

Many other types of chromium treatment have also been investigated. Researchers have found that photoreduction treatment of low concentration Cr(VI) contaminated wastewater, can be accomplished using silica granules coated with TiO_2 (Saeki, Kadono, and Nabeshima 2010). Ion-exchange resins have been successfully used to remove Cr(VI) from aqueous solution (Li et al. 2018; Pakzadeh 2010; Rafati et al. 2010). In another study, silica sand (with groundwater treatment residuals coating) was used as a Cr(VI) adsorbent. Cr(VI) adsorption was improved with high ionic strength and low pH of solution. At a pH of 4, the maximum computed adsorption capacity was 0.27 mg g^{-1} (Kan et al. 2017). Activated carbon made from mango kernel has also been investigated as a Cr(VI) adsorbent. At 35° C and a pH of 2, a maximum adsorption capacity of 7.8 mg g^{-1} was reported (Rai et al. 2016). In other research, two GACs were studied for artificial groundwater Cr(VI) removal. The removal decreased significantly

with increase in pH from 4 to 7.5, but the performance of the GAC was improved with DO removal from the experimental systems (Han, Schlautman, and Batchelor 2000).

2.4.2 Abiotic Treatment Technologies for Nitrate

Archna et al. (2012) provide a very useful overview of nitrate treatment technologies. Since nitrate is a very soluble and stable ion with little inclination to adsorb or co-precipitate, conventional treatment technologies are not applicable. There are several methods of treatment that may be used. Processes that have had full-scale application for removal of nitrate include biological de-nitrification, ion exchange and reverse osmosis, with limited full-scale application potential for the other discussed methods (Archna, Sharma, and Sobti 2012). Jensen, et al. (2012) discuss three categories of drinking water treatment options: nitrate removal (such as ion exchange, reverse osmosis and electrodialysis), nitrate reduction (biological and chemical denitrification, and hybrid systems. A residual waste stream contains the nitrate with removal treatment options, and other nitrogen species result from nitrate transformation through reduction options (Jensen et al. 2012).

Reducing nitrate with metals accomplishes chemical denitrification. Various metals, including iron and aluminum, have been investigated. Meanwhile, nitrate reduction catalysts of metals like copper, etc. can be used (Jensen et al., 2012). Zero-valent iron (ZVI) reduction of NO_3^- has been shown to proceed rapidly via NO_2^- to NH_4^+ with the proposed overall reaction pathway (Liu et al., 2014):

$$NO_3^- + 4Fe^0 + 10H^+ \rightarrow 4Fe^{2+} + NH_4^+ + 3H_2O$$

Denitrification with powdered aluminum, at a pH of 10.25 mainly produced ammonia which air stripping removed (Archna, Sharma, & Sobti, 2012). Catalysts of Pd and Cu combined can also reduce nitrate to nitrogen (Archna et al., 2012). Reactive media that contained organic carbon or an organic carbon + ZVI mixture was demonstrated to be effective for NO_3^- and ClO_4^- removal from water (Y. Liu et al. 2014).

Unlike the nitrate removal technologies, in chemical treatment, other nitrogen species result from nitrate's conversion and no waste streams result. Excess reduction of nitrate to ammonia instead of nitrogen gas, partial denitrification, and inadequate removal of nitrate (disinfection chlorine can convert nitrate from nitrite), are problems with potable water chemical denitrification. Jensen et al., 2012 reported on other research showing that control could be had, based on catalyst use and nitrate to iron ratio, on whether ammonium or nitrogen gas was the denitrification end product (Jensen et al., 2012). In treatment of potable water, for nitrate removal in the U.S., there have been no installed full-scale systems of chemical denitrification. In the generic denitrification mechanism, nitrate receives electrons from the donating metals. The following equation shows nitrate reduction (similar to biological reduction).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

However, in chemical denitrification, unlike biological, ammonium (most reduced form) is often the end product (Jensen et al., 2012).

$$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$$

Another process that can be used to address nitrate contamination is reverse osmosis, however, membrane fouling can be a concern. Treatment using electrodialysis methods utilize membranes and electric currents to remove nitrate. Other processes include catalytic denitrification, electrocatalytic reduction, and ion exchange. Nanofiltration is also a nitrate treatment alternative (Archna et al. 2012).

Reverse osmosis nitrate removal in point-of use and municipal applications can be a feasible alternative (Jensen et al., 2012). This process uses pressure and semipermeable membranes to impede contaminants while water passes on. Appropriate disposal is required for concentrate containing high nitrate and other salts. Rejection rates as high as 93% for sodium nitrate are documented (Jensen et al., 2012).

Electrical methods also exist. Under electrodialysis/electrodialysis reversal/selective electrodialysis, Jensen et al., 2012 describe the use of electrical current passed through cation and anion exchange membranes. Anions move towards the anode past the anion exchange membrane but are stopped—into the recycle waste stream—by the cation exchange membrane. Cations are removed analogously. Nitrate selective membranes, permit treatment with the absence of significant alteration of balance of the water's other ions (Jensen et al. 2012).

Ion exchange (IX) is the most common nitrate removal method in treatment of potable water, with multiple operating full-scale installations. In conventional IX, the exchange resin used is strong base anion (SBA) exchange resin. After raw water pretreatment, chloride is displaced by nitrate at surface sites on contact with the resin (Jensen et al. 2012). Archna et al. (2012) discussed ion exchange resins (strong base anion) that exchanged bicarbonate or chloride

ions for nitrate. The exhausted resin was regenerated (Archna et al., 2012). Weak base anion exchange resins can also be used to remove nitrate (Jensen et al. 2012).

Although IX and membrane filtration can be used for drinking water treatment of nitrate, their use to treat contaminated groundwater is limited by high cost. In addition, the methods require the water to be pumped from the ground for treatment. Conversely, biological reduction or denitrification can be accomplished both with in-situ and ex-situ treatment.

2.4.3 Abiotic Treatment Technologies for Chlorate/Perchlorate

Morss (2003) discussed four treatment technology types for perchlorate remediation in groundwater: ion exchange, granular activated carbon (GAC), reverse-osmosis (RO), and fluidized bed reactor (FBR) systems involving microorganisms.

According to Morss (2003) regenerable and non-regenerable ion-exchange systems can be successfully used. Ion-exchange, also discussed by Ye et al. (2012), is stated to be a technology that is effective for removal of perchlorate in trace quantities from drinking water. The authors reviewed research on the topic and summarize that in terms of promise, ion-exchange is shown as a paramount drinking water perchlorate removal technology. Its implementation, however, still has some serious drawbacks. High cost makes one time use economically unsustainable. Furthermore, perchlorate concentrated brine must be disposed of and competition of other anions affects perchlorate adsorption capacity. They recommend further improvement to perchlorate adsorption/desorption, in terms of the exchangers having different

functional groups and matrices, to further use the potential of perchlorate removal using ion exchange technology (Ye et al. 2012).

Morss (2003) mentions that low concentrations of perchlorate can be removed from water using GAC, in a cost-effective process. The capacity of the GAC is dependent on the selected activated carbon type and the perchlorate concentration compared to other ions in the water. The surface adsorption and therefore the capacity of some carbons can be increased by surface functional groups (i.e. carboxyl), which permit ion exchange with perchlorate (Morss 2003). Ye et al. (2012) reviewed water perchlorate reduction and removal technologies. The first major technology they discussed was adsorption. They addressed other research on modified activated carbons and conclude that carbon modification with cationic surfactants is necessary to enhance adsorption with perchlorate. The modifying cationic surfactants, however, may cause cost increase and secondary pollution. Variables that have strong effects on breakthrough time and kinetics include, solution pH and coexisting anions. Also, in this treatment, regenerative brine and spent carbon are problems that must be addressed. Research on perchlorate removal with other new absorbents is also presented, but the authors conclude that they "still have a long way to go" prior to being taken seriously as an activated carbon alternative. Additionally, further research is still needed on many influencing factors (Ye et al. 2012).

For RO perchlorate removal, Morss (2003) discussed the use of a semi permeable membrane for selective removal of varied inorganics in water. High-pressure RO membranes yielded roughly 99.9% perchlorate removal with a brine stream with concentrated perchlorate. Nanofiltration membranes usually required membrane surface pH shifts for effective perchlorate

removal. Low-pressure RO membranes generally rejected 95% of perchlorate but, additional treatment was expected to be needed to meet specific standards (Morss 2003). Ye et al. (2012) also discussed membrane filtration. They state the important role of membrane filtration, in drinking water perchlorate removal, using nanofiltration, reverse osmosis and ultrafiltration membranes. Again, after a review of research, the authors mention that drinking water perchlorate removal, using membrane filtration technology that is pressure driven, is a technology that is promising. Its main impediment to large scale system application is that the perchlorate is simply being transferred to another stream, with the rejection stream needing additional treatment (Ye et al. 2012). The authors mention that conventional membrane filtration may be less effective than electrodialysis for perchlorate, however, electrodialysis has very high operation costs (Ye et al., 2012).

Ye et al. (2012) also discuss chemical reduction of perchlorate. Metal and metal ion reductants are perchlorate treatment options. Considering research on the topic, they observe that perchlorate reduction has a large kinetic barrier. They also mention that iron removal treatment will also be needed as finished water will now have residual iron. Also, they mention that further research is needed on certain important factors. Hydrogen gas, the next reducing agent discussed, is apparently concluded to have high perchlorate reduction kinetic barriers, but catalysts shorten reaction times. However, the cost of the chemicals, their toxicity, and their low-level perchlorate contaminated groundwater treatment effectiveness are major limitations. Therefore, the large-scale field applications of these technologies are questionable. H_2 is economically justifiable, but also has flammability issues. The authors discuss/recommend further work on materials selection for an improved reduction rate of perchlorate. It is desirable

that the new catalysts be taken advantage of, as they provide high efficiency and shorter reaction time, among other advantages (Ye et al. 2012).

The final non-biological perchlorate reduction method that will be discussed here, electrochemical reduction, is also based on information from Ye et al. (2012). Ye et al. (2012) mention that the approach results in complete, catalyst free, perchlorate destruction. The authors summarize that acidic medium is often where perchlorate is electrochemically reduced, and eroded electrode is the technology's major drawback. They mention that use of "a new electrode material" is desirable. Lowered treatment costs and higher reduction efficiency would be a breakthrough. Additional pilot scale tests are also recommended (Ye et al. 2012).

2.5 Summary of Chemical Treatment Technologies

All of the contaminants investigated in this research: chromate, nitrate, chlorate and perchlorate are oxyanions. Since all are in higher states of oxidation, it is thermodynamically possible to reduce each of those compounds. Both chemical and biological reductions are achievable, but the kinetics of reduction and its feasibility vary significantly. At room temperature, perchlorate is very difficult to chemically reduce (Vellanki, Batchelor, and Abdel-wahab 2013). Nitrate chemical reduction, although it is indeed promising, has been limited to the applied research level and has not had field scale applications (Capodaglio, Petr, and Raboni 2015). Conversely, for effluents' chromates removal, chemical reduction with ensuing precipitation is a conventional method (Garbisu et al. 1998). Chemical treatment has been applied for chlorate removal from water, however catalytic chemical methods require extreme conditions. For example, high temperatures and large quantities of catalyst lead to high costs.

These methods did not remove 100% of chlorate (Jung et al. 2017). All these oxyanions have been shown to degrade biologically, which will be discussed in a subsequent section.

2.6 Chemical Reduction of Cr(VI) Using CaS_x

Calcium polysulfide (CaS_x) contains anionic polysulfide chains with 2 to 7 atoms of sulfur, but the pentasulfide (CaS_5) form is predominant (Pesticide Research Institute for the USDA National Organic Program 2014). Calcium polysulfide (CaS_x) is a liquid that is orange in color and has a characteristic smell of rotten eggs. Hydrogen sulfide (which is flammable and highly toxic) is produced when the substance contacts acids and decomposes. Calcium polysulfide also attacks metal and in water, the solution is a medium strong base. Its relative density is 1.28 (water = 1), and its water solubility is miscible. It is highly toxic to aquatic organisms (Centers for Disease Control and Prevention 2015). Figure 4 is a sketch of CaS_5 as re-drawn from NIH, 2018.

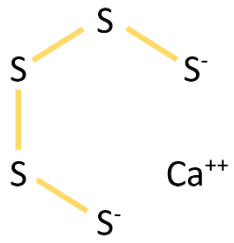


Figure 4: Sketch of Calcium Polysulfide (CaS_5), re-drawn from (NIH 2018)

In aqueous solution, polysulfides are unstable and volatile. At values of pH greater than 9, the majority of polysulfides (long chain) are stable (Chrysochoou & Ting, 2011). As pH is reduced, the polysulfide chains break down to lesser lengths (Chrysochoou & Ting, 2011). The equations below are taken from that source.

$$HS^- + S^0 \rightarrow S_2^{2-} + H^+ \qquad pKa = 12.16$$

$$HS^- + 2S^0 \rightarrow S_3^{2-} + H^+ \qquad pKa = 10.85$$

$$HS^- + 3S^0 \rightarrow S_4^{2-} + H^+ \qquad pKa = 9.86$$

$$HS^- + 4S^0 \rightarrow S_5^{2-} + H^+ \qquad pKa = 9.18$$

Generation of sulfides from polysulfides is known (Chrysochoou & Ting, 2011). At pH 12 only about 10% of the S (total) is polysulfides: S_5^{2-} (about 6%), S_6^{2-} (4%) and other species (below 1%). At or below a pH of 7, less than 1% of total S is polysulfides. Below a pH of 8, the two species that dominate are HS^- and H_2S. Sulfide and polysulfides both had thiosulfate ($S_2O_3^{2}$) as a prominent oxidation product. Thiosulfate production is dependent on the solution oxygen content and pH. Thiosulfate has the capability of reducing Cr(VI) (Chrysochoou & Ting, 2011). Table 4, taken from Vanýsek, n.d., presents standard reduction potentials, or possible standard reduction potentials for many sulfur compounds.

Table 4: Standard, or Possible Standard Reduction Potentials for Many Sulfur Compounds (Vanýsek n.d.)

Reaction	E°/V
$S + 2\ e \leftrightarrow S^{2-}$	– 0.47627
$S + 2H^{+} + 2\ e \leftrightarrow H_2S(aq)$	0.142
$S + H_2O + 2\ e \leftrightarrow SH^{-} + OH^{-}$	– 0.478
$2\ S + 2\ e \leftrightarrow S_2^{2-}$	– 0.42836
$S_2O_6^{2-} + 4\ H^{+} + 2\ e \leftrightarrow 2\ H_2SO_3$	0.564
$S_2O_8^{2-} + 2\ e \leftrightarrow 2\ SO_4^{2-}$	2.010
$S_2O_8^{2-} + 2\ H^{+} + 2\ e \leftrightarrow 2\ HSO_4^{-}$	2.123
$S_4O_6^{2-} + 2\ e \leftrightarrow 2\ S_2O_3^{2-}$	0.08
$2\ H_2SO_3 + H^{+} + 2\ e \leftrightarrow HS_2O_4^{-} + 2\ H_2O$	– 0.056
$H_2SO_3 + 4\ H^{+} + 4\ e \leftrightarrow S + 3\ H_2O$	0.449
$2\ SO_3^{2-} + 2\ H_2O + 2\ e \leftrightarrow S_2O_4^{2-} + 4\ OH^{-}$	– 1.12
$2\ SO_3^{2-} + 3\ H_2O + 4\ e \leftrightarrow S_2O_3^{2-} + 6\ OH^{-}$	– 0.571
$SO_4^{2-} + 4\ H^{+} + 2\ e \leftrightarrow H_2SO_3 + H_2O$	0.172
$2\ SO_4^{2-} + 4\ H^{+} + 2\ e \leftrightarrow S_2O_6^{2-} + H_2O$	– 0.22
$SO_4^{2-} + H_2O + 2\ e \leftrightarrow SO_3^{2-} + 2\ OH^{-}$	– 0.93

Half-cell reactions with larger positive E° values have greater reduction driving force (Brown et al. 2003).

Cr(VI) is reduced to Cr(III) by calcium polysulfide. Ready precipitation as iron chromium hydroxide (with orders of magnitude lesser solubility than pure chromium hydroxide) or chromium hydroxide, follows. The redox equilibrium reaction suggested (Zhong et al. 2009) can be written as:

$$2CrO_4^{2-} + 3CaS_5 + 10H^{+} \rightarrow 2Cr(OH)_{3(s)} + 15S(s) + 3Ca^{2+} + 2H_2O$$

Additionally, Cr(VI) reduction and immobilization is enhanced by a reduced reactive barrier in the matrix of the sediment. That barrier is established when other possibly sediment-present electron acceptors (like sorbed Fe^{3+}) can react with calcium polysulfide. The authors (using columns and batch tests) studied vadose zone foam delivery of calcium polysulfide to sediments and evaluated the thus delivered calcium polysulfide's Cr(VI) immobilization. Surfactant solutions and calcium polysulfide were used to generate the foams. Their laboratory-based experimental results showed efficient delivery of calcium polysulfide to unsaturated sediments for in situ Cr(VI) immobilization was possible. Also minimized was the reaction front Cr(VI) mobilization which results from water-based single phase solution delivery of calcium polysulfide. They found that calcium polysulfide foam delivery is an approach that is promising for remediation and immobilization of Cr(VI) in the vadose zone, but more development is required for methodology assessment for three-dimensional system heterogeneous soils (Zhong et al. 2009).

Based on observed kinetic analysis, Chrysochoou and Ting, (2011) sought to determine pH and O_2 influence on aqueous/adsorbed Cr(VI) reduction, using calcium polysulfide. In the 5.5 – 8.5 pH range, calcium polysulfide reduction of Cr(VI) followed a reaction model which was second-order while there was a first-order reaction for sulfide. Observed kinetic rates increased with Cr(VI) adsorption to goethite. Due to H_2S prevalence, which is highly reactive, and increased H^+ availability, there was an exponential increase in reaction rate with pH decrease under anaerobic conditions. Compared to anaerobic conditions, there was a decrease in reaction rate in aerobic conditions, where the maximum reaction rate occurred at a pH of 7. At that pH, there was a correlation between the observation of the reductants sulfide and thiosulfates and the

maximum reaction rate. With oxygen present, only thiosulfates production was observed for pure calcium polysulfide; however, a sulfate, sulfite and thiosulfates mixture resulted from sulfide conversion. Of sulfate, sulfite, thiosulfate and sulfide, Cr(VI) can only be reduced by sulfide and thiosulfate. Furthermore, at values of pH above 7, calcium polysulfide-Cr(VI) solutions were found to have thiosulfate. The implication of these findings as noted by the authors is that subsurface reductive ability can be maintained longer by calcium polysulfide than by sulfide, if the pH is above neutral. They also noted that, with the exception of acidic soils that are well-buffered, alkalinity caused by CPS will probably favor such subsurface pH conditions (Chrysochoou and Ting 2011).

Graham et al. (2006) reported that pseudo-first-order, in Cr(VI), kinetics were followed for Cr(VI) reduction by CaS_x interaction. The rate constant was 0.077 min^{-1}—a roughly 9 min half-life (Graham et al. 2006).

In Pakzadeh and Batista (2011), calcium polysulfide (CaS_5) was successfully used for the removal of Cr(VI) from ion-exchange (IX) brines. The research results led to the following conclusions: For pH of 1.6 - 10.3, there was 100% - 93.5% Cr(VI) to Cr(III) reduction, respectively. However, the pH range of 8 - 10.3 was best for combined reduction/precipitation (maximum removal of chromium). The chromium removal efficiency by CaS_5 coagulation increased slightly with increase of brine ionic strength from 0.1 M to different values up to 2.1 M. Increase over 1.5 M ionic strength was not observed to significantly improve efficiency of removal. There was no effect on chromium removal efficiency for alkalinity (as $CaCO_3$) increases to 5 g/L from 0.01 g/L. For typical Cr(VI) concentrations in IX brine, the required

CaS_5/Cr(VI) molar ratio ranged between 0.6 to 1.4, for a treatment goal of < 5mg/L Cr(VI). To reach 0.1 mg/L Cr(VI), it would be necessary to have higher molar ratios, such as 3.7-1.7. Lower molar ratios corresponded to higher Cr(VI) initial concentration. Total chromium removal was correlated strongly with the brines' oxidation/reduction potential (measured by probe). The maximum removal of total chromium occurred under reducing conditions, when E_h numbers were brought down to within -0.1 and 0 V. The decrease to the lowest oxidation-reduction potential value of -0.32 V resulted from a CaS_5 addition at a ratio higher than 4 (CaS_5/Cr(VI)) and total chromium removal was caused to slightly decrease. Therefore, due to higher sludge production and lower efficiency of reduction, CaS_5 addition in excess doses is not recommended. The amount of added CaS_5 was directly proportional to the amount of generated sludge solids and there was observed scale formation (Pakzadeh & Batista, 2011).

Calmet® was the calcium polysulfide product used in this research. Some relevant properties are provided in Table 5.

Table 5: Relevant Properties of Calcium Polysulfide (Calmet®) Solution Used. Taken from (Tessenderlo Kerley Inc. 2018)

Parameter	Value	Units	Notes
Formula	CaSx	—	—
Chemical:		% by Wt.	
Calcium Polysulfide, CaSx		24 - 29	Synonym Common Name: Lime Sulfur, Calcium Sulfide
Water		Remaining %	
pH	11.5 - 11.7		Typical
Melting Point/Freezing Point	18 to 25	ºF	Typical
Relative Density	1.27		Typical
Solubility	Miscible		

2.7 Biotic Treatment of Oxyanions

2.7.1 Biotic Treatment of Chromate

The typical stages in the microbial removal of Cr(VI) from solutions are: chromium binding to the surface of the cell, chromium translocation into the cell and Cr(VI) to Cr(III) reduction (Barrera-Díaz et al., 2012). Huang et al. (2017) investigated the taxa of bacteria which resist/reduce chromium, using laboratory isolation, literature survey and genome mining. They found that 1877 out of 7887 mined genome species contained the chromate ion transporter protein (*ChrA)* gene. Long ChrA protein is more predominant numerically than short ChrA protein, and as a chromate ion transporter has clear functions (Huang et al. 2017). ChrA proteins can efflux from the cell cytoplasm excess chromate ions, using proton motive force as the driver. The three genera having ChrA that were most abundant, were *Pseudomonas*, *Bacillus* and *Vibrio*. The top three abundant genera of the 81 species with the Cr(VI) reductase gene were found to be

Bifidobacterium, *Bordetella* and *Bacillus*. The genome mining found 30 genera to have both genes, while the literature survey resulted in 43; 4 genera overlapped (Huang et al. 2017).

Singh et al. (2015) presented information on the bioreduction of Cr(VI). In their work, the obligate thermophilic methanogen, *Methanothermobacter thermautotrophicus*, was investigated and the substrate used was H_2/CO_2. Potassium dichromate Cr(VI) concentrations ranging from 0.2 to 5 mM (10.40 to 259.98 mg/L) as Cr(VI) were considered. The concentrations at or below 0.4 mM (20.80 mg/L) showed complete reduction and those at or above 1 mM (52.00 in mg/L) showed decreasing percent reductions with increasing Cr(VI) concentration. The authors inferred that this implies a toxic effect at that range. They also note that at those Cr(VI) concentrations, there was inhibition to methanogenesis and there was impaired cell growth. Increased initial Cr(VI) concentrations also decreased the rate of bioreduction. Most of the reduced Cr(III) produced was as an amorphous chromium hydroxide precipitate with a small aqueous Cr(III) fraction. The authors mention that extra- and intracellular mechanisms of chromium reduction are both suggested. They also note the possible bioremediation application of these microorganisms, particularly at sites for radioactive waste disposal, which have high temperature (Singh et al. 2015).

In research highly relevant to the current work, Wen et al. (2017) investigated the use of Emulsified Vegetable Oil (EVO) for removal of hexavalent chromium in column experiments. Reducing conditions were generated by an injection of EVO as it caused terminal electron acceptor depletion (O_2 and Fe(III)), and Fe(II) release. The Cr(VI) concentration was then decreased drastically. For EVO and EVO amended with either acetate (which may speed up

Fe(III) leaching through pH reduction) or colloidal $Mg(OH)_2$ (for pH decrease buffering), Cr(VI) contamination was removed by all three treatments. The simulated groundwater Cr(VI) was removed and was stably immobilized as compounds of Cr(III) in the sediments. The amendments enhanced Fe(III) bioreduction and thereby facilitated Cr(VI) removal performance by EVO, although the column with only EVO treated Cr(VI) for a longer period. This was attributed to amendments increasing iron reduction early on; excess Fe(II) was eluted without being used in Cr(VI) reduction. The addition of EVO decreased microbial community diversity and richness while iron reduction and organic fermentation related microbes accumulated. The authors discuss the bioreduction of iron using soybean oil according to the following equation (citing Hiortdahl and Borden, 2013):

$$C_{56.3}H_{99.6}O_{6.0}\text{ (soybean oil)} + 106.6H_2O + 312.8Fe^{3+}{}_{solid} \rightarrow 56.3CO_2 + 312.8Fe^{2+} + 312.8H^+$$

The authors also indicate that Fe(II) could reduce Cr(VI) according to the following:

$$3Fe^{2+} + Cr^{6+} \rightarrow 3Fe^{3+} + Cr^{3+}$$

The authors attribute the Cr(VI) reduction to the presence of biogenic Fe(II), (Wen et al. 2017).

Of critical interest in this research are the concentrations of contaminants which can be degraded biologically, as well as the concentrations of contaminants at which inhibition of microbial activity may occur. Huang et al. (2017) cite research by Narayani and Shetty (2013), in which Cr(VI)-resistant bacteria so far had reported minimum inhibitory concentration values of $K_2Cr_2O_7$ from 147 mg/L to 140,000 mg/L. *Arthrobacter spp.* are isolates which are Cr(VI)-

reducing and resistant. An isolated *Arthrobacter sp.* from soil with long-term contamination was found to have 100,000 mg/L Cr(VI) toleration, on plate. It was also found to reduce up to 50 mg/L of hexavalent chromium (Huang et al. 2017). Also, two isolated *Arthrobacter* strains, from samples of chromite mine overburden, could tolerate up to 613.5 and 925.4 mg/L of Cr(VI) and have a capacity for reduction of 64% and 67%, respectively – for 104 mg/L Cr(VI). This genus, in genome mining, was not an identified resistant and reducing Cr(VI) taxa. This may be because of yet limited genome data or alternative Cr(VI) pollution reduction or resistance mechanisms (Huang et al. 2017).

2.7.2 Biotic Treatment of Nitrate

Organic carbon denitrification, mediated by bacteria, is proven to reduce nitrate to N_2, according to the following steps (Y. Liu et al. 2014):

$$NO_3^-{}_{(aq)} \rightarrow NO_2^-{}_{(aq)} \rightarrow NO_{(\text{enzyme complex})} \rightarrow N_2O_{(gas)} \rightarrow N_{2(gas)}$$

In biological denitrification, nitrates/nitrites can replace oxygen as the terminal electron acceptor leading to ATP generation (Archna et al., 2012). Many bacteria in different genera reduce ionic nitrogenous oxides to gaseous products and can grow anaerobically. This is called dissimilatory nitrate reduction (Archna et al., 2012). Cell mass synthesis and existing cell mass maintenance in the organism is supported by energy from donor to acceptor electron transfer. The synthesis conditions of denitrification-associated-enzymes are partially aerobic or anaerobic (Archna et al., 2012). An enzyme system catalyzed each step in the reduction from nitrate to N_2. For most bacteria, the nitrate to nitrite dissimilatory reduction was important. That was because

the reaction was an increased substrate level phosphorylation reaction which involved conservation of energy (Archna et al. 2012). In this respiratory process, an energy source (oxidizable substrate) was needed. Apparently, residual organic presence and possible bacterial contamination limited biological denitrification (Archna et al., 2012). In *Azospira* sp. perc1ace, it was concluded that nitrate reductases were located in the membrane cell fractions (Nam et al. 2016).

Archna et al. (2012), stated that heterotrophic biological denitrification is more widely applied than autotrophic denitrification, based on literature review. Some European countries have confirmed the full-scale feasibility, both technical and economic, of heterotrophic denitrification. The rate of the autotrophic reaction is low, leading to large reactor volume requirements and increased capital costs (Archna et al. 2012). Denitrifying bacteria are mostly heterotrophic. The oxidizable substrates that they utilize are complex organic substances, e.g. methanol, ethanol, methane, carbon monoxide and acetic acid (Archna et al., 2012), and nitrogen is converted from nitrate. Heterotrophic denitrification was investigated at pilot scale with fluidized and packed columns (Archna et al., 2012). Two weeks of start-up time was required to establish sufficient bacterial populations. Unit reactor volume denitrification rates were 160 g N/m^3 h and 12 g N/m^3 h for fluidized sand bed and packed bed reactors, respectively. The reduction of nitrate was to a concentration of roughly 45 mg/L (Archna et al., 2012).

According to Archna et al. (2012), autotrophic denitrification can be accomplished by certain bacteria from the *Paracoccus, Thiobacillus, Thiosphaera* as well as other genera. The energy sources (reductants) used are hydrogen or several reduced sulfur compounds like S^0, S^{2-},

SO_3^{2-}, $S_2O_3^{2-}$, or $S_4O_2^{2-}$. Another autotrophic denitrification energy source is ferrous iron, which can be used by *Gallionella, Ferrobacillus, Leptothrix* and *Sphaerotillus* genera bacteria. The microbial cell synthesis carbon source was bicarbonate or carbon dioxide for autotrophic growth conditions (Archna et al. 2012). A 24mg/L to 1mg/L reduction of nitrate was reported using *Thiobacillus denitrificans*, packed bed reactors and an electron source of elemental sulfur (Archna et al., 2012).

In their overview of nitrate treatment technologies, Archna et al. (2012) also discussed the use of a membrane bioreactor for denitrification, citing work by McAdam and Judd (2007) and Ergas and Rheinheimer (2004); a removal of over 99% of the influent 200 mg/L nitrate was achieved.

Research has indicated that nitrate and/or products of denitrification had toxic effects on methanogenic bacteria, possibly as well as other microbial community members (Klüber and Conrad 1998).

Table 6, reproduced from Glaser (1920) shows various species of organism exposed to different molecular concentrations of KNO_3 and $NaNO_3$ and their growth (good, weak or none), in addition to information on nitrate-nitrite reduction.

Table 6: Reduction Test Results (Nitrate-Nitrite), reproduced from (Glaser 1920)

Organism Species		0.0002 M	0.0005 M	0.0008 M	0.001 M	0.01 M	0.1 M	0.5 M	1 M	2 M	4 M
								W.G.	W.G.	N.G	N.G.
Spirillum metchnikovi	$NaNO_3$	0	+	+	+	+	+	0	0	0	0
	KNO_3	0	+	+	+	+	+	0	0	0	0
								W.G.	W.G.	N.G	N.G.
										N.G	N.G.
Bacillus prodigiosus	$NaNO_3$	0	0	+	+	+	+	+	+	0	0
	KNO_3	0	0	+	+	+	+	+	+	0	0
										N.G	N.G.
								W.G.	W.G.	W.G.	N.G.
Bacillus coli communsis	$NaNO_3$	0	0	0	0	+	+	+	0	0	0
	KNO_3	0	0	0	0	+	+	+	0	0	0
									W.G.	W.G.	N.G.
										W.G.	N.G.
Coccobacillus acridiorum. Variety "Souche Sidit"	$NaNO_3$	0	0	0	0	+	+	+	+	0	0
	KNO_3	0	0	0	0	+	+	+	+	0	0
										W.G.	N.G.
									W.G.	W.G.	W.G.
Coccobacillus acridiorum. Variety "Souche Cham"	$NaNO_3$	0	0	0	0	+	+	+	0	0	0
	KNO_3	0	0	0	0	+	+	+	0	0	0
									W.G.	W.G.	N.G.
									W.G.	W.G.	W.G.
Bacillus anthracis	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
								W.G.	W.G.	W.G.	N.G.
										W.G.	N.G.
Staphylococcus pyogenes albus	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
										W.G.	N.G.
		W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	N.G.	N.G.	N.G.
Streptococcus disparis	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
		W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	N.G.	N.G.
Checks (no bacteria)	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0

+: nitrates reduction to nitrites

Cipher [0]: lack of that reduction

WG: weak growth

NG: no growth

Table 6 shows that various organisms exhibit good growth over a wide range of concentrations, while only one, *Streptococcus disparis*, shows weak growth even at the lowest concentrations presented.

2.7.3 Biotic Treatment of Chlorate/Perchlorate

Microbial respiration is the primary means of the terrestrial decomposition of perchlorate (Youngblut et al. 2016). During anaerobic respiration, (per)chlorate (chlorate and perchlorate) are the terminal electron acceptors used by dissimilatory (per)chlorate reducing bacteria (Youngblut et al., 2016). The typical characteristics of perchlorate respiration are: specialized reductases being present, Cld detoxification of ClO_2^-, and horizontal transfer of enzyme-encoding genomic units (Youngblut et al., 2016). With canonical (per)chlorate respiring organisms, the two ions are first reduced to ClO_2^-, followed by dismutation to O_2 and Cl^-. The same microorganism simultaneously respires the O_2. These chemotrophs combine anaerobic and aerobic metabolisms due to the biogenesis of O_2 (Youngblut et al. 2016). Canonical (per)chlorate reducers all are anaerobes (facultative) or microaerophilic, in line with their transient O_2 production (Youngblut et al. 2016). Interestingly, with an exception, nitrate respiration producing N_2 is possible by all canonical dissimilatory (per)chlorate-reducing bacteria, while nitrate reduction by only a small amount of dissimilatory chlorate-reducing bacteria is possible (Youngblut et al. 2016).

While still not proved to occur environmentally, symbiotic (per)chlorate reduction involves one organism reducing (per)chlorate and another organism removing ClO_2^-. Another process, already seen in the laboratory but not in an environmental sample, is cryptic perchlorate

reduction, which incompletely reduces ClO_4^- and then ClO_2^- is removed by chemical reactions (Youngblut et al. 2016).

Liebensteiner, et al. (2016) discuss microorganisms that reduce chlorate and perchlorate. Figure 5 shows various alternatives for the reduction of perchlorate to chloride. The authors citing Leibensteiner, et al. (2013), mention that there are perchlorate-reducing microorganisms with an absence of a functionally efficient chlorite dismutase. In one case, at least, reduced sulfur compounds scavenge the chlorite, which is enzymatically formed, thereby enabling continuous chlorine oxyanion reduction and energy conservation (Liebensteiner, Oosterkamp, and Stams 2016).

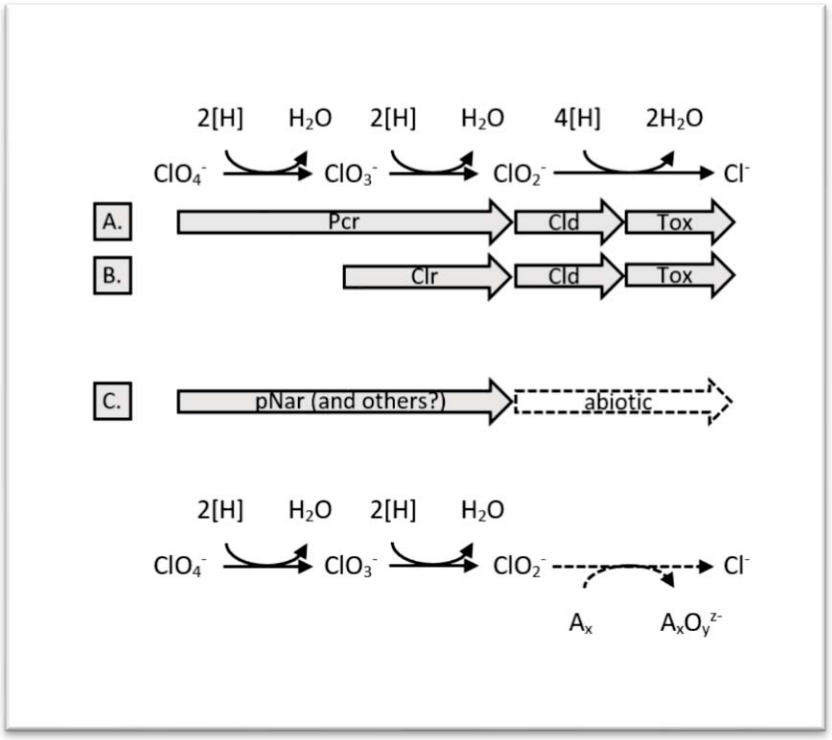


Figure 5: "(A) Complete microbial perchlorate reduction involving perchlorate reductase (Pcr) and chlorite dismutase (Cld) in perchlorate-reducing bacteria. (B) Alternatively, chlorate-reducing microorganisms employ a chlorate reductase (Clr) combined with chlorite dismutase. The disproportionation of chlorite temporarily forms dioxygen that is reduced by a terminal oxidase (Tox) to water. (C) In the absence of chlorite dismutase, alternative ways of complete perchlorate reduction were observed. Molybdenum enzymes (e.g., periplasmic Nar-type reductases) other than chlorate and perchlorate reductase are able to reduce chlorine oxyanions to chlorite; possibly followed by abiotic chlorite elimination (e.g., observed for reduced sulfur compounds). "A_x" and "$A_xO_y^{z-}$" stand for the reduced and oxidized forms of any potential reductant" Figure reproduced and subtitle taken verbatim from (Liebensteiner et al. 2016)

Chlorite disproportionation forms a covalent O-O bond. With no oxygen present, heme b oxidoreductases (which are consistently called "chlorite dismutase" incorrectly) accomplish this. It is one of not many such enzymatic reactions with that outcome—other than photosynthesis and an alternative nitrite reduction pathway that is proposed (Liebensteiner et al. 2016). On the other

hand, a dismutase is “any of a group of enzymes that have the ability to catalyze the reaction of two molecules of the same compound to yield two molecules in different oxidation states” (Dismutase n.d.).

As introduced previously, Morss (2003) discussed the use of FBR systems in perchlorate treatment. It was mentioned that in this method, perchlorate is destroyed instead of concentrated (as in IX). This process takes only minutes, using “accelerated growth microorganisms” that are naturally occurring. They are attached to a GAC or sand media bed that is hydraulically fluidized. The biochemical reaction results in chloride ions and oxygen. Residual biosolids (non-hazardous) are the only byproducts of the process.

Wang and Coates (2017) discusses the applications in biotechnology of chlorate and perchlorate reduction by microbes. Different bioreactor types can accomplish ex-situ or in-situ bioremediation of perchlorate. Autotrophic reactors at laboratory-scale which use (as electron donor) hydrogen, reduced iron, or compounds of sulfur (Wang and Coates 2017) have been produced. However, most fluidized and fixed-bed reactors at industrial-scale are run as heterotrophic reactors and use, as electron donor, either acetate or simple alcohols (Wang and Coates, 2017). The most successful to emerge for perchlorate treatment have been heterotrophic fluidized bed (ethanol electron donor). They have up to 34 million L/day in capacity and reduce perchlorate to below the California MCL of 6 µg/L (Wang and Coates 2017).

In research of relevance to the current work, Hunter (2002) conducted research to emulate a permeable in-situ barrier, using laboratory soil columns injected with vegetable oil, in order to treat flowing groundwater for chlorate and perchlorate. The author found that it was

appropriate to consider this a substrate for the reduction to chloride of chlorate and perchlorate by native microorganisms. Chlorate at 0.2 mM and perchlorate at 0.2 mM were reduced by about 96% and 99%, respectively, forming chloride as a product. Additionally, nitrate and chlorate were both removed, when 1.4 mM of nitrate was added. Microcosm incubations, under helium, completely reduced 6 mM perchlorate and 24 mM chlorate to chloride. The author also noted that perchlorate-reducing microorganisms are environmentally plentiful (Hunter 2002). As shown by this research and of current interest, soil columns may be supplemented with vegetable oil and used for the reduction of chlorate and perchlorate. Nitrate was also not found to interfere with the reduction of chlorate. And high levels of the contaminants may be effectively converted to chloride. This would imply positive results for the current research, if the additional oxyanions cause no problems.

Investigating types of bacteria capable of chlorate and perchlorate reduction is also of interest. Wallace et al. (1996) sought to identify such a bacterium. The bacterium in question was able to reduce perchlorate at more than 7000 ppm in wastewaters and was a municipal anaerobic digester isolate. It can use dissimilatory chlorate or perchlorate reduction for growth and energy and is gram-negative and obligately anaerobic. Based on data, they say it was indicated that it was a *Wolinella succinogenes* strain, capable of using for terminal electron acceptor either chlorate or perchlorate (Wallace et al. 1996).

Van Wijk et al. (1998) address chlorate and chlorite toxicity to certain species. Select information is presented in Table 7.

Table 7: Chlorate/Chlorite Growth Inhibition Toxicity Test Results. Reproduced from (van Wijk, Kroon, and Garttener-arends 1998).

With	Organism	Nitrogen source	Test duration	EC_{50} (mM)	C (mM)	NOEC (mM)
Chlorate	*Bacillus subtilus*	NO_3[a]	8 h			
	(Gram-positive bacteria)	NH_4			3.74	1.87
	Pseudomonas putida	NO_3	8 h			≥ 3.74
	(Gram-negative bacteria)	NH_4				≥ 3.74
Chlorite	*Bacillus subtilus*	NO_3[a]	8 h			
	(Gram-positive bacteria)	NH_4		0.03	0.01	< 0.01
	Pseudomonas putida	NO_3	8 h	0.05	0.02	< 0.02
	(Gram-negative bacteria)	NH_4		0.02	0.02	< 0.02

Note. Organisms were cultured in medium or media containing either ammonium or nitrate as sole nitrogen source.

[a]Did not grow sufficiently to permit proper testing.

Bacteria respiration inhibition tests were also considered by the authors and the results are presented in Table 8.

Table 8: Chlorate/Chlorite Respiration Inhibition Test Results. Reproduced from (van Wijk et al. 1998).

With	Organism	Nitrogen source	EC_{50} (mM)	LOEC (mM)	NOEC (mM)
Chlorate	*Bacillus subtilus*	NO_3	–	9.35	4.67
	(Gram-positive bacteria)	NH_4		4.67	2.34
	Pseudomonas putida	NO_3	–	9.35	3.74
	(Gram-negative bacteria)	NH_4		8.97	4.49
Chlorite	*Bacillus subtilus*	NO_3		8.79	4.40
	(Gram-positive bacteria)	NH_4	0.56	0.32	< 0.32
	Pseudomonas putida	NO_3	4.85	1.09	0.55
	(Gram-negative bacteria)	NH_4			10.55

Note. Organisms were tested in medium containing either ammonium or nitrate as sole nitrogen source.

Soudi et al. (2017) also provide a useful compilation of literature on bacterial tolerance to chlorate and perchlorate. *Escherichia Coli* was reported to grow with 2.5% sodium perchlorate, *Staphylococcus pyogenes aureus* grew slowly with 7.5% sodium perchlorate, but did not grow with 10%. *Aspergillus niger* at 1% sodium perchlorate showed strong mycelial growth and at 4% sodium perchlorate about quarter of the growth (Al Soudi et al. 2017). *Haloarcula*, *Haloferax* and *Halomonas,* which are halotolerant, were shown, in the presence of sodium perchlorate (0.4 M), to have strong growth, and *Haloferax* weak growth at 0.6 M. *Methanobacterium*, *Methanosarcina* and *Methanothermobacter* strains demonstrated methanogenesis with 1% perchlorate, not greater (Al Soudi et al. 2017). However, these methanogens did appear to metabolize with 5% perchlorate, (not 10%) when they were adapted to perchlorate salts at greater concentrations. Magnesium and sodium perchlorates, only at 0.1 M or less were seen to

result in methanogenesis when permafrost isolated *Methanobacterium* and *Methanosarcina* were studied (Al Soudi et al. 2017). Another study reportedly found that at 0.4% perchlorate no growth was detected with a consortium that was facultatively anaerobic (Al Soudi et al. 2017).

In their own work, Al Soudi et al. (2017) investigated salinotolerant bacterial isolates from extreme, rare environments and found that almost all of the examined isolates grew in both 0.1 and 0.5 M perchlorate salts, some even at 1.0 M. Bacterial growth in up to 2.5 M chlorate was also demonstrated. It was noted that chlorate salts tolerance was much higher than perchlorate salts tolerance. Since at greater than 1 M concentrations of NaCl and $MgSO_4$ all isolates could grow, cation effects were not singly responsible for the growth limits with perchlorate salts. The microbes were reaching their Na tolerance limits in the case of 2.75 M sodium chlorate (Al Soudi et al. 2017). For overall less tolerant isolates, K perchlorate addition showed resultant strong growth. There was an evident mirroring of highest chlorate and perchlorate tolerance among isolates, indicating a growth inhibition common mechanism (Al Soudi et al. 2017).

Sheth (2010) was partially concerned with biodegradation remediation of perchlorate. Perchlorate reducing bacteria are involved in the biological reduction or biodegradation of perchlorate. Found in soil environments, these bacteria which are facultative anaerobes are surprisingly widespread; included are *Dechloromonas*, *Dechlorospirillum* and *Azospira* strains (Sheth 2010). Examples of the required electron donors for the reduction include: acetate, citrate, ethanol, glucose, lactate, hydrogen gas and vegetable oil. The bacteria can then oxidize the electron donor, while perchlorate, (which can serve as a terminal electron acceptor) is

reduced to chlorate. A chlorate reductase enzyme then reduces chlorate to chlorite. The chlorite dismutase enzyme is the catalyst in the oxygen and chloride producing breakdown of chlorite (Sheth 2010). In batch studies, using a perchlorate-degrading strain, (*Azospira suillum* JPLRND), the perchlorate utilizing maximum specific growth rate was 0.16 per hour compared to 0.22 when oxygen was behind the growth (Farhan and Hatzinger 2009). Another laboratory-scale study used a stirred tank bioreactor and *Proteobacterium* ARJR SMBS for perchlorate degradation. Anaerobically grown and under anoxic conditions, the perchlorate degradation rate averaged a value of 17.24 mg L^{-1} day^{-1} at a pH optimum of 7.5. In synthetic effluent there was a 0.83 – 1.2 h^{-1} maximum observed anoxic growth rate, while in real effluent it was 0.45 – 0.59 h^{-1} (Anoop Raj and Muruganandam 2013).

The Michaelis-Menten Model is used to describe enzyme kinetics (Berg, J. M., Tymoczko, J. L. 2002) and applies to biological reduction reactions. The equation (Biaglow, Erickson, and McMurran 2010) follows:

$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

V_0 = Initial reaction velocity

V_{max} = Maximum reaction velocity

K_M = Substrate concentration at which half of the enzyme active sites are occupied by substrate molecules, also known as half saturation constant

[S] = Substrate concentrations

For certain enzyme concentrations, the reaction velocity is almost linearly proportional to the substrate (for small substrate concentrations), and independent of the substrate concentrations (for high concentrations) (Berg, J. M., Tymoczko, J. L. 2002). These would represent first and zero-order reactions, respectively. Typical environmental perchlorate (parts-per-billion) reduction kinetics are known to be first-order with respect to the perchlorate concentration, for example (Logan et al. 2001). Half-saturations constants are useful to understand overall kinetics and to know if the reaction will have considerably slower or faster kinetics at certain concentrations of the contaminant of interest. This is because K_M is the concentration after which the reaction proceeds at half of its maximum velocity. For environmental applications, lower K_M values translate to smaller reactor sizes or smaller degradation times; that is, the reaction will proceed at high rates and will slow down only when the concentrations are very small.

2.7.4 Organic Substrates Utilized

Three compounds are to be used in this research as the organic sources promoting biodegradation. EOS PRO (a known remediation compound), industrial sugar wastewater (wastewater from a nearby fruit juice industry) and edible molasses.

2.7.4.1 EOS PRO

Table 9 provides relevant properties of EOS PRO.

Table 9: EOS PRO Properties (taken directly from Elkins 2016)

Property	Typical
Refined and Bleached US Soybean Oil (% by wt.)	59.8
Rapidly Biodegradable Soluble Substrate (% by wt.)	4
Other Organics (emulsifiers, food additives, etc.) (% by wt.)	10
Specific Gravity	0.96 - 0.98
pH (Standard Units)	6 - 7
Median Oil Droplet Size (microns)	1
Organic Carbon (% by wt.)	74
Mass of Hydrogen Produced (lbs. H_2 per lbs. EOSPRO)	0.25

2.7.4.2 Industrial Sugar Wastewater

No organics composition analysis results were available for the industrial sugar wastewater and none were conducted, but the authors were informed that it is composed of off-specification fruit juice.

2.7.4.3 Molasses

The molasses used was Golden Barrell® Unsulfured Blackstrap Molasses, the only ingredient of which is molasses. From other research the following information on blackstrap molasses was obtained. Tables 10 - 12 presents various of the contained compounds.

Table 10: Blackstrap Molasses Chemical Constituents (Reproduced from Abou-Zeid, Khan, and Abulnaja 1993)

Constituents	(%)
Moisture	22.30
Total Sugars	50.00
Sucrose	32.00
Reducing Sugars	18.00
Total Nitrogen	0.88
Ash	8.00

The main sugars, amino acids, etc. are presented in Table 11.

Table 11: Paper Chromatographic Detection of Sugars, Amino Acids and Organic Acids Present in Blackstrap Molasses (Data Reproduced and Title Taken Verbatim from Abou-Zeid et al. 1993)

Sugars	Amino Acids	Organic Acids
Arabinose	Alanine	Aconitic Acid
Fructose	Aspartic Acid	Citric Acid
Glucose	Cystine	Malic Acid
Raffinose	Glutamic Acid	
Sucrose	Glycine	
	Histidine	
	Leucine	
	Lysine	
	Methionine	
	Proline	
	Serine	
	Threonine	
	Tyrosine	
	Valine	

For blackstrap molasses ash elemental analysis, Table 12 presents elements, whose presence were indicated/not detected.

Table 12: Blackstrap Molasses Ash Elemental Analysis (Reproduced from Abou-Zeid et al. 1993)

Elements	mg/100 g ash
Na	0.24
K	3.46
Mg	0.28
Ca	1.49
Fe	—
Li	trace
Sr	trace
Ba	—
Al	trace
Cu	trace
Ni	—
Co	—
Pb	trace

2.7.5 Potential Release of Contaminants

A key concern with treatment processes is that while one or multiple contaminants are being treated, other contaminants may be inadvertently released or formed. For example, it has been found that with microorganisms, such as the dissimilatory arsenate reducing prokaryotes group, arsenate is used as electron acceptor and arsenic is solubilized from sediments (Barua, Barua, and Adhikari 2016). Additionally, it is widely recognized that organic matter is important

in its electron donor capacity, in reducing (microbially mediated) As(III) or As-host minerals which include Fe(III), which leads to solid phase As mobilization (Al Lawati et al. 2013). Evidently, the reducing conditions that prove favorable for the current contaminants being investigated can also be undesirable.

CHAPTER 3

METHODOLOGY

This chapter describes the experimental approach used in this research. Jar tests were performed to compare the impacts of $FeSO_4$ and CaS_x on Cr(VI) removal. These tests were then used to guide column experiments on Cr(VI) reduction using an actual contaminated groundwater. In the investigation of biological treatment of multiple oxyanions, microcosm tests were conducted examining the effects of types and doses of added substrates on the degradation of Cr(VI), nitrate, chlorate and perchlorate. Subsequent column testing was also performed to determine the effect of retention time and contaminant concentrations on the biological reduction process.

Soils from the contaminated site were obtained by rotary drilling. All groundwater was also obtained from the site. There were two strata of soils/groundwater that were principally used. The deeper soil (UMCF) was finer and clay-like. The deeper groundwater was associated with this depth. The shallower soil (QAL) was coarser and sandier. The shallower groundwater was associated with this depth.

In both cases the column testing was intended to simulate in-situ treatment. The start of the contact soil in the laboratory columns saturated with groundwater would represent the presence of a treatment injection well in the field. That is where the chemical or biological treatment would occur. The column effluent would represent a monitoring sampling well, downflow in the field, that would be tested for contaminants after underground treatment. The goal was to have similar retention times within the columns as those found at the site. For

example, higher flowrates exist in the sandy layer than in the claylike layer of soil. Packing variation of columns, changing pressures, and changes in the columns in terms of possible blocking of pores (from chemical precipitation or microbial growth) or development of cracks, can cause significant variation in flowrates and associated contact times among the columns.

3.1 Chemical Reduction Tests

These investigations were divided into two parts, jar tests and column tests. The jar tests investigated the use of calcium polysulfide, (Calmet®, ~27%) and ferrous sulfate in order to select the reductant that would reduce/coagulate Cr(VI) the most effectively and efficiently. After jar testing was accomplished, CaS_x was selected as the reductant that would be further investigate in laboratory columns intended to simulate in-situ treatment.

3.1.1 Jar Tests Using $FeSO_4/CaS_x$

3.1.1.1 Overview of Cr(VI) Reduction Jar Testing

The chemical Cr(VI) reduction jar tests were divided into two stages. The preliminary stage investigated various doses of CaS_x and $FeSO_4$ as reductants. The secondary stage widened the stoichiometric doses analyzed and also further investigated the effects of solids/added soil on the treatment.

For all jar tests, actual groundwater contaminated with Cr(VI) taken from two different horizons, QAL (shallow alluvial horizon) and UMCf (deep clayey horizon) were collected. High chromium concentrations were achieved by spiking the groundwater with Cr(VI) standard to roughly 10,000 µg/L. Low concentrations were achieved by either leaving the groundwater as

is, or spiking to about 500 µg/L. At the lower spiked QAL water concentration, analytical measurement interferences were present. The solution, was diluting QAL water 100 times prior to Cr(VI) addition. Total dissolved chromium (TDC) analysis resulted in no known interference.

A Phipps and Bird Batch Tester (Richmond, VA), and one-liter glass beakers were used for the jar batch chemical Cr(VI) removal tests with CaS_x and $FeSO_4$. Using as guidance the published work of Pakzadeh and Batista (2011) and Qin et al. (2005), (Pakzadeh and Batista 2011; Qin et al. 2005) batch tests were planned using ratios of each reducing agent to chromium, previously tested by those scientists. The stoichiometric requirements are 1.5 mol CaS_x/mol Cr(VI) and 3 mol Fe/mol Cr(VI), for calcium polysulfide and ferrous sulfate, respectively.

The desired dosage of coagulant was added to the beaker and for one minute, the beaker contents (coagulant, water and any suspended solids) were stirred rapidly (100 rpm). Slow mixing followed, by lowering the mixer speed to 30 rpm for a 30-minute period. The mixer was stopped and formed solids were allowed to settle after transferring the beaker contents to a graduated cylinder. After a settling time of ten minutes the volume of solids was recorded. Since soil can retain coagulant-formed precipitates during in-situ treatment, obtaining clear effluent was not the goal in this study, although it is often desired in water treatment. However, it was important to determine the amount of sludge formed during the batch tests because generation of too much solids may result in clogging around the groundwater well.

Approximately 100 mL of the settling graduated cylinder supernatant was transferred to vials for pH, total chromium and turbidity measurements. Using nitric acid (trace metal quality),

roughly 25 mL of supernatant were preserved for total dissolved chromium analysis with ICP. The graduated cylinder's remaining content was centrifuged at 3000 rpm for 30 minutes to obtain a clear liquid. Filtration of the carefully poured supernatant was accomplished using a 0.45 µm membrane filter and either vacuum or syringe filtration. Filtered supernatant was then analyzed for Cr(VI) and perchlorate, a co-contaminant in the groundwater. The sludge generated in the process was quantified using suspended solids testing, by drying a known amount of sample at 105°C and weighing the remaining solids in an aluminum dish. Beaker walls and mixer blades were inspected for scale formation, which may have formed as a result of reduction/precipitation. Jar tests were single point, only one jar had a duplicate for QC.

3.1.1.2 Preliminary CaS_x/$FeSO_4$ Jar Testing

To simulate areas on the site where Cr(VI) concentrations are very high, actual site groundwater was spiked with roughly 10,000 µg Cr(VI)/L. For lower Cr(VI) concentrations the groundwater was not spiked. The selected testing ratios were 2-3 times stoichiometric for CaS_x and 10-30 times stoichiometric for $FeSO_4$. Table 13 shows the preliminary stage batch test matrix.

Table 13: Preliminary Stage Cr(VI) Chemical Reduction Matrix for Batch Tests with CaSx, and $FeSO_4$

	High Concentration of Cr(VI)		Low Concentration of Cr(VI)	
Multiple of Stoichiometric Ratio	**mL of CaS_x/ 1000 L GW**	**mL of $FeSO_4$/ 1000 L GW**	**mL of CaS_x/ 1000 L GW**	**mL of $FeSO_4$/ 1000 L GW**
2X	336		34	
3X	505		50	
10X		4472		224
30X		13416		671

Raw CaS_x = undiluted, as it comes from manufacturer.

For these tests, the coagulant doses were added to 250 or 500 mL of an actual groundwater, containing Cr(VI). Additional testing was performed on groundwater with added UMCf soil to investigate the effects on treatment.

3.1.1.3 Secondary CaS_x/$FeSO_4$ Jar Testing

Using the previously described methods, six secondary batch test sets were performed, using six beakers in each set. Hexavalent chromium was used to spike QAL and UMCf groundwater to either 10,000 µg Cr(VI)/L or 500 µg Cr(VI)/L for high and low concentration testing, respectively. The batch testing coagulant doses ranged from 1.5X – 5X the stoichiometric requirements for CaS_5, and 5X – 50X times the stoichiometrically required amount for $FeSO_4$. The experimental matrix is shown in Table 14.

Table 14: Stage 2 Chemical Treatment Batch Test Matrix for QAL and UMCf at high and low Cr(VI) Concentrations, using CaS_x and $FeSO_4$ as Reductants

Set #	Cr Level/Test Volume	Groundwater Source and Concentration	Multiple of Stoichiometric Requirement (CaS_x)	Multiple of Stoichiometric Requirement ($FeSO_4$)
Set 1	High Cr (Target starting: 10000 µg Cr^{+6}/L) (250 mL GW/beaker)	QAL (10500 µg Cr^{+6}/L)	1.5X, 2X, 3X, 4X and 5X, replicate 2X	5X, 10X, 20X, 30X and 50X, replicate 10X
Set 2		UMCf (9800 µg Cr^{+6}/L) with 1 g of dry soil/L		
Set 3		QAL (10500 µg Cr^{+6}/L) Filtered groundwater with and without 1 g of dry QAL soil/L	5X and 10X, replicate 5X (3 beakers without soil and 3 beakers with soil)	N/A
Set 4		UMCf (9800 µg Cr^{+6}/L) Filtered groundwater with and without 1 g of dry QAL soil/L	N/A	5X and 10X, replicate 5X (3 beakers without soil and 3 beakers with soil)
Set 5	Low Cr (Target starting: 500 µg Cr^{+6}/L) (500 mL GW/beaker)	QAL at 500 µg Cr^{+6}/L	1.5X, 2X, 3X, 4X and 5X, replicate 2X	5X, 10X, 20X, 30X and 50X, replicate 10X
Set 6		UMCf at 500 µg Cr^{+6}/L		

As shown in Table 14, sets 1-4 of the batch tests were conducted for high concentrations of chromium. UMCf groundwater was found by the preliminary test to have turbidity that was very low and the $FeSO_4$ and CaS_x dosages did not result in good coagulation. In set 2, therefore, soil from boreholes drilled in the area was added to maintain 1 g of dry UMCf soil per liter of UMCf groundwater. In order to examine the effect (in tests with high Cr(VI) concentration) of suspended solids (turbidity), test sets 3 and 4 were done. A coffee filter was used to filter both depths of groundwater and approximately 10,000 µg Cr(VI) per liter concentration was attained through Cr(VI) addition. Out of the six tests in set 3, three were done with coarsely filtered

groundwater (250 mL). The three remaining batch tests were performed again with groundwater that was coarsely filtered, however, 1 g dry soil per liter of groundwater was attained by adding soil (QAL or UMCf). Sets 5 and 6 were spiked with low Cr(VI). Tables 15 and 16 are the testing matrices.

Table 15: High and Low Cr(VI) Concentration Test Matrix for QAL and UMCf, using CaS_x and $FeSO_4$ as Reductants

Calcium Polysulfide with QAL and UMCF			Ferrous Sulfate with QAL and UMCf		
Multiple of Stoichiometric Requirement	**Coagulant Dose (mL of CaSx/1000 L GW)**		**Multiple of Stoichiometric Requirement**	**Coagulant Dose (mL of $FeSO_4$/1000 L GW**	
	High Concentration	Low Concentration		High Concentration	Low Concentration
1.5	252		5	2236	
1.5	252		5	2236	
2	336	34	10	4472	224
2	336	34	10	4472	224
3	505	50	20	8945	447
3	505	50	20	8945	447
4	673	67	30	13417	671
5	841	84	50	22361	1118
5	841	84	50	22361	1118

Table 16: Test Matrix for Filtered QAL and UMCf Groundwater and for Filtered with Added Soil using CaS_x and $FeSO_4$ as Reductants

Groundwater Type	Multiple of Stoichiometric Requirement	Volume of CaS_x (mL/1000 L QAL GW)	Volume of $FeSO_4$ (mL/1000 L UMCf GW)
Filtered through coffee filter	5	842	2236
	5	842	2236
	10	1682	4472
Soil added (1 g/L) to the filtered water	5	842	2236
	5	842	2236
	10	1682	4472

Due to possible coagulant interferences with the Hach-tested Cr(VI) readings, Total Dissolved Chromium (TDC) values (using ICP) are only reported here. Batch sample concentrations of TDC were measured after they had been settled 10 minutes, but without filtration. Acid digestion was performed on still-turbid samples prior to ICP analysis

3.1.2 Column Tests Using CaS_x

3.1.2.1 Column Layout

For this portion of the research, three columns were prepared and packed with dry, actual soils from a contaminated site. One column (QAL) contained alluvial soil and two columns (UMCF-A and B) contained clay-like soil. The measurements of the soil strata in Figure 1 are approximate, since decisions were made during soil addition to try to achieve the best layout. The cover soil (above the injection ports) was intended to prevent upward flow of the injected coagulant, while the lower soil was intended to be the contact soil (about 25 inches). The

injection ports were filled with glass beads to facilitate coagulant injection. Above the glass beads was a small size gravel with coarse sand layer. This was covered by the "barrier," about 6 inches of soil covered with gravel. Since the top soil was the barrier above the injection point, it was not considered in the contact soil. The layers of gravel were intended to prevent the migration of the fine soil particles between layers.

The QAL column soil was fed downflow, using only gravity feed, and both UMCf columns were fed downflow, using pumps and in-house built pressure regulators, with a pressure of 15 psi. Actual groundwater from the site was used as column feed. The column schematic is shown in Figure 6.

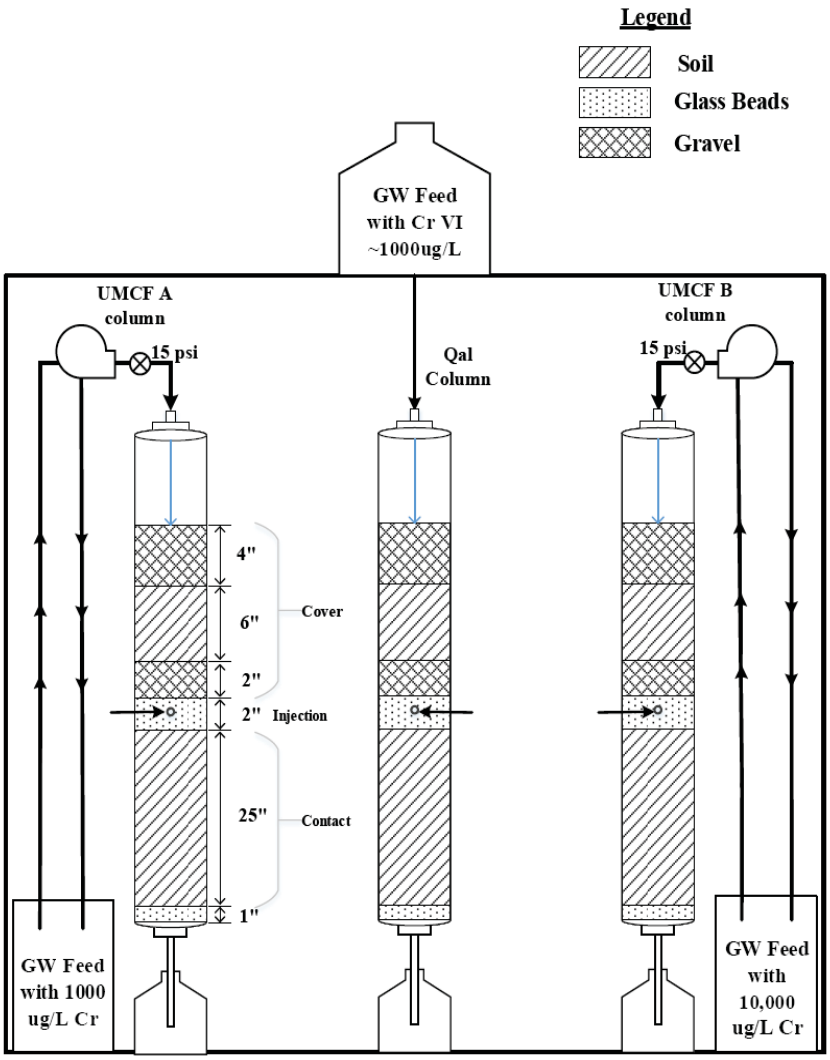


Figure 6: Column Layout for Cr(VI) Reduction Testing Using CaS_x as Reductant

3.1.2.2 Testing Procedures

Throughout the collection of reported data, UMCf-A and QAL columns received influent groundwater spiked to approximately 1 mg Cr(VI)/L and UMCf-B received groundwater spiked

with roughly 10 mg Cr(VI)/L. The reductant used was CaS_x. All reductant additions were done via manual injection with a syringe.

Initially, only groundwater spiked with Cr(VI) was fed to the columns, with no CaS_x. The effluent chromium concentration was near that of the influent, after two days. This indicated that the pore spaces and the soil had approximately reached their capacity for chromium sequestration and testing could begin. Table 17 shows each column's operation timeline.

Table 17: Column Operation Schedule for Cr(VI) Reduction Testing, Using CaS_x as Reductant

Operation Phase	**Days**		
	QAL 1000 µg/L	**UMCf-A 1000 µg/L**	**UMCf-B 10000 µg/L**
Prior to Starting CaS_x Addition	36	34	24
Injection of Calcium Polysulfide	1-30	1-17	1-27
No Injection with Same Cr(VI) Concentration	31-43	18-29	28-39
No Injection with Cr(VI) Increased by 10X	44-51	30-53	40-43

CaS_x injection was only started between days 24 and 36, upon stabilization. Migration of soil fines and effluent valve clogging were noted prior. The need for daily effluent valve cleaning was noted to allow for proper column operation. For data analysis, initiation of CaS_x addition was considered as Day 1. Considering results of the batch tests, a two times stoichiometric dose of CaS_x was selected, but 20X and 40X stoichiometric were used (for low and high Cr(VI) concentration columns, respectively) to address potential mixing issues in fine soils. The doses

were based on initial daily flowrates and the given Cr(VI) concentrations. The estimated amount for the QAL column was 2 mL CaS_x. Two daily injections were made of 1 mL each. For UMCF-A one daily injection of 0.3 mL was used and for UMCf-B one daily injection of 1 mL was utilized. This was based on higher QAL flowrates than UMCf. The only exception was that at the start of chemical treatment, UMCf-B was injected with 0.8 mL of CaS_x then for the next three days no injections took place. On day 5 the daily injections of 1 mL began.

Pumps were stopped during the CaSx injections. It was not atypical to lose some of the injection CaS_x while replacing the rubber injection port stopper, etc. Towards the end of the testing, after the termination of CaS_x addition, because chromium breakthrough was not occurring, the chromium influent concentration was increased tenfold, to speed up the process. The columns effluents were continuously collected. Sampling was conducted once, daily. The overall sample was considered a composite sample and a small grab sample was also collected daily at a proximal time. It is proposed that the grab samples might have higher Cr(VI) concentrations than the composite, since they were collected prior to CaS_x injection. Cr(VI) spiked groundwater was prepared then added.

3.1.2.3 Analyses

The measured parameters were hexavalent chromium (in both grab samples and daily composite samples) flowrate, pH, throughput volume, and in composite samples total dissolved chromium. The grab samples were filtered/analyzed for Cr(VI) on the given day by the Hach 8023 method. Nitric acid was used to preserve the composite samples for later ICP analysis at Utah State University Analytical Laboratories (USUAL). The composite samples were settled,

although unfiltered and relatively clear. This was thought to result from filtering by column soil, which would presumably similarly take place in the field. At each addition of new groundwater to the feed tank, the tank's chromium concentration was measured. Column tests were single point, since one sample was taken at a time and each column had a different soil or Cr(VI) concentration. Duplicate analysis was only done for QC.

3.2 Biological Reduction Tests

Testing was performed to determine whether the contaminants of the groundwater, (i.e. chromate, perchlorate, nitrate, chlorate), all of which are electron acceptors for bacteria, could be reduced biologically. The electron donors used in the microcosm testing were: EOS-PRO, industrial sugar wastewater, and molasses, (with COD values of 2,000,000, 99,440, and 1,053,000 mg/L, respectively). Microcosms were initially tested and the information from that stage was used in the column testing, to simulate in-situ groundwater treatment. In the columns, only EOS-PRO and industrial sugar wastewater were used as substrates.

3.2.1 Microcosm Tests Using Multiple Organic Substrates

3.2.1.1 Microcosms

A total of 128 microcosms were readied for in this experiment. The microcosms included the microcosms of the selected substrate and each one's controls (blanks and no phosphate nutrient). The carbon substrates were: EOS-PRO, Industrial sugar wastewater (ISW), EOS-PRO + ISW, and Molasses. Borosilicate glass bottles (125 mL), sterilized by autoclave, were used for all of the microcosm tests. For all tests, wet soil (30 grams) was placed in each bottle. The desired substrate amount, groundwater and nutrients, with a total combined volume of 100 mL were then

added. Considering the concentrations of chlorate, chromium, nitrate and perchlorate, a volume of substrate to achieve one hundred times the stoichiometric demand was added. To provide conditions that were anaerobic/anoxic, aluminum rings and butyl rubber caps were used to crimp close the microcosm bottles. Oxygen was not removed from the headspace.

Sixteen microcosms/replicates were prepared with 60mL EOS-PRO/L of GW, 16 were prepared with 50mL ISW + 40mL EOS-PRO/L of GW, 16 were prepared with 60mL ISW/L of GW, 8 were prepared with 40mL (molasses) + phosphate/L of GW, 4 blanks with no substrate were prepared, 4 were prepared which had molasses without phosphate, and 4 ISW without phosphate. Phosphate was added as a nutrient to molasses and ISW microcosms, increasing dilution, so EOS-PRO and mix microcosms received 15% and 10% by volume additions of DI water.

Microcosms were mixed continuously at 30 rpm on a rotary shaker, at room temperature. The tests were all done in duplicate. At time intervals, bottles and duplicates were sacrificed and desired analyses were performed, unless specified otherwise. For a minimum of 6-8 hours after microcosm removal from the rotor, solids were allowed to settle. It was essential to allow for settling since the soil was very fine. Membrane filters, 0.2 μm (Pall Laboratories), were used to filter the decanted liquid. Chlorate, COD (a surrogate test for the organic substrate content of the microcosms), Cr(VI), nitrate and perchlorate were analyzed in the filtered samples. Some sample microcosms were sampled and then placed back on the rotors for later re-sampling. Generally, however, the contents of the sacrificed bottles were centrifuged, then filtered, and analyzed for the Cr(VI), etc.

On days 7 and 11 the COD of the microcosm was not measured since the bottle was the same as that sampled from day 3. From day 19, each bottle was not sacrificed as before; after that, previously sampled microcosms were tested for COD. Twenty mL of liquid would be removed, and the bottle would be returned to the shaker for later sampling. When there was no observed significant degradation, resampling was done. This procedure was to maintain sufficient samples for longer incubation. With continuous sampling, oil from the microcosms is lost and there were lower COD levels in second re-samplings of microcosms. To sample, bottles were allowed to sit 8 hours to allow fines to settle prior to removing the top clear liquid. The loss of oil resulted from the removal (with the liquid) of the oil film which formed at the top during settling. Resampling of the same microcosms was done on days: 3, 7, 14, 19, 26, 44, 50, 71, 82, 92, 99.

3.2.1.2 Addition of Nutrients

Since the phosphorous content of the molasses and ISW microcosms was not adequate to support microbial growth, phosphorous (phosphate buffer) was added to these microcosms. EOS-PRO already contains phosphate, so none was added. Since there was nitrate in significant amounts in the contaminated groundwater and soils used (a potential bacterial nitrogen source), no nitrogen source was added initially. Upon degradation of nitrate, however, in some microcosms, nitrogen and phosphorous were supplied by adding di-ammonium phosphate.

3.2.1.3 Control Microcosms

The three control microcosm types introduced were: (1) Blank: no carbon substrate addition, (2) ISW with no phosphate, (3) Molasses with no phosphate. The blank microcosms were to test

for the biodegradation rates without external carbon substrate/electron donor, while the introduction of microcosm controls without phosphate were to investigate for phosphate's impact on oxyanion biodegradation.

3.2.1.4 Microbial Characterization

Microcosm content, roughly 30 mL, was shipped overnight to Research and Testing Laboratories (Lubbock Texas), using autoclaved containers for analysis of bacterial communities. Evaluated were archaea, bacteria and Cr(VI) reducing bacteria total number.

DNA was extracted from the present microorganisms utilizing Illumina next-technology (Anon 2015), which uses clonal amplification as well as sequencing via synthesis. Specific for archaea and bacteria, 515F-806R was the initial primer chosen for the study. Based on Somenahally et al. 2013, 27YMF and 534 R were the primers for the chromium reducing bacteria. After the generation of sequences, data examination was done for short, singleton, noisy and bad read sequences removal. A 4% divergence was used to cluster the quality-checked sequences utilizing USEARCH clustering algorithm (Anon 2015). A National Center of Biotechnology Information (NCBI) derived in-house-maintained database was used to identify the obtained sequences. Each organism's percentages, up to species level identification, were included in the final results that were obtained.

3.2.1.5 Analyses

Chlorate, COD, Cr(VI), nitrate, perchlorate as well as phosphate were measured in microcosms. The ion-chromatograph method used, was found to have poor chlorate detection,

therefore some samples were sent to (Silver State Labs) to check chlorate degradation. Nitric acid (pH = 2) was used to preserve TDC ICP analysis samples. Ion chromatography was used to measure nitrate concentrations in molasses microcosms, since the molasses color would interfere with the colorimetric method.

3.2.2 Column Investigations

3.2.2.1 Column Layout

Four columns were built to investigate oxyanion bio-reduction at varying flowrates (Figure 7). Two columns (UMCf-A and UMCf-B) were packed with dried deeper clay-like UMCf soil and two (QAL-A and QAL-B) were packed with shallower alluvial soil. The columns were 1.27 m long and 5.08 cm in diameter. Table 18 shows the columns' soil characteristics.

Table 18: Soil Parameters Used in the Columns Packed for Biological Reduction Investigations

Parameter, Units	**Soil Type**	
	QAL	**UMCf**
Volume of column occupied by soil (excluding the top cover), cm^3	2329	2329
Weight of soil used in column, g	2213	3026
Bulk density of dry QAL and UMCf, g/cm^3	1.3	0.9
Volume of solids only in the soil (estimated based on bulk density), cm^3	1147	803
Estimated porosity of the column (%)	48	66

Field porosity values varied from: 35-61% (QAL) and 51-66.8% (UMCf). Again, the laboratory measured values fell within field measured ranges.

The soils which were dried, were saturated with electron donor/nutrient free groundwater prior to the biodegradation testing. The columns were fed actual groundwater from a contaminated site in downflow mode. Given the fine nature of the UMCf material, pressure was needed to achieve reasonable flowrates. A pressurization of 15 psi (103.4 kPa) was used during saturation (UMCf columns) that was lowered to 10 psi (68.9 kPa) for the start of column operation. Pressurization was achieved by using a booster pump (Aquatec CDP6800) fitted with an in-house built pressure regulator. The QAL columns were initially gravity fed. Due to significant flow decrease, QAL columns were then pressurized after day 28. When QAL columns were also pump pressurized, the gravity influent tank was replaced with an analogous pump configuration. Although the pressures were intended to be 10 psi (68.9 kPa) and 5 psi (34.5 kPa) for the UMCf and QAL columns, respectively, when regulators were in use, there was sometimes variation due to clogging and adjustments, etc. so cleaning and adjustment were needed.

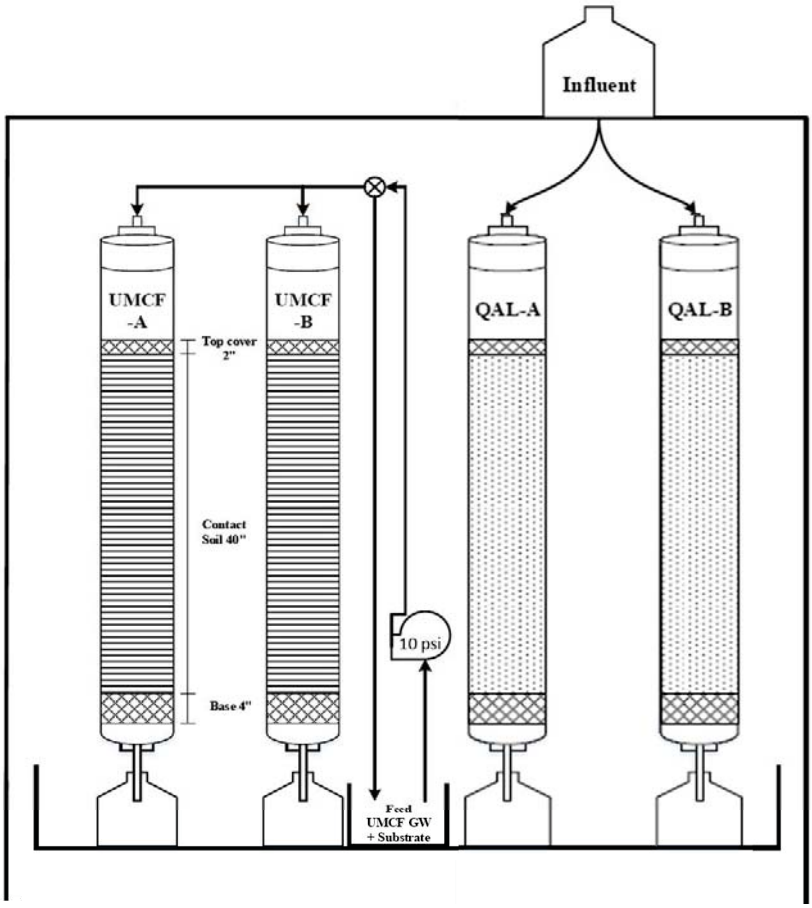


Figure 7: Initial Column Layout for Biological Reduction Tests, QAL, Columns are Gravity fed (day 1-28). After day 28, QAL Gravity Feed was Replaced by an Analogous Pump Pressure System.

3.2.2.2 Testing Procedures

Table 19 shows column operation details. Of note are the changing feed compositions over time.

Table 19: Biological Reduction Column Operation Information

QAL		UMCf	
Days	**Feed Variation**	**Days**	**Feed Variation**
1-2	7% ISW and 2% EOS-PRO in 91% GW (45880 mg/L COD equivalent)	1-7	7% ISW and 2% EOS-PRO in 91% GW 45880 mg/L COD equivalent)
3-5	Dilution of the previous feed by GW	8-10	Dilution of the previous feed by GW
6-8	GW only	11-13	GW only
9-14	7% ISW and 2% EOS-PRO in 91% GW (45880 mg/L COD equivalent)	14-19	7% ISW and 2% EOS-PRO in 91% GW 45880 mg/L COD equivalent)
15-17	0.2% EOS-PRO in 99.8% GW (4000 mg/L COD equivalent)	20-31	0.2% EOS-PRO in 99.8% GW (4000 mg/L COD equivalent)
18-29	GW only		
30-36	0.4% EOS-PRO (8000 mg/L COD equivalent)	32-40	0.4% EOS-PRO in 99.6% GW (8000 mg/L COD equivalent)
37-160	1.5% ISW and 0.4% EOS-PRO and 1.9% Phosphate in 96.2% GW (9260 mg/L COD equivalent)	41-165	1.5% ISW and 0.4% EOS-PRO and 1.9% Phosphate in 96.2% GW (9260 mg/L COD equivalent)

One QAL column had issues with clogging. Effluent valve cleaning and five days of operation with only groundwater were used to resolve the issue. Therefore, there was a five-day offset between UMCf and QAL column initial substrate addition. Shallower groundwater (Well C-S) was used for the alluvial soil and deeper groundwater (Well C-D) was used for the clay-like soil. Groundwater was used as collected. The substrates used for both soil types were either ISW, EOS-PRO, both or neither. The substrates were mixed with the groundwater in the influent feed tanks and fed downflow through the column tops. Initially the feed was not prepared daily, but was later on, to minimize degradation in the influent tanks. Ice packs were generally used and changed, daily to minimize degradation in the influent tanks and sample collection vessels.

Initially, there was no addition of extra phosphate; later, during low strength substrate feeding, 120 mg/L as PO_4 was added.

3.2.2.3. Analyses of Column Influent and Effluent/Data Collection

Sampling was conducted daily. Parameters tested included, chlorate, Cr(VI), nitrate, perchlorate, phosphate. Data was also collected on flowrate, and throughput volume. Where identified, data from total collection volumes below 80 mL were not included. Composite samples were used for the total dissolved chromium (TDC) measurements and Cr(VI) measurements utilized grab samples. Filtered samples were used for Cr(VI) measurements, and settled (but not filtered) effluent samples were used for TDC measurements. Although duplicates of each column were run, data were plotted for each column as single point results, but error bars were included for replicate analyses.

3.3 Overall Analytical Methods

Table 20 presents the analytical methods that were used throughout this research.

Table 20: Analytical Procedures in this Study

Parameter	Method details/ Reagents used	Hach method or EPA method	Equipment
COD	Ultra Low Range	8000	Spectrophotometer (Hach DR 5000)
	Low Range		
	High Range		
	High Range Plus		
Ammonia	Low Range, High Range	10031	Spectrophotometer (Hach DR 5000)
Nitrate	NitraVer® 3	10020	Test 'N Tube™ Vials
Chlorate/ Perchlorate	KOH (eluent)	314	Ion Chromatograph (Dionex ICS-2000)
Phosphate	PhosVer® 3	8048	Spectrophotometer (DR 5000)/Colorimeter (Hach Dr 900)
Sulfate	SulfaVer® 4	8051	Spectrophotometer (DR 5000)
Total Iron	FerroVer®	8008	Spectrophotometer (DR 5000)
Hexavalent Chromium	ChromaVer® 3	8023	Spectrophotometer (Hach DR 5000)
			Colorimeter (DR 900)
pH	pH buffer solution	8156	pH meter
Total metal (trace and major metal)		200.7	Thermo ICP 6300
Total Dissolved Chromium		200.7	Thermo ICP 6300

The analysis of total metals on some treated samples, although somewhat increasing the purview of this research, enabled the monitoring of other constituents such as arsenic, which as previously mentioned may increase in solubility as a result of reductive treatment.

Hexavalent chromium was extensively monitored, but since there were found to be interferences with the colorimetric test method used, ICP chromium results were often reported where available. Since this analysis was often performed without acid digestion, the results were labeled "Total dissolved chromium" since it was a measure of all chromium species in solution.

CHAPTER 4

COMPARISON OF CHEMICAL REDUCTANTS FOR HEXAVALENT CHROMIUM TREATMENT AND INVESTIGATION OF IN-SITU APPLICATION

4.1 Abstract

Hexavalent chromium (Cr(VI)) is a contaminant that poses significant health impacts. Groundwater hexavalent chromium contamination is a widespread concern. The objective of this research was to compare two chemical reductants in the treatment of Cr(VI) and to investigate the in-situ treatment potential of the selected reductant. Jar tests were employed, which consisted of treating an actual Cr(VI) containing groundwater with calcium polysulfide (CaS_x) and ferrous sulfate ($FeSO_4$). Both coagulants showed the ability to reduce Cr(VI). Tests with roughly 10 mg Cr(VI)/L were more successful than for lower concentrations. In those higher concentration tests, stoichiometric doses of 3X calcium polysulfide resulted in Cr(VI) reductions of 89-93% and 10X doses of $FeSO_4$ promoted reductions of 73-97%. The presence of suspended solids in the groundwater was found to have a positive effect on chromium removal. As anticipated, perchlorate, a co-contaminant, was not shown to have been reduced as a result of these reductants. CaS_x underwent additional testing in laboratory scale columns. The results showed very effective and sustainable treatment of Cr(VI), with many effluent total dissolved chromium readings at or below detection (1 µg/L) for all columns. This investigation supports the use of CaS_x as a reductant for in situ Cr(VI) groundwater treatment.

Keywords: Hexavalent chromium reduction, Groundwater remediation, In Situ chromium treatment, Calcium polysulfide, Ferrous Sulfate

4.2 Introduction

Groundwater contaminated with Cr(VI) is found around the world (Graham et al. 2006; Sharma et al. 2012; U.S. Department of Health and Human Services 2018). Hexavalent chromium can occur naturally (McNeill et al. 2012), or result from human activities. It can result from tanneries and the manufacturing of chrome sulfate (Sharma et al. 2012). The US EPA drinking water maximum contaminant level (MCL) for total chromium is 100 ppb, while multiple states have total chromium limits established at 50 ppb (U.S. Department of Health and Human Services 2018). The two oxidation states of chromium that are predominant are trivalent and hexavalent chromium (+3 and +6) (McNeill et al. 2012). Trivalent chromium is insoluble and less harmful than hexavalent chromium (Graham et al. 2006). Through inhalation, compounds of hexavalent chromium have been demonstrated to cause cancer of the lungs, in humans, and are listed to be known human carcinogens by "The Report on Carcinogens" (U.S. Department of Health and Human Services 2018). The effects of the oral consumption of Cr(VI) were still under evaluation (McLean et al. 2012). Hexavalent chromium is also a strong oxidizing agent, is mutagenic, carcinogenic and through aquatic and soil environments, it diffuses quickly. Cations of Cr(III) are not environmentally very mobile and toxic and insoluble precipitates are formed by Cr(III). Therefore, toxicity and mobility are decreased when Cr(VI) is reduced to Cr(III) and its removal from effluent is simplified (Barrera-Díaz et al. 2012).

Currently, the reduction of aqueous solution Cr(VI) is effectively accomplished by biological, chemical and electrochemical methods (Barrera-Díaz et al. 2012). Chemicals that have had success in reducing Cr(VI) to Cr(III) include, ferrous sulfate (Qin et al. 2005), calcium

polysulfide (Pakzadeh and Batista 2011) and zerovalent iron (Prasad, Das, and Golder 2011). In addition, both ferrous sulfate and calcium polysulfide have been found to significantly decrease Cr(VI) content and leachability in soil contaminated with Cr(VI), with calcium polysulfide proving the superior of the two for stabilization (Zhang et al. 2018). Citing Dermatas et al. 2006 and Chrysochoou and Johnston, 2015, it was proposed that the two coagulants would reduce Cr(VI) according to the following equations (Zhang et al. 2018):

$$3Fe^{+2} + HCrO_4^- + 7H^+ \leftrightarrow 3Fe^{+3} + Cr^{3+} + 4H_2O$$

$$2\ CrO_4^{-2} + 3CaS_5 + 10H^+ \leftrightarrow 2Cr\ (OH)_3 + 15S + 3Ca^{2+} + 2H_2O$$

It was desirable to identify the superior reductant for the groundwater conditions and to investigate its application in a simulated laboratory environment. The objectives of this research were to compare the treatment of Cr(VI) contaminated groundwater using ferrous sulfate and calcium polysulfide. Jar tests were used to study required doses, treatment effects and sludge production. Stoichiometric doses and sludge production were key considerations based on the potential for clogging with in situ treatment. Column studies were used to apply the selected reductant (CaS_x) and to investigate treatment levels and flow conditions in order to provide insight into field applications and the effectiveness of in-situ remediation.

4.3 Experimental

4.3.1 Chemical Reduction Tests

These investigations were divided into two parts, jar tests and column tests. The jar tests investigated the use of calcium polysulfide, (Calmet®, ~27%) and ferrous sulfate in order to

select the reductant that would reduce/coagulate Cr(VI) the most effectively and efficiently. After jar testing was accomplished, CaS_x was selected as the reductant that would be further investigate in laboratory columns intended to simulate in-situ treatment.

4.3.1.1 Jar Tests Using $FeSO_4$/CaSx

Overview of Cr(VI) Reduction Jar Testing

The chemical Cr(VI) reduction jar tests were divided into two stages. The preliminary stage investigated various doses of CaS_x and $FeSO_4$ as reductants. The secondary stage widened the stoichiometric doses analyzed and also further investigated the effects of solids on the treatment.

For all jar tests, an actual groundwater contaminated with Cr(VI) was taken from two different horizons. QAL (shallow alluvial horizon) and UMCf (deep clayey horizon) were collected. High chromium concentrations were achieved by spiking the groundwater with Cr(VI) standard to roughly 10,000 µg/L and low concentrations were achieved by either leaving the groundwater as is, or spiking to about 500 µg/L. At the lower spiked QAL water concentration, analytical measurement interferences were clearly present. The solution to the interference was diluting QAL water 100 times prior to Cr(VI) addition. Total dissolved chromium (TDC) had no known interference.

A Phipps and Bird Batch Tester (Richmond, VA), and one-liter glass beakers were used for the jar batch chemical Cr(VI) removal tests with CaS_x and $FeSO_4$. Using as guidance the published work of Pakzadeh and Batista (2011) and Qin et al. (2005), batch tests were planned

using ratios of each reducing agent to chromium, previously tested by those scientists. Based on the equations, the stoichiometric requirements are 1.5 mol CaS_x/mol Cr(VI) and 3 mol Fe/mol Cr(VI), for calcium polysulfide and ferrous sulfate, respectively.

The desired dosage of coagulant was added to the beaker and for one minute, the beaker contents (coagulant, water and any suspended solids) were stirred rapidly (100 rpm). Slow mixing followed. The mixer speed was lowered to 30 rpm for a 30-minute period. The mixer was stopped and formed solids were allowed to settle after transferring the beaker contents to a graduated cylinder. After a settling time of ten minutes the volume of solids was recorded. Since soil can retain coagulant-formed precipitates during in-situ treatment, obtaining clear effluent was not the goal in this study, although it is often desired in water treatment. However, it was important to determine the amount of sludge formed during the batch tests because generation of too much solids may result in clogging around the groundwater well.

Approximately 100 mL of the settling graduated cylinder supernatant was transferred to vials for pH, total chromium and turbidity measurements. Using nitric acid (trace metal quality), roughly 25 mL of supernatant were preserved for total dissolved chromium analysis with ICP. The graduated cylinder's remaining content was centrifuged at 3000 rpm for 30 minutes to obtain a clear liquid. Filtration of the carefully poured supernatant was accomplished using a 0.45 μm membrane filter. Filtered supernatant was then analyzed for Cr(VI) and perchlorate, a co-contaminant in the groundwater. The sludge generated in the process was quantified using suspended solids testing, by drying a known amount of sample at 105°C and weighing the

remaining solids in an aluminum dish. Beaker walls and mixer blades were inspected for scale formation. Jar tests were single point, only one jar had a duplicate for QC.

Preliminary $CaS_x/FeSO_4$ Jar Testing

To simulate areas on the site where Cr(VI) concentrations are very high, actual site groundwater was spiked with roughly 10 mg Cr(VI)/L. For lower Cr(VI) concentrations, the groundwater was not spiked. The selected testing ratios were 2-3 times stoichiometric for CaS_x and 10-30 times stoichiometric for $FeSO_4$. Table 21 shows the preliminary stage batch test matrix.

Table 21: Preliminary Stage Cr(VI) Chemical Reduction Matrix for Batch Tests with CaSx, and $FeSO_4$

	High Concentration of Cr(VI)		Low Concentration of Cr(VI)	
Multiple of Stoichiometric Ratio	**mL of CaS_x/ 1000 L GW**	**mL of $FeSO_4$/ 1000 L GW**	**mL of CaS_x/ 1000 L GW**	**mL of $FeSO_4$/ 1000 L GW**
2X	336		34	
3X	505		50	
10X		4472		224
30X		13416		671

Raw CaS_x = undiluted, as it comes from manufacturer.

For these tests, the coagulant doses were added to 250 or 500 mL of an actual groundwater, containing Cr(VI). Additional testing was performed on groundwater with added UMCf soil solids to investigate their effects on treatment.

Secondary $CaS_x/FeSO_4$ Jar Testing

Using the previously described methods, six secondary batch test sets were performed, using six beakers in each set. Hexavalent chromium was used to spike QAL and UMCf groundwater to either 10,000 µg Cr(VI)/L or 500 µg Cr(VI)/L for high and low concentration testing, respectively. The batch testing coagulant doses ranged from 1.5X – 5X the stoichiometric requirements for CaS_5, and 5X – 50X times the stoichiometrically required amount for $FeSO_4$. The experimental matrix is shown in Table 22.

Table 22: Stage 2 Chemical Treatment Batch Test Matrix for QAL and UMCf at high and low Cr(VI) Concentrations, using CaS_x and $FeSO_4$ as Reductants

Set #	Cr Level/Test Volume	Groundwater Source and Concentration	Multiple of Stoichiometric Requirement (CaS_x)	Multiple of Stoichiometric Requirement ($FeSO_4$)
Set 1	High Cr (Target starting: 10000 µg Cr^{+6}/L) (250 mL GW/beaker)	QAL (10500 µg Cr^{+6}/L)	1.5X, 2X, 3X, 4X and 5X, replicate 2X	5X, 10X, 20X, 30X and 50X, replicate 10X
Set 2		UMCf (9800 µg Cr^{+6}/L) with 1 g of dry soil/L		
Set 3		QAL (10500 µg Cr^{+6}/L) Filtered groundwater with and without 1 g of dry QAL soil/L	5X and 10X, replicate 5X (3 beakers without soil and 3 beakers with soil)	N/A
Set 4		UMCf (9800 µg Cr^{+6}/L) Filtered groundwater with and without 1 g of dry QAL soil/L	N/A	5X and 10X, replicate 5X (3 beakers without soil and 3 beakers with soil)
Set 5	Low Cr (Target starting: 500 µg Cr^{+6}/L) (500 mL GW/beaker)	QAL at 500 µg Cr^{+6}/L	1.5X, 2X, 3X, 4X and 5X, replicate 2X	5X, 10X, 20X, 30X and 50X, replicate 10X
Set 6		UMCf at 500 µg Cr^{+6}/L		

As shown in Table 22, sets 1-4 of the batch tests were conducted for high concentrations of chromium. UMCf groundwater was found by the preliminary test to have turbidity that was very low and that the $FeSO_4$ and CaS_x dosages did not result in good reduction, based on percent removals. In set 2, therefore, soil from boreholes drilled in the area was added to maintain 1 g of dry UMCf soil per liter of UMCf groundwater. In order to examine the effect (in tests with high Cr(VI) concentration) of suspended solids (turbidity), test sets 3 and 4 were done. A coffee filter

was used to filter both depths of groundwater and approximately 10 mg Cr(VI) per liter concentration was attained through Cr(VI) addition. Out of the six tests in set 3, three were done with coarsely filtered groundwater (250 mL). The three remaining batch tests were performed again with groundwater that was coarsely filtered, however, 1 g dry soil per liter of groundwater was attained by adding soil (QAL or UMCf). Sets 5 and 6 were spiked with low Cr(VI). Tables 23 and 24 are the testing matrices.

Table 23: High and Low Cr(VI) Concentration Test Matrix for QAL and UMCf, using CaS_x and $FeSO_4$ as Reductants

Calcium Polysulfide with QAL and UMCF			Ferrous Sulfate with QAL and UMCf		
Multiple of Stoichiometric Requirement	**Coagulant Dose (mL of CaS_x/1000 L GW)**		**Multiple of Stoichiometric Requirement**	**Coagulant Dose (mL of $FeSO_4$/1000 L GW**	
	High Concentration	Low Concentration		High Concentration	Low Concentration
1.5	252		5	2236	
1.5	252		5	2236	
2	336	34	10	4472	224
2	336	34	10	4472	224
3	505	50	20	8945	447
3	505	50	20	8945	447
4	673	67	30	13417	671
5	841	84	50	22361	1118
5	841	84	50	22361	1118

Table 24: Test Matrix for Filtered QAL and UMCf Groundwater and for Filtered with Added Soil using CaS_x and $FeSO_4$ as Reductants

Groundwater Type	Multiple of Stoichiometric Requirement	Volume of Raw CaS_x (mL/1000 L QAL GW)	Volume of $FeSO_4$ (mL/1000 L UMCf GW)
Filtered through coffee filter	5	842	2236
	5	842	2236
	10	1682	4472
Soil added (1 g/L) to the filtered water	5	842	2236
	5	842	2236
	10	1682	4472

Due to possible reductant interferences with the Hach colorimetric Cr(VI) readings, Total Dissolved Chromium (TDC) values (using ICP) were only reported here. The interferences, for example, may have resulted from the color of the reductants themselves, or the presence of interfering compounds which would affect the reactions that generate the color to be detected. Batch sample concentrations of TDC were measured after they had been settled 10 minutes, but without filtration. Acid digestion was performed on still-turbid samples prior to ICP analysis

4.3.1.2 Column Tests Using CaS_x

Column Layout

For this portion of the research, three columns were prepared and packed with actual soils from a contaminated site. One column (QAL) contained alluvial soil and two columns (UMCF-A and B) contained clay-like soil. Each column was packed with soil. The measurements of the soil strata in Figure 1 are approximate, since decisions were made during soil addition to try to

achieve the best layout. The lower soil was intended to be the contact soil (about 25 inches) where the reduction process was to take place. The injection ports, above the lower soil, were filled with glass beads to facilitate reductant injection in the void spaces they provided. The glass beads were covered by the "barrier," about 6 inches of soil, intended to prevent upward flow of the injected reductant. Layers of gravel/sand were used to prevent the migration of fine soil particles between layers.

The QAL column soil was fed downflow, using only gravity feed, and both UMCf columns were fed downflow, using pumps and in-house built pressure regulators, with a pressure of 15 psi. Actual groundwater from the site was used as column feed. The column layout is shown in Figure 8.

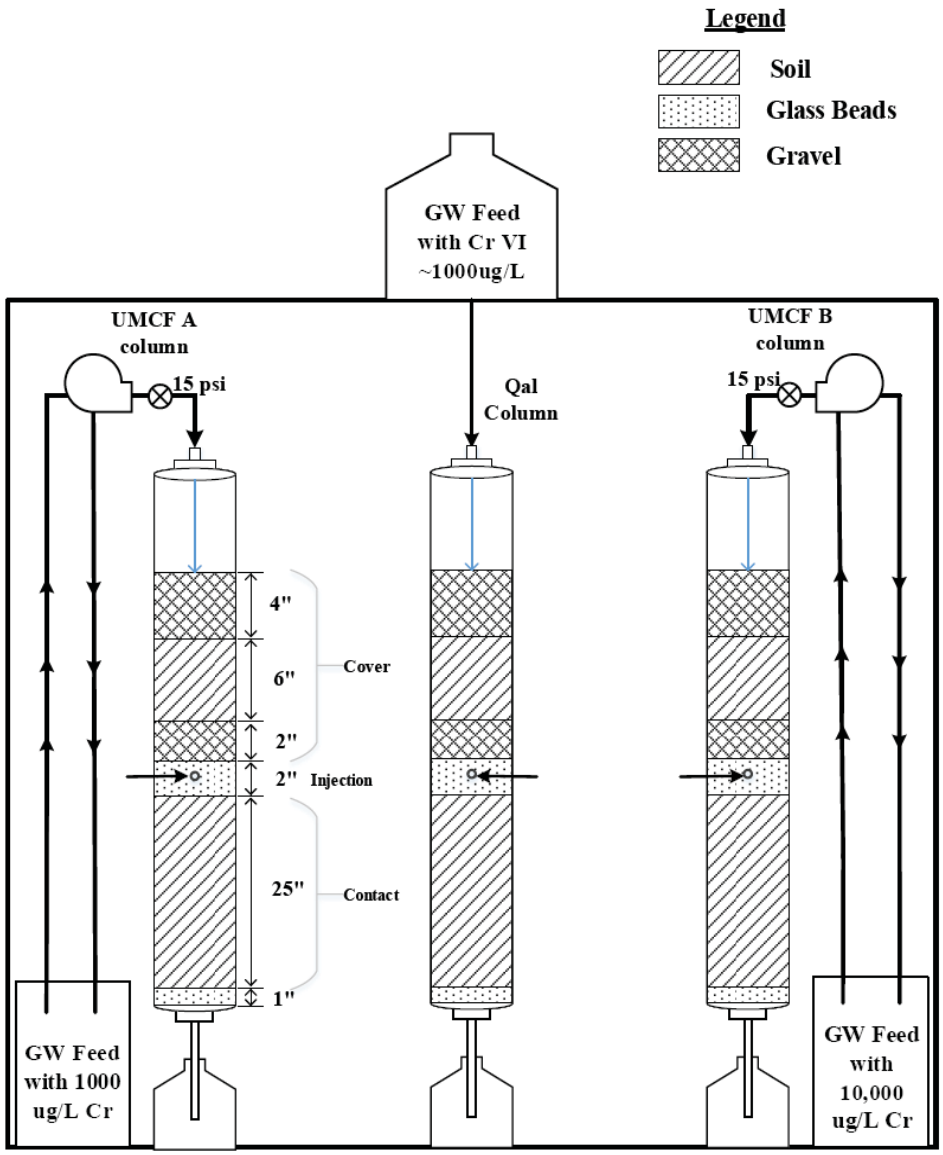


Figure 8: Column Layout for Cr(VI) Reduction Testing Using CaS_x as Reductant

Testing Procedures

Throughout reported data collection, UMCf-A and QAL columns received influent groundwater spiked to approximately 1 mg Cr(VI)/L and UMCf-B received groundwater spiked with roughly 10 mg Cr(VI)/L. The treatment chemical used was CaS_x. All coagulant additions were done via manual injection with a syringe.

Initially, only groundwater spiked with Cr(VI) was fed to the columns, with no CaS_x. The effluent chromium concentration was near that of the influent, after two days. This indicated that the pore spaces and the soil had approximately reached their capacity for chromium sequestration and testing could begin. Table 25 shows each column's operation timeline.

Table 25: Column Operation Schedule for Cr(VI) Reduction Testing, Using CaS_x as Reductant

Operation Phase	**Days**		
	QAL	**UMCf-A**	**UMCf-B**
	1000 µg/L	**1000 µg/L**	**10000 µg/L**
Prior to Starting CaS_x Addition	36	34	24
Injection of Calcium Polysulfide	1-30	1-17	1-27
No Injection with Same Cr(VI) Concentration	31-43	18-29	28-39
No Injection with Cr(VI) Increased by 10X	44-51	30-53	40-43

CaS_x injection was only started between days 25 and 37, upon stabilization. Migration of soil fines and effluent valve clogging were observed prior. The need for daily effluent valve cleaning was noted to allow for proper column operation. For data analysis, initiation of CaS_x addition

was considered as Day 1. Considering results of the batch tests, a two times stoichiometric dose of CaS_x was suitable. For the columns 20X and 40X, stoichiometric doses were used (for low and high Cr(VI) concentration columns, respectively) since column testing may have less optimal mixing conditions compared to jar testing. The estimated amount for the QAL column was 2 mL CaS_x. Two daily injections were made of 1 mL each. For UMCF-A one daily injection of 0.3 mL was used and for UMCf-B one daily injection of 1 mL was utilized. This was based on higher QAL flowrates than UMCf. The only exception was that at the start of chemical treatment, UMCf-B was injected with 0.8 mL of CaS_x then for the next three days no injections took place. On day 5 the daily injections of 1 mL began.

Pumps were stopped during the CaS_x injections. It was not atypical to lose some of the injection CaS_x while replacing the rubber injection port stopper, for example. Towards the end of the testing, after the termination of CaS_x addition, because chromium breakthrough was not occurring, the chromium influent concentration was increased tenfold, to speed up the process. It was of interest to determine how much residual treatment capacity was available and this made the process occur within a reasonable timeframe. The columns effluents were continuously collected. Sampling was conducted once, daily. The overall sample was considered a composite sample and a small grab sample was also collected daily at a proximal time. It is proposed that the grab samples might have higher Cr(VI) concentrations than the composite, since they were collected prior to CaS_x injection. Cr(VI) spiked groundwater was prepared then added.

Analyses

The measured parameters were hexavalent chromium (in both grab samples and daily composite samples) flowrate, pH, throughput volume, and in composite samples total dissolved chromium. The grab samples were filtered/analyzed for Cr(VI) on the given day with the colorimetric Hach method 8023 (1,5-Diphenylcarbohydrazide Method). Nitric acid was used to preserve the composite samples for later ICP analysis at Utah State University Analytical Laboratories (USUAL). The composite samples were unfiltered, settled samples, and were relatively clear. This was thought to result from filtering by column soil, which would presumably similarly take place in the field. At each addition of new groundwater to the feed tank, the tank's chromium concentration was measured. Column tests were single point, since one sample was taken at a time and each column had a different soil or Cr(VI) concentration. Duplicate analysis was only done for QC.

4.3.1.3 Analytical Methods

Hexavalent chromium was measured using the 1,5-Diphenylcarbohydrazide colorimetric Hach Method 8023. Ion chromatography was used to measure perchlorate (Dionex ICS-2000). Total Dissolved Chromium (TDC) and total metals (trace and major metals) were measured using Inductively Coupled Plasma (Thermo ICP 6300) at Utah State University Analytical Laboratories.

4.4 Results and Discussion

4.4.1 Jar Tests

4.4.1.1 Preliminary $CaS_x/FeSO_4$ Jar Testing

Table 26 shows the results for the preliminary Cr(VI) chemical reduction testing. All Cr(VI) results were obtained using a colorimetric method and thus were subject to scrutiny for interferences (Hach Method 8023). Since colorimetric analyses rely on reactions which generate certain colors, the presence of other compounds may slow or alter those reactions, or introduce new colors, thereby changing the intensity of color or the wavelength which is absorbed. There were found to be interferences, particularly with QAL groundwater. As a result, the low chromium dose preliminary batch results were not considered.

Table 26: High Cr(VI) Preliminary Chemical Coagulation Results, Using CaS_x and $FeSO_4$ as Reductants

Groundwater/Cr Levels		Coagulant	Multiple of Stoichiometric Ratio	Initial Cr (µg/L Cr^{+6})	Final Cr (µg/L Cr^{+6})	% removal
QAL	High Cr	CaS_x	2X	10200	20	99.8
			3X		0	100
		$FeSO_4$	10X		20	99.8
			30X		50	99.5
UMCf	High Cr	CaS_x	2X	10200	9400	7.84
			3X		9000	11.7
		$FeSO_4$	10X		9400	7.84
			30X		800	92.1
UMCf	High Cr (w/1g dry UMCf soil/L)	CaS_x	2X	10200	10	99.9
			3X		30	99.7
		$FeSO_4$	10X		45	99.6
			30X		30	99.7

As seen in Table 26, there were very high Cr(VI) percent removals noted for QAL groundwater but generally bad removals for UMCf groundwater (no added soil), except for the higher dose $FeSO_4$. The poor removals from UMCf groundwater were thought to be due to the low turbidity of the water. To evaluate this possibility, other tests were conducted with 1 gram of soil (dry UMCf) added per liter of groundwater (UMCf) and are also presented in Table 26. The addition of soil improved results, with all percent removals noted at above 99.5%. In comparison, Wang et al. (2013) found that with the flocculant polyethyleneimine-sodium xanthogenate, a slight decrease was found for Cr(VI) removal and a slight increase for total chromium removal in the presence of turbidity. The authors proposed that turbidity-generated flocs may have had a sweeping effect for a certain insoluble Cr(III) ion compound (increasing total chromium

removal). The turbidity components resulted in consumption of the flocculant, while only providing weak Cr(VI) ion adsorption capacity, decreasing the removal of Cr(VI) slightly (Wang et al. 2013). In the case of the UMCf tests, it is possible that the added soil solids in the current research did contribute some adsorption capacity, thereby increasing the Cr(VI) removal. It is also possible that the solids in both the QAL and the amended UMCf samples generated flocs when treated, which in turn had adsorptive capacity for Cr(VI).

4.4.1.2 Secondary Stage Results: Jar Testing Utilizing CaS_x and $FeSO_4$ for High and Low Cr(VI) Concentration Reduction in Groundwater

As mentioned, Total Dissolved Chromium (TDC) values (measured using ICP) were only reported here. Table 27 shows a results summary of various reductant doses for high concentration chromium removal from groundwater, as well as turbidity data after 10 minutes of settling.

Table 27: Coagulation Batch Test Results for High Chromium Groundwater (250 mL)

Coagulant	Multiple of Stoichiometric Ratio	Coagulant Dose (mL/1000 L GW)	QAL (Initial Cr: 10500 µg Cr^{+6}/L)		UMCf (Initial Cr: 9800 µg Cr^{+6}/L)	
			Turbidity (NTU)	TDC (µg/L)	Turbidity (NTU)	TDC (µg/L)
CaS_x (27% by wt.)	1.5X	253	246	9260	256	8740
	1.5X	253	251	9160	284	8770
	2X	337	174	4190	201	7910
	2X	337	181	4680	216	8600
	3X	505	153	770	178	1030
	3X	505	170	1640	222	750
	4X	673	160	830	217	730
	5X	842	146	860	284	820
	5X	842	159	970	200	560
$FeSO_4$ (6% as Fe)	5X	2236	154	9280	216	8620
	5X	2236	148	9310	231	8490
	10X	4472	166	310	287	2670
	20X	8945	103	270	28	90
	20X	8945	111	320	31	80
	30X	13417	93	140	139	260
	50X	22361	70	150	87	112
	50X	22361	66	160	79	110

In the CaS_x reduction/coagulation results, it was clear that for both groundwater types, there was no really dramatic TDC removal until a 3-times stoichiometric dose was used. The ferrous sulfate dose needed for appreciable removals of TDC was 10 or 20 times the stoichiometric dose. The contradiction with the preliminary Cr(VI) results implies that chromium is either present in a valence other than Cr(VI) or that the previous Cr(VI) test results were affected by interferences. Previous research, with similar contact times, using the TCLP on chromium in ion exchange

brines showed that for initial concentrations of 9 - 93.2 mg/L Cr(VI), 0.6 – 1.4 times the molar ratio of CaS_5/Cr(VI) were used to reach the 5 mg/L limit. To meet the 0.1 mg/L MCL, molar ratios of 3.7-1.7 were needed. Therefore, for 9 mg Cr(VI)/L (close to the high concentration Cr(VI) here), the values ranged from 0.6 to 3.7 times the molar ratio (Pakzadeh and Batista 2011). Since the stoichiometric demand is expected to be 1.5 mol CaS_x;1 mol Cr(VI) this translates to 0.4 – 2.47 times the stoichiometric dose. In the current work, at 2-3 times the stoichiometric dose, removals were becoming clear, albeit not as drastic. In other research, again with regenerant brine, 100% of an initial 20 mg Cr(VI)/L was removed with as little as 1.9 mM $FeSO_4$, at pH 6.4 (De Araujo Silva 2018), which is only a 1.65 times the stoichiometric ratio. Clearly the current results are not as successful in chromium reduction as the previous references. The reasons will be discussed further below. Based on the use of 2X stoichiometric CaS_x and 10X stoichiometric $FeSO_4$, the required mass ratio of CaS_x to $FeSO_4$ for such treatment would be about 1 to 7.5.

To investigate the impact of solids on chromium removal, coagulation tests were done for QAL (with CaS_x) and UMCF (with $FeSO_4$). The chromium results, as well as data on solids' volume and weight are shown in Table 28. More difficulty in CaS_x sludge volume measurement was noted due to slower settling. To measure the weights of solids, all the contents of the batch tests were centrifuged at 3000 rpm for 10 minutes; the settled sludge was then moved to aluminum dishes to weigh. During transfer processes a small part of CaS_x sludge was lost.

Table 28: Effects of Solids on High Chromium Batch Tests in Groundwater (250 mL) Using CaS_x and $FeSO_4$

Conditions	Groundwater	Multiple of Stoichiometric Ratio	Coagulant Dose (mL/1000 L GW)	Final Total Cr (µg/L)	Solids	
					Volume (mL)	Weight (g)
CaS_x in QAL GW (Cr Initial = 10500 µg Cr^{+6}/L)	Filtered with Coffee Filter	5X	842	8920	<1	0.0237
		5X	842	8760	<2	0.0201
		10X	1682	8670	<3	0.0300
	1 g Soil Added to Filtered GW	5X	844	5740	4	0.2756
		5X	844	6230	4	0.2212
		10X	1682	5250	4	0.2559
$FeSO_4$ in UMCf GW (Cr Initial = 9800 µg Cr^{+6}/L)	Filtered with Coffee Filter	5X	2236	7720	1	0.0247
		5X	2236	7470	1	0.0154
		10X	4472	4660	4	0.0355
	1 g Soil Added to Filtered GW	5X	2236	4810	5	0.2851
		5X	2236	3740	5	0.2608
		10X	4472	2390	6	0.2925

Notes: In batches, one g/L of solids was added to the 250 mL of groundwater used

After settling for 10 minutes, the sludge volume was recorded

It is apparent from Table 28 that the addition of soil did improve total chromium removal but surprisingly, neither scenario showed very effective Cr removal results. Similarly, in the ferrous sulfate tests using UMCf, there was a total chromium removal improvement with added soil, but again, neither procedure was very effective. Interestingly, the QAL water, with its original solids, was treated more effectively than both the filtered QAL and the filtered QAL with added solids, implying that the type of solids influences the effects, as well. Previous research showed varying maximum Cr(III) sorption on different soils. Maximum sorbed Cr(III) amounts ranged from 101 to 431 mmol kg^{-1} for different soils, while roughly 63 mmol kg^{-1} was the maximum sorbed Cr(VI) amount, averaged over soils, for the tested conditions (Cifuentes, Lindemann, and

Barton 1996). Additionally, other research showed that nearly the entire Cr(III) in solution readily adsorbed onto a studied soil for given conditions (Nikagolla et al. 2013). This provides another mechanism for removal in the presence of soil: reduction/adsorption, which will be discussed further.

The next coagulant results investigated groundwater with low level chromium content. Results are shown in Table 29. Measurements for total dissolved chromium were done using ICP with unfiltered samples and are presented in place of Cr(VI) results. During the research, QAL groundwater was found to interfere with chromium readings using Hach colorimetric tests for Cr(VI). Dilutions of 100X were needed to address the interference. However, at low initial Cr(VI) concentration, this was not practical. Cr(VI) results have been omitted here and only total dissolved chromium results have been reported. No soil was added in UMCf batches.

Table 29: Low Cr(VI) Concentration Batch Tests Using CaS_x (27% by Wt.) and $FeSO_4$ (6%) in Groundwater (500 mL)

Coagulant	Multiple of Stoichiometric Ratio	Coagulant Dose	QAL (Initial Cr: 520 µg Cr^{+6}/L)	UMCf (Initial Cr: 550 µg Cr^{+6}/L)
		(mL/ 1000 L GW)	Final TDC (µg/L)	Final TDC (µg/L)
CaSx (27% by wt.)	2X	17	430	470
	2X	17	440	460
	3X	26	410	460
	3X	26	410	460
	5X	43	400	460
	5X	43	400	450
			Final TC (µg/L)	Final TDC (µg/L)
Ferrous Sulfate (6%)	10X	515	220	450
	10X	515	230	450
	25X	1278	180	430
	25X	1278	220	440
	50X	2575	160	420
	50X	2575	170	430

The data in Table 29 suggest that at these lower initial chromium concentrations, the removal with all the tested CaS_x doses was not very successful. This was observed with both groundwater types. On the other hand, for the ferrous sulfate results shown, while neither groundwater was treated very effectively, there was a noticeably better total chromium removal in the QAL water.

In these tests, CaS_x sludge did not settle well. Comparatively, the settlement of $FeSO_4$ sludge was faster and the sludge was fluffier. Possible scale formation was investigated in beakers and on stirrer blades after the completion of batch tests. Neither high nor low

concentration tests were seen to involve scaling, meaning that all the significant solids were either suspended, or had settled in the beakers.

Other results from analyses conducted in the final batch testing are presented below. Table 30 shows pH results.

Table 30: Batch Test pH Results, Using CaS_x (27%) and $FeSO_4$ (6%)

Conditions	Multiple of Stoichiometric Ratio	Calcium Polysulfide		Multiple of Stoichiometric Ratio	Ferrous Sulfate		Groundwater Treatment
		QAL	UMCf		QAL	UMCf	
High Cr (10,500 µg Cr^{+6}/L)	1.5X	8.01	7.78	5X	6.30	5.90	—
	1.5X	8.12	7.85	5X	6.15	6.10	—
	2X	8.04	7.81	10X	5.98	6.84	—
	2X	8.06	7.97	10X	6.28	6.25	—
	3X	8.18	7.92	20X	5.99	5.42	—
	3X	8.20	7.93	20X	6.01	5.50	—
	4X	8.26	7.88	30X	5.16	5.18	—
	5X	7.85	7.99	50X	8.08	8.10	—
	5X	8.21	8.03	50X	7.45	8.09	—
Low Cr (500 µg Cr^{+6}/L)	1.5X	7.60	7.52	5X	7.40	7.22	—
	1.5X	7.62	7.48	5X	7.44	7.32	—
	2X	7.60	7.37	10X	7.29	6.98	—
	2X	7.56	7.48	10X	7.32	7.08	—
	5X	7.59	7.41	50X	7.10	6.74	—
	5X	7.57	7.47	50X	7.14	6.75	—
High Cr	5X	8.29	—	—	—	6.98	[a]
	5X	8.30	—	—	—	6.28	[a]
	10X	8.22	—	—	—	6.42	[a]
	5X	8.12	—	—	—	6.81	[b]
	5X	8.10	—	—	—	5.98	[b]
	10X	8.06	—	—	—	6.62	[b]

[a] Filtered through coffee filter

[b] 1 g soil added to the filtered groundwater

The pH values ranged between 5.16 and 8.29. CaS_x testing had consistently higher pH values than those of $FeSO_4$. This was not surprising since CaS_x itself has a pH of 11.5 – 11.7 (Tessenderlo Kerley Inc. 2018). There was also no obvious change in pH when soil was added

to either groundwater. Clearly all CaS_x pH readings fell within the secondary MCL range of 6.5 – 8.5 (US EPA 2017c), while the $FeSO_4$ readings fell either within that range or below by up to more than one pH unit.

As previously mentioned, however, the pH values may have impacted the precipitation of Cr(III). In previous research using calcium polysulfide, pH ranging from 1.6 – 6.4 resulted in complete Cr(VI) reduction. By a pH of 10.3, the efficiency was down to 96%. The best pH range for total chromium removal was found to be 6.5 - 10.3 (with approximately 94% removal). It was noted that at pH less than 6.5 and greater than 10.3, total chromium removal is very poor. The removal efficiency goes down to 15% from 94% when pH goes to 4.5 from 6.5 (Pakzadeh and Batista 2011). Therefore, there should not have been reduction in efficiency for CaS_x samples in the current work. Again, in other research on Cr(VI) reduction using ferrous sulfate, 100% removal was found at pH values ranging from 3.5 to 7.2 and was still 99% at a pH of 9.6 (these were for 3.08 mM $FeSO_4$ additions). While it was Cr(VI) that was mentioned, the author cites other research stating that more than 96% of total chromium was removed for pH values ranging from 4 to 7.7 (De Araujo Silva, 2018). Therefore, pH should not have been a major factor in the $FeSO_4$ treatment process.

Table 31 presents turbidity data which helped to assess the quality of the coagulation process.

Table 31: Low 500 µg Cr^{+6}/L, Initially – Batch Tests Turbidity Results in Settled Supernatant, Using CaS_x (27%) and $FeSO_4$ (6%) as reductants

Calcium Polysulfide			Ferrous Sulfate		
Multiple of Stoichiometric Ratio	**QAL**	**UMCf**	**Multiple of Stoichiometric Ratio**	**QAL**	**UMCf**
1.5X	174	54	5X	201	28
1.5X	181	48	5X	216	31
2X	153	66	10X	178	87
2X	170	70	10X	217	79
5X	146	103	50X	284	139
5X	159	93	50X	200	169

It is clear from Table 31 that for both reductants, turbidity was consistently higher in QAL samples than in UMCf samples. UMCf samples seem to have higher turbidity at higher coagulant doses, but there does not appear to be a very obvious change in turbidity as coagulant doses are changed with QAL samples.

Finally, results for perchlorate concentrations are presented in Table 32.

Table 32: High Cr(VI) – 10,500 µg Cr^{+6}/L, Initially – Perchlorate Results, Using CaS_x (27%) and $FeSO_4$ (6%)

Calcium Polysulfide			Ferrous Sulfate		
Multiple of Stoichiometric Ratio	**Perchlorate (mg/L)**		**Multiple of Stoichiometric Ratio**	**Perchlorate (mg/L)**	
	QAL	**UMCf**		**QAL**	**UMCf**
2X	1266.12	1399.27	10X	1194.35	1342.04
5X	1212.15	1384.61	50X	1153.11	1326.04

From Table 32, it appears that increased reductant dose had little effect on measured perchlorate concentrations. This is not surprising since it has long been reported that perchlorate is not reduced or precipitated by common reducing agents or cations, respectively (Urbansky 1998).

4.4.2 Column Tests

4.4.2.1 Hexavalent Chromium

Figure 9 shows the grab sample Cr(VI) concentrations for QAL, UMCf-A and B columns. CaS_x was added on day 1, via column injection. Previous data are not shown.

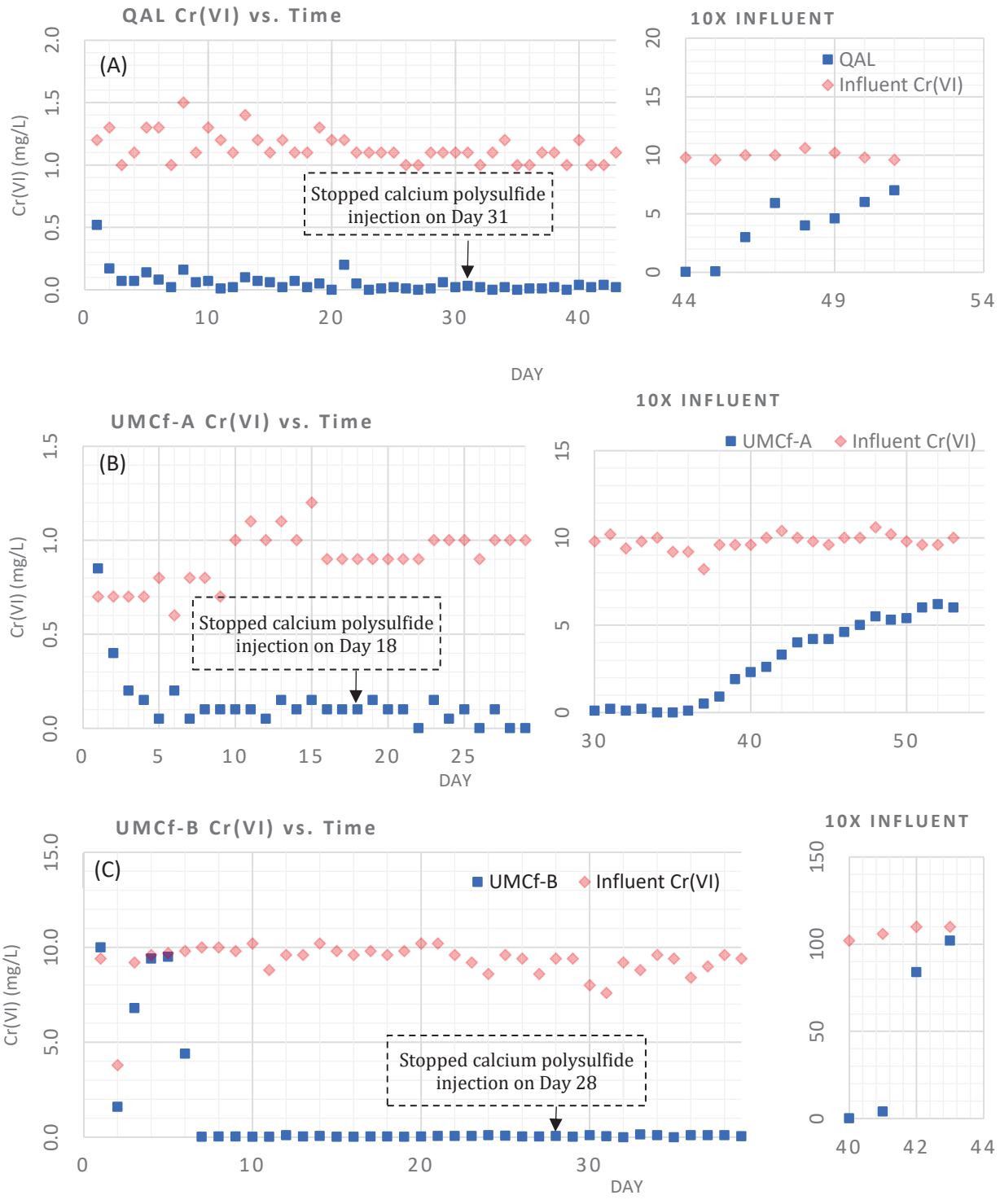
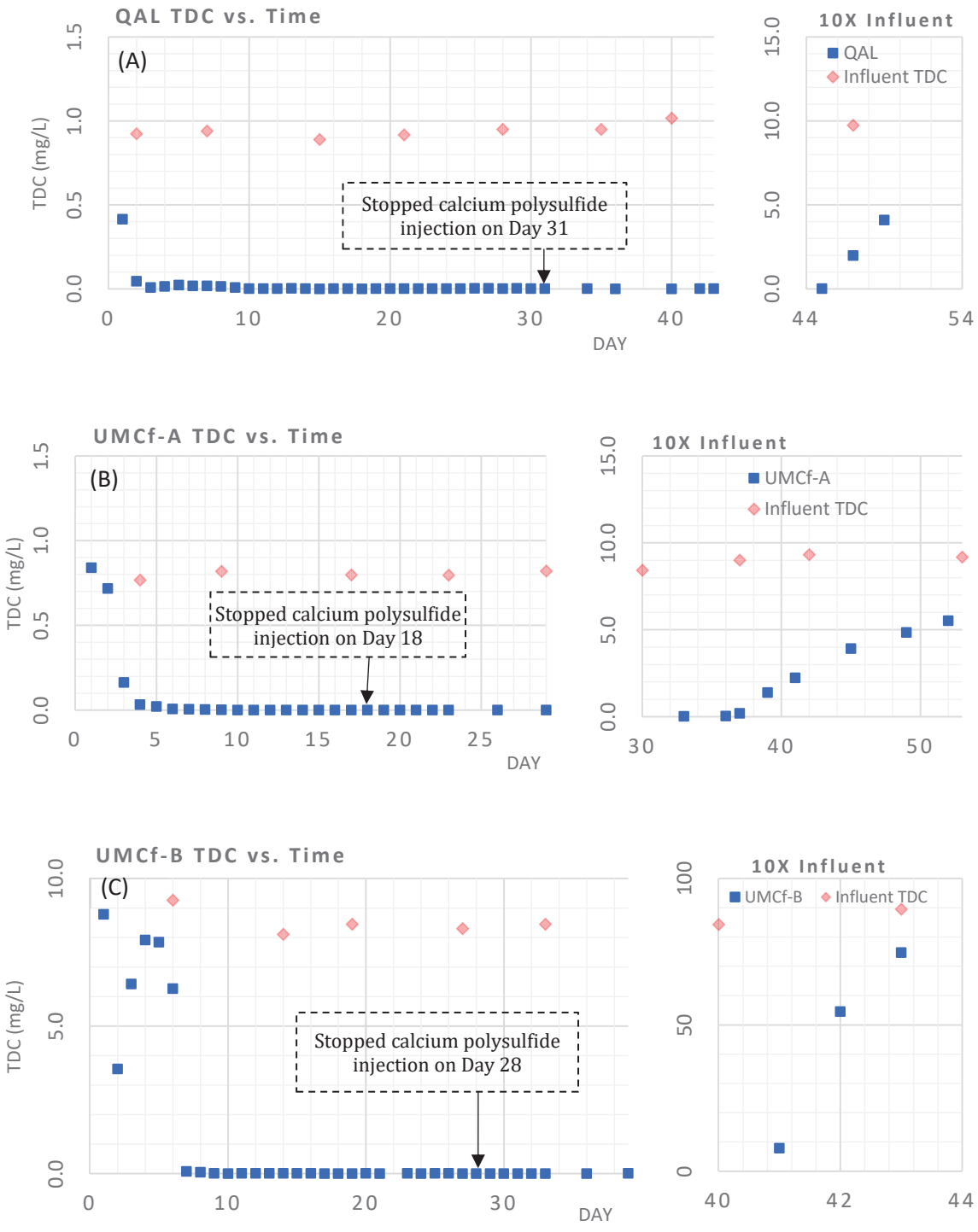


Figure 9: Influent and Effluent Cr(VI) Concentrations in Grab Samples (A) QAL (B) UMCf-A (C) UMCf-B

By day 4, the QAL and UMCf-A columns' effluent water had less than 100 µg/L Cr(VI). UMCf-B decreased dramatically by day 2 but rose again to influent levels when no CaS_x was added for three days. Upon regular addition of CaS_x on day 5, the reduction began and the effluent was at 20 µg/L of Cr(VI) by day 7 and remained very low. For each column, data points with complete removal were obtained. It is clear that for all of the columns, once treatment began, there was very effective removal of Cr(VI) in the tested effluent. After the cessation of CaS_x addition, Cr(VI) continued to be removed, even after increasing the influent concentration by an order of magnitude. It is likely that the increased stoichiometric dose, intended to be 20X and 40X more than the 2X stoichiometric dose from the jar testing (for low and high Cr(VI) concentrations, respectively), had an impact on the improved treatment when compared to the jar tests. In previous work, a dose of 12 times stoichiometric CaS_x applied to Cr(VI) contaminated soil had also been found to maintain its treatment ability over time (Chrysochoou, Johnston, and Dahal 2012). Given that, and the demonstrated ability of calcium polysulfide to reduce and stabilize Cr(VI) (Pakzadeh and Batista 2011; Zhang et al. 2018) these results were in line with expectations. The columns continued to reduce Cr(VI) after stopping CaS_x addition for 12 – 18 days and only began to break through when the Cr(VI) concentration was increased tenfold, in the case of UMCf-A, six days into the increase. The maintained treatment ability will be discussed further. This fact supports this treatment for in-situ remediation, as non-continuous well injections of CaS_x would appear likely to still be effective.

4.4.2.2 Total Dissolved Chromium (TDC)

Figure 10 shows the dissolved chromium in the columns. TDC measurements were made on composite samples that were not filtered but settled.



Note: Data points that were below the detection limit of o.oo1 mg/L were plotted as zero.

Figure 10: Total Dissolved Chromium Concentrations Measured in Settled, but Unfiltered Composite Samples in (A) QAL, (B) UMCf-A and (C) UMCf-B

Once again, the TDC reduction in all columns was clear and sustained over time. By day 3, the TDC effluent concentration for QAL was 7 µg/L, and by day 4 UMCf-A was 31 µg/L. UMCf-B reached 72 µg/L by day 7, in spite of the initial treatment discontinuity. Even after termination of the CaS_x addition, successful treatment continued for at least 11 to 18 days in the columns. In the case of UMCf-A there was still a 98% removal of TDC even 7 days into the concentration increase. These factors showed the resilience of the treatment process.

Although TDC was analyzed in composite samples as opposed to the grab sample Cr(VI) results, the low TDC concentrations (generally below Cr(VI) measurements) demonstrated that: (a) the low Cr(VI) concentrations likely did not result from colorimetric interferences; (b) Cr(VI) was not simply transformed into another soluble form of chromium but was either filtered out by the column or was found as precipitates in the effluent. Finding low TDC in the composite samples gave strong confidence in the absence of dissolved chromium species in the column effluents.

Based on Graham, et al., (2006), the proposed precipitates would have been iron chromium hydroxide $Cr_{(x)}Fe_{(1-x)}(OH)_3$ or chromium hydroxide, after Cr(VI) reduction to Cr(III) (Zhong et al. 2009).

$$2CrO_4^{2-} + 3CaS_5 + 10H^+ \rightarrow 2Cr(OH)_{3(S)} + 15S_{(S)} + 3Ca^{2+} + 2H_2O \text{ (Zhong et al. 2009)}$$

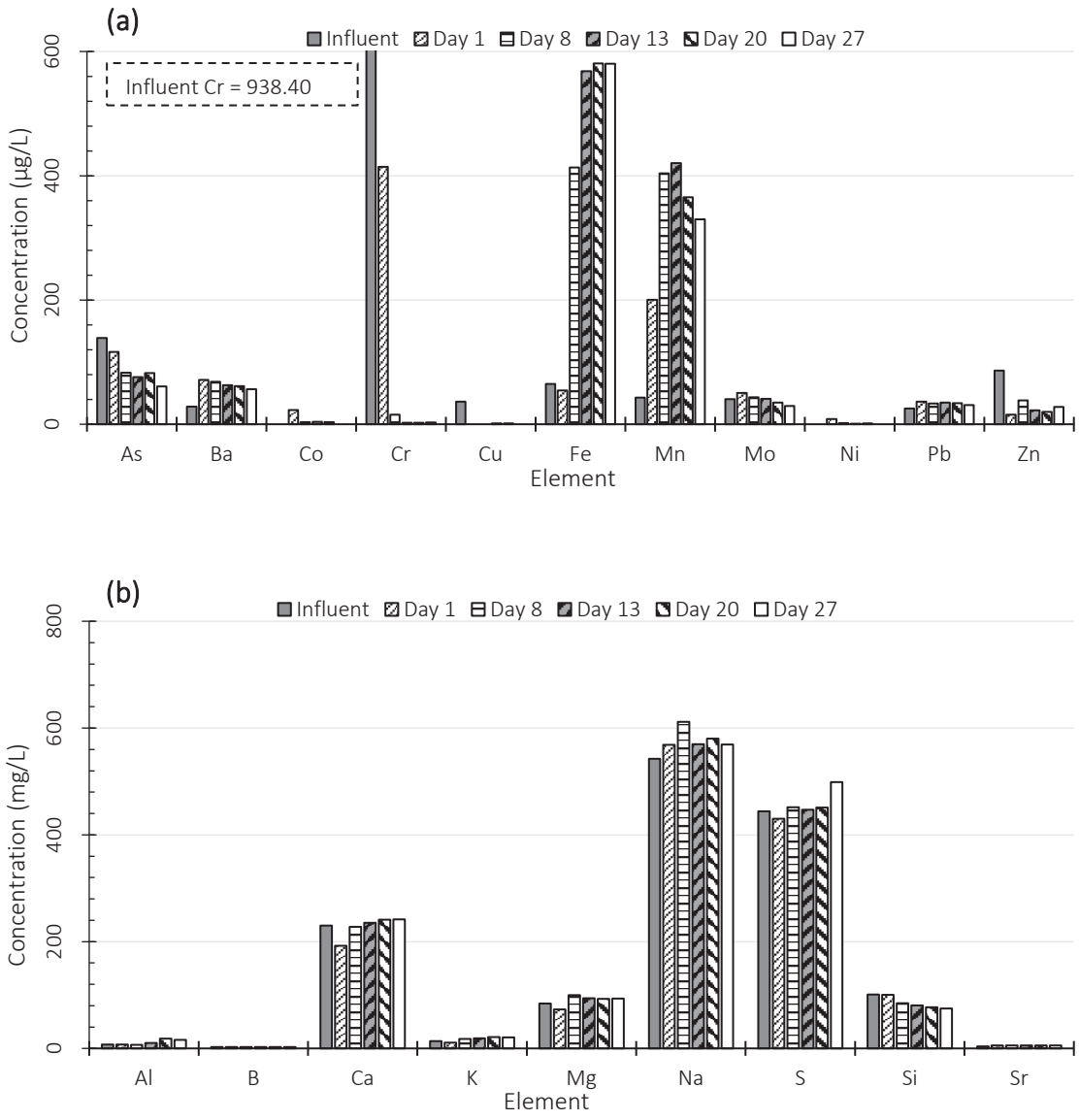
In addition to the increased stoichiometric doses used in the columns, adsorption and contact time are thought to have improved the TDC column treatment, as compared to the jar test. Contact time would provide for more Cr(VI) reduction, and Cr(III) species in solution

would have more opportunity to precipitate or adsorb to soil particles, and not be detected in the effluent.

Injection port and effluent white solids were observed, but not reported on. These may have included some chromium precipitates but were at least partially expected to have been some form of calcium precipitates as well. In either case, precipitation was in fact the treatment objective here, as it was the intended mechanism for Cr(VI) removal from the groundwater flows.

4.4.2.3 Metal Scans

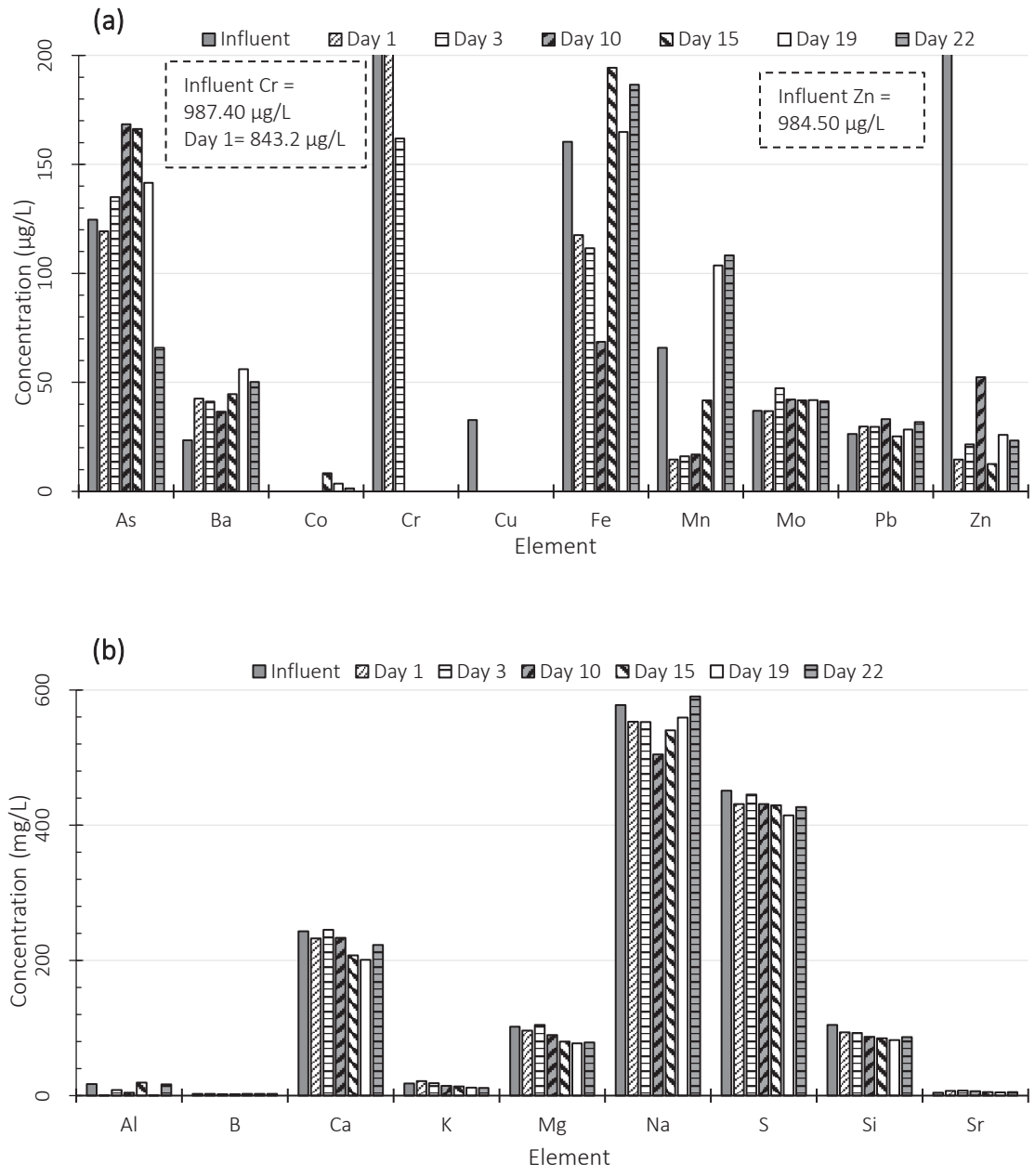
To investigate the potential issue of the solubilization of other toxic metals during treatment, some metal scans were performed using Inductively Coupled Plasma (Thermo ICP 6300). Figures 11-13 show the metal scan results for the QAL, UMCf-A and UMCf-B columns, respectively.



Note: Detection limits = 0.001 mg/L for As, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn, Al, B, Ca, Mg, Na, Si, and Sr; 0.005 mg/L for K and 0.01 mg/L for S

Figure 11: Total Metal Concentrations in QAL in (a) µg/L (b) mg/L.

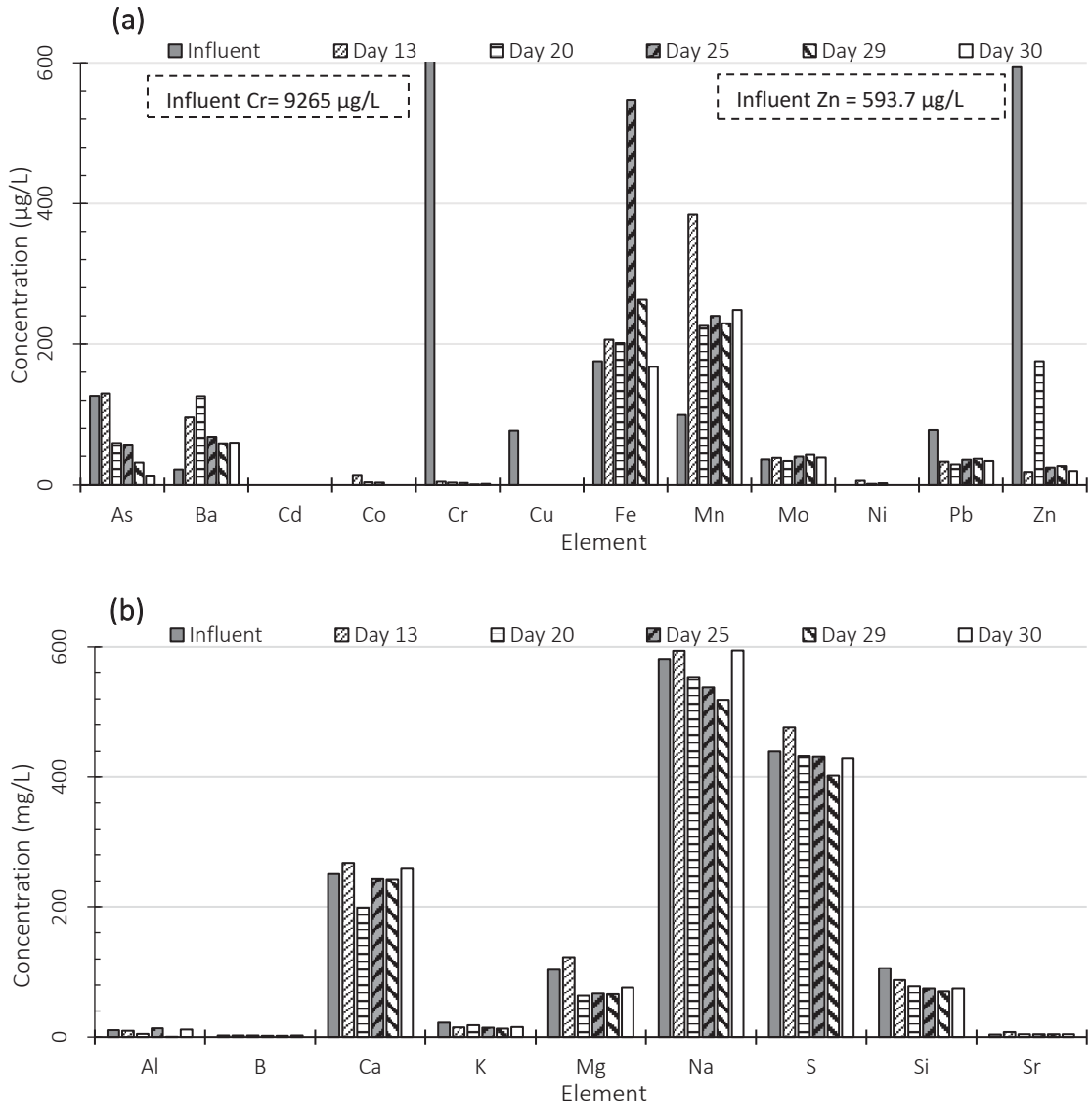
Apart from the obvious reduction in chromium over time, it was also noted that the QAL column showed slightly decreasing concentrations of arsenic and relatively steady concentrations of lead. This was reassuring since solubilization of these elements is undesirable and it would be a matter of concern if the effluent values were higher than the influent values (although the influent value may have varied over time). In these cases, the effluent values were lower or close to the influent values indicating that no obvious solubilization had occurred. There were notable general increases in iron and manganese showing that these elements were apparently leaching or reduced from the soil over time. Both of those have only secondary MCL levels of 300 µg/L for Fe and 50 µg/L for Mn, (US EPA 2017c), but those levels were surpassed.



Note: Detection limits = 0.001 mg/L for As, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn, Al, B, Ca, Mg, Na, Si, and Sr; 0.005 mg/L for K and 0.01 mg/L for S

Figure 12: Total Metal Concentrations in the UMCf-A in (a) µg/L (b) mg/L

For the UMCf-A column, chromium showed a large reduction over time, arsenic increased slightly then decreased, lead remained steady and again iron and manganese generally increased over time, but only Mn was ever above its MCL.



Note:

Detection limits = 0.001 mg/L for As, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn, Al, B, Ca, Mg, Na, Si, and Sr; 0.005 mg/L for K and 0.01 mg/L for S

Figure 13: Total Metal Concentrations in the UMCf-B in (a) µg/L (b) mg/L

For the UMCf-B column, chromium was always far below the influent value, as generally was zinc showing that some zinc treatment was evident. Arsenic decreased over time, lead remained stable and iron peaked to a level above its MCL, then decreased slightly. The solubilization of metals will be discussed further.

4.5 Analysis of Results

4.5.1 Stoichiometric Demand

This research investigated the treatment of groundwater contaminated with Cr(VI) using CaS_x and $FeSO_4$ as reducing agents. It was proposed that lower doses of CaS_x than of $FeSO_4$ would result in equal or lower chromium concentrations in treated water. The results have shown that with high initial chromium concentrations, far lower stoichiometric doses of CaS_x are needed compared to $FeSO_4$ (roughly 2-3 times stoichiometric for CaS_x and 10-20 times stoichiometric for $FeSO_4$). In term of mass demands of the reductants, this indicates that roughly 7.5-10 times more $FeSO_4$ was used, compared to CaS_x, for those doses. In reference, CaS_x is only about 6 times more expensive than $FeSO_4$ (Alibaba 2019a, 2019b), so the chemical cost of CaS_x would still be roughly equal or lower overall, in spite of its higher unit cost. Additionally, shipping of CaS_x would be more economical due to the smaller mass requirements. Even when densities are taken into account (Liquid CaS_x solution vs. solid $FeSO_4$) at least three times as much $FeSO_4$ must be transported (Alibaba 2019a, 2019b).

4.5.1.1 Ferrous Sulfate

In the current study, the pH values of the $FeSO_4$ samples were in the 5.4 - 8.1 range, so it does not appear that pH would have adversely affected treatment. Previous research found

strong reductions of Cr(VI) by Fe(II) in the pH range of 3 to 9 (Chen et al. 2015). In contrast to the 10 to 20 times stoichiometric doses of $FeSO_4$ found to be optimal for treatment of chromium at ~10 mg/L, near complete chromium removal was possible (Hwang et al. 2002) with slightly more than a 1 times stoichiometric dose. Those solutions had 2 mg/L of chromium and were deoxygenated. Other research agreed, showing that a stoichiometric dose (1 times the Fe:Cr ratio) of $FeSO_4$ resulted in a 97.5% removal of 0.52 mg/L Cr(VI) in capped experiments (Guan et al. 2011). In other research, 1 mg/L Cr(VI) was reduced using a 1 times stoichiometric dose of Fe(II) (Buerge and Hug 1997). In light of these previous findings, an explanation was sought for the higher $FeSO_4$ requirements in the current investigation.

The presence of other oxyanions or dissolved oxygen (DO) may have reduced the effectiveness of $FeSO_4$ reduction of Cr(VI). For example, previous research found slightly lower chromium removal effectiveness in the presence of a 1:1 molar ratio of arsenate to Cr(VI). Increasing the dose of Fe alleviated the problem, particularly at lower pH values (Guan et al. 2011). In the present work, arsenic was detected in groundwater samples, but in the 1-2 μM range, meaning that this was unlikely to have been a major factor.

Another possible interference was the reaction of Fe(II) with dissolved oxygen (DO) in the water, which has been found to potentially impact Cr(VI) reduction at pH values above 7 (similar to many of the current pH values, particularly in low Cr(VI) dose samples). In this reaction, DO can compete with Cr(VI) in oxidizing Fe(II). In that way, less Cr(VI) is reduced by Fe(II) (Qin et al. 2005). Researchers have also pointed to this competing side reaction as a concern (Guan et al. 2011). It has been stated that a moderately alkaline pH increased the rate of

competitive DO oxidation of Fe(II) and therefore decreased the reaction of Fe(II) with Cr(VI) (Kim et al. 2002). This is due to the fact that the concentration of the $Fe(OH)_2$ iron species rises steeply between pH 5 – 8 and that form of Fe(II) is much more readily oxidized than other forms (Morgan and Lahav 2007). DO oxidation of Fe(II) requires a 4:1 molar ratio of Fe(II):O_2 (Qin et al. 2005) which is equivalent to 7 mg Fe(II)/1 mg O_2. Given the open to the atmosphere conditions of the current tests, the roughly 25° C temperature, and the 600 m elevation, the approximate initial DO levels (not measured) of the tests would have been roughly 7.7 mg/L (Tchobanoglous et al. 2014), with potential for re-aeration. The presence of the initial DO alone could therefore have increased the stoichiometric requirement of Fe(II) by 1.7 to 33 times, for high and low Cr(VI) concentrations respectively. This provides an explanation for the need for higher $FeSO_4$ doses. Similar to the current findings, Qin et al (2005) showed that roughly 3.1 to 15.5 times the stoichiometric Fe(II) dose resulted in almost complete Cr(VI) reduction when DO was present at 3 to 4 mg/L (Qin et al. 2005). These findings also give additional insight into why similar stoichiometric doses of $FeSO_4$ were far less effective at low chromium concentrations than at high concentrations. $FeSO_4$ dosing was determined based on Cr(VI) concentrations and did not consider the presence of DO. DO concentrations should have been similar for all Cr concentrations. At lower Cr(VI) levels, less $FeSO_4$ was added and the DO would have caused more interference than at high Cr(VI) concentrations where more $FeSO_4$ was added. In support of that conclusion, it was previously found that at low Cr concentrations (40 µg/L), a 78 times stoichiometric Fe dose was used, under conditions where oxygen was not removed (Martin, Asghar, and Germain 2018).

4.5.1.2 Calcium Polysulfide

When CaS_x was used, pH levels were again in the optimal range for treatment. The pH values in the current analyzed CaS_x tests all fell within the range of 7.37 – 8.30. In previous research, when observed kinetic reaction rates were studied for Cr(VI) and calcium polysulfide in aerobic systems, the rates began to decrease at or below a pH of 6 – 6.5 (Chrysochoou and Ting 2011). Other research using calcium polysulfide also showed optimal total chromium removal in the pH range of 6.5 to 10.3 (Pakzadeh and Batista 2011), confirming that pH was at optimal levels.

This research demonstrated that a 2-3 times stoichiometric dose was required for treatment of ~10 mg/L Cr(VI), using CaS_x. Previous work suggested a 1.1 times stoichiometric dose for 67 mg/L Cr(VI) reduction (Graham et al. 2006), which agreed well with the 1 time stoichiometric dose previously suggested (Graham et al. 2006) and the 0.4 to 2.47 times stoichiometric doses found for Cr(VI) concentrations from 9 to 93 mg/L (Pakzadeh and Batista 2011). These previous results are very similar to the current findings. However, in the case of low initial Cr(VI) concentrations, there was once again unsatisfactory treatment even at up to 5 times stoichiometric CaS_x doses.

The higher CaS_x requirements at low Cr(VI) concentrations were thought to be due to the Cr(VI) concentration and the inhibitory presence of DO. It was also noted in previous work that higher initial Cr(VI) concentrations required lower calcium polysulfide/Cr(VI) molar ratios (Pakzadeh and Batista 2011). With respect to DO inhibition, it was previously found that DO

reaction with sulfide was the likely dominant influence in slowing the observed kinetic reaction rate of the calcium polysulfide reduction of Cr(VI) (Chrysochoou and Ting 2011).

4.5.1.3 Implications

This research provided chemical dosing information for Cr(VI) reduction in complex, contaminated groundwater using both $FeSO_4$ and $CaS_{x,}$. It also gave new insight into the impacts of dissolved oxygen and pH on Cr(VI) reduction, since the reactors were open and not deoxygenated. The adverse effect of dissolved oxygen on the reduction process, particularly at higher DO to reductant ratios in the close to neutral pH ranges have been demonstrated.

4.5.2 Reduction Rates

Determining the rates at which reductions take place is another approach to evaluating treatment results, with important design implications for reactor sizing or groundwater contact times. Figure 14 shows reduction rates calculated for the chemical jar tests. These rates were based on the change in chromium concentration and the approximate 41-minute contact time, from reductant addition through the settling of precipitates.

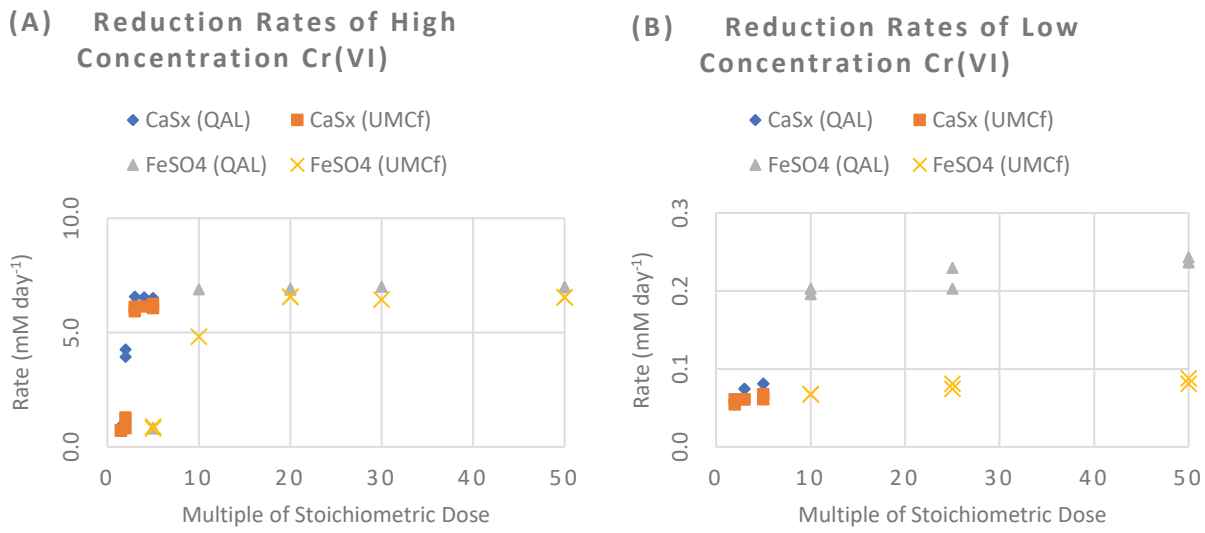


Figure 14: Chromium Reduction Rates Using CaS_x and $FeSO_4$ as Reductants at Various Stoichiometric Ratios (Fe:Cr and CaS_5:Cr) for Initial Cr(VI) Concentrations of (A) 10,000 µg/L and (B) 500 µg/L

4.5.2.1 Low Initial Cr(VI) Concentrations

The lowest rates for both reductants, 2.8 to 12.6 mg L^{-1} day^{-1} (0.05 to 0.24 mM day^{-1}) were found with low initial Cr(VI) concentrations. The low initial Cr(VI) concentrations were a major reason for these lower rates. The rates were simply calculated as $\frac{\Delta\, Concentration}{\Delta Time}$ and smaller concentration changes lead to smaller rates. In addition, as previously mentioned, DO was thought to have been responsible for the poor reduction of low Cr(VI) samples, since at low Cr(VI) concentrations, the smaller reductant doses were more likely to be overwhelmed by the presence of oxygen. In aerobic uncovered experiments with low initial Cr(VI) concentrations, similar rates have been found. For example, with 2 mg/L (40 µM) Cr(VI), reduction rates of

roughly 0.1 mM day^{-1} using calcium polysulfide were demonstrated (Chrysochoou and Ting 2011).

In the case of Fe(II), observed rates of 0.09 mM day^{-1} with effective removal; 0.0005 mM day^{-1} with ineffective removal for 0.1 mg/L (1.9 µM) Cr(VI) (Gröhlich et al. 2017) or 0.06 mM day^{-1} for 0.16 mg/L (3 µM) Cr(VI) (Kim et al. 2002) have been found. In other low Cr(VI) concentration research (0.52 mg/L or 10 µM), removal with ferrous sulfate showed much higher reduction rates of roughly 2.88 mM day^{-1} and 1.44 mM day^{-1}. While dissolved oxygen was not controlled in their work, the reaction flasks were capped (Guan et al. 2011), which may have limited oxygen presence/interference and explains the higher Cr(VI) reduction rates. In another anoxic experiment using Fe(II) at 2 mg/L (38 µM) Cr(VI) concentrations, a reduction rate of about 1 mM day^{-1} was found (Hwang et al. 2002). These rates indicate that less oxygen and increased Cr(VI) concentrations lead to increased reduction rates. The low reduction rates of the current work are very similar to these previous results and are thought to result from both low Cr(VI) concentrations and DO interference.

4.5.2.2 High Initial Cr(VI) Concentrations

The highest rates were observed for high initial chromium concentrations with large reductant doses and were generally in the 208 to 364 mg L^{-1} day^{-1} (4 to 7 mM day^{-1}) range. Compared to these high Cr(VI) concentration reduction rates, previous studies found an approximate 1.55 mM day^{-1} reduction rate of 13.4 mg/L (258 µM) Cr(VI) when using calcium polysulfide (Graham et al. 2006). Other work showed a reduction rate of 23 mM day^{-1} of 50 mg/L (960 µM) Cr(VI) using calcium polysulfide (Anunike 2015). For ferrous sulfate reduction,

a roughly 17 - 33 mM day^{-1} total chromium reduction rate for 17 mg/L (327 μM) Cr was found (Chang 2003). These values conform reasonably well with the current rates.

4.5.3 Influence of Solids

Another key factor in the reduction of Cr(VI) was found to be the presence of solids, which improved treatment results. Previous work has shown that Cr(VI) adsorption to goethite, found in soil environments, led to higher observed kinetic rates of reduction between Cr(VI) and calcium polysulfide (Chrysochoou & Ting, 2011). At low pH values (up to pH 6), previous research showed that clay minerals could catalyze the reduction of Cr(VI) by various organic compounds (reductants). This activity decreased at higher pH values. Although more slowly, the minerals could even directly reduce Cr(VI). Reactive moieties (such as Fe sites) were responsible for the reduction (Deng et al. 2003). Other researchers found a positive relationship between the reduction of Cr(VI) in soils and the contents of organic matter, dissolved organic matter, Fe(II) as well as the clay fraction and bacterial community (Xiao et al. 2012). Clearly the first four of those parameters are probable abiotic influences in the current work. In the present research, sorption and catalytically increased reduction rates are plausible mechanisms for the improved chromium removal found in the presence of solids. Although the reduction mechanisms were not investigated, this work contributed additional data regarding the beneficial effects of solids/soils on the reduction of Cr(VI).

4.5.4 Maintained Reducing Conditions

The current column results have shown reduction of Cr(VI) in groundwater for different influent chromium concentrations. The effectiveness of calcium polysulfide for the stabilization

of Cr(VI) contaminated soil has been well documented (Chrysochoou et al. 2012; Zhang et al. 2018). In addition to effective treatment, dissolved chromium removal was maintained even after the addition of CaS_x was discontinued. Extended calcium polysulfide reduction periods have been previously demonstrated (Jagupilla 2011). This has significant implications for in-situ treatment applications, since intermittent CaS_x additions would still be expected to continuously treat contaminated groundwater. The continued treatment is postulated to have resulted from the presence of reductants which built up in the columns during the course of the CaS_x addition. Chrysochoou and Johnston (2015) investigated the prolonged reducing conditions of Cr(VI) contaminated soil treated with calcium polysulfide. The treated soil was found to have elemental sulfur and thiosulfate present. It was stated that soluble thiosulfate, can sorb, for example, to iron oxides and thus be retained in the solid phase. It was noted that chromate is not reduced by thiosulfate, however, Fe(III) can be. The presence of Fe(II) is another potential contributor to maintained reducing conditions (Chrysochoou and Johnston 2015). In other research, even though ferrous sulfate was more effective at Cr(VI) reduction, sodium thiosulfate was also found to be effective for reducing Cr(VI) to Cr(III) in artificial soil (Kostarelos et al. 2009). The presence of reduced iron, elemental sulfur and thiosulfate are all likely to have contributed to reducing conditions in the current columns and the continued reduction of Cr(VI) after CaS_x additions had ended.

4.5.5 Mobilization of Other Metals

Finally, the analysis of other metals in the column effluents showed that in general there were increases in Fe and Mn. These results are in line with previous effective attempts at Cr(VI) treatment of soil, also using calcium polysulfide, which resulted in some increased Fe and Mn

levels but below detection level Pb concentrations (Chrysochoou et al., 2012). Other research has shown aquifer mobilization of As, Fe and Mn (U.S. DOE 2008). In general, the current work resulted in fairly steady levels of Pb and decreasing As levels (although in one column levels increased). Arsenic solubilization was a particular concern, since As is least soluble when it is in its most oxidized state. Therefore, as it is reduced from As(V) to As(III), its solubility increases. Because of its high pH and reducing ability, CaS_x is known to make As more mobile (US EPA, 2018). The data presented here show that for two out of three of the tested scenarios, the leaching of As does not appear to be a major concern, although caution is recommended based on specific site and treatment conditions.

4.6 Conclusions

The purpose of this work was to investigate and compare the application of calcium polysulfide and of ferrous sulfate for Cr(VI) reduction, and to apply the selected reductant in a simulated in-situ treatment test. Jar tests were conducted using calcium polysulfide and ferrous sulfate, and laboratory scale columns were investigated with CaS_x as the reductant. Testing was conducted at high chromium concentrations (roughly 10 mg Cr(VI)/L) and low concentrations (roughly 0.5 or 1 mg Cr(VI)/L).

4.6.1 Summary and Implications

Overall, this research has shown that with high initial chromium concentrations, $FeSO_4$ doses of 10 – 50 times the stoichiometric dose resulted in reduction rates only slightly higher than for CaS_x in the 3-5 times stoichiometric range, thereby highlighting the greater efficacy of CaS_x. Measured reduction rates calculated matched expected literature values quite well. The

required stoichiometric doses and resulting reduction rates provided additional insight into the effects of Cr(VI) concentrations, as well as potential interferences from other compounds, such as DO on treatment economics. Clearly the smaller doses of CaS_x would result in lower chemical and transportation costs. Since smaller CaS_x doses still resulted in similar reduction rates as $FeSO_4$, the required reactor sizes (or groundwater contact times) in the field would still be similar for both reductants. Investigations of sludge production also showed CaS_x treatment produces similar or smaller amounts of sludge compared to $FeSO_4$, so landfilling requirements have also been considered (data shown above). Based on the Cr(VI) concentrations in the field and for the given groundwater matrix, specific reductant stoichiometries may be selected to provide adequate reduction rates. For example, if a specific contact time is available for treatment (based on groundwater flow conditions), it would be necessary to ensure that the reduction reactions will have adequate time to take place. The current results will also provide the fundamental data for such determinations.

4.6.2 Major Conclusions

The following conclusions were drawn from the jar testing:

1. TDC in high Cr(VI) samples was reduced noticeably at 2X – 3X stoichiometric doses for CaS_x and 10X – 20X for ferrous sulfate (5X was the previous increment). Even at the highest doses (5X and 50X for CaS_x and $FeSO_4$, respectively), TDC still remained but was lower with $FeSO_4$.
2. Based on treatment using 2X and 10X stoichiometric doses for CaS_x and $FeSO_4$, respectively, about 7.5 times more mass of $FeSO_4$ would be used.

3. TDC removal was very poor for low initial Cr(VI) concentrations, apparently due to dissolved oxygen interference. DO is thought to have competed against Cr(VI) and oxidized the Fe(II) and sulfide reductants.
4. The presence of solids in the water had a positive impact on TDC reduction, but the type of solids (naturally occurring or added soil) also played a role. The mechanisms for the increased removal are thought to be sorption and catalytically increased reduction rates.

The column investigation lead to the following conclusions:

1. Soon after the start of CaS_x addition, all columns resulted in dramatic and sustained reduction of Cr(VI) and TDC in effluent samples.
2. Even after CaS_x was no longer being added, treatment continued in the columns until an increased Cr(VI) influent dose led to contaminant breakthrough. The presence of reduced iron, elemental sulfur and thiosulfate in the columns are thought to have led to these reducing conditions.
3. None of the three columns investigated showed increased values of effluent lead in the water, only one exhibited a modest peak of arsenic, while the other columns had slight decreases over time.

In summary, for jar tests, Cr(VI) was removed by both CaS_x and $FeSO_4$. Reduction was far superior for high Cr(VI) concentrations. Some results showed appreciable Cr(VI) treatment at 2X – 3X CaS_x stoichiometric doses and 10X $FeSO_4$ stoichiometric doses. At those doses, a 7.5 times greater mass of $FeSO_4$ is required, compared to cascade. CaS_x at higher doses was then used in the chemical Cr(VI) column reduction studies that followed, to simulate in-situ field

treatment. At these higher doses, strong and sustained Cr(VI) and total dissolved chromium removal were observed. The improved treatment may have been based on the increased doses, contact time and soil sorption potential. The treatment also demonstrated resilience as it continued even after the cessation of CaS_x addition.

This research has added to the body of knowledge on calcium polysulfide reductant doses and their effects on Cr(VI) reduction. It has also shown that the presence of dissolved oxygen and solids has influence on the effectiveness of treatment. Furthermore, the types of solids present can result in important effects on treatment, as well. Finally, this research has demonstrated that for a complex contaminated groundwater, calcium polysulfide appears to be a very capable and resilient reductant for in-situ Cr(VI) reduction applications.

CHAPTER 5

BIODEGRADATION OF PERCHLORATE IN THE PRESENCE OF CO-OCCURRING OXYANIONS

5.1 Abstract

Perchlorate is a contaminant of concern in groundwater and poses human health risks through its interference with iodine uptake. In some groundwaters, perchlorate may occur together with several-co-occurring oxyanions. This research investigated the simultaneous reductive treatment of perchlorate, as well as chlorate, chromate (Cr(VI)) and nitrate (which were also present) using various organic substrates. Such water matrices can pose treatment difficulties, as oxyanions may interfere or compete for microbial reduction. Microcosm and column studies were implemented using actual contaminated groundwater and soil. It was found that degradation of all oxyanions was possible and the reduction generally followed the order: Cr(VI) > Nitrate > Chlorate > Perchlorate. There were however clear exceptions and differences in the order of reduction when using different substrates and soil/groundwater. The investigated organic substrates have proved effective for reducing all the oxyanions except perchlorate. Although in general a reduction order was observed, there was no universal characterization of reduction order. Different substrates and soil/groundwaters lead to different results and varying treatment sequences.

Keywords: Perchlorate, Biodegradation, Chlorate competition, Hexavalent chromium

5.2 Introduction

The presence of contaminated sites is a concern in the United States and around the world. There are estimated to be roughly more than 126,000 facilities or contaminated sites in the U.S. which have not reached closure, with a completion cost estimated between $110 and $127 billion. A number of facilities significantly threaten or impact systems of public water supply (National Research Council 2013). Oxyanions are a class of compounds that must be addressed. There has been wide detection of nitrate (NO_3^--N) and hexavalent chromium (Cr(VI)) in groundwater in recent decades (Xia et al. 2013), and groundwaters and surface waters the world over have shown high concentrations of perchlorate (ClO_4^-) and chlorate (ClO_3^-) (Logan 1998). Septic systems and fertilizers are primary sources of groundwater nitrate, which in excess in drinking water, can cause methemoglobinemia (McCasland et al. 2012). Stainless steel production, electroplating, wood preservation and textile manufacturing all utilize chromium compounds, like Cr(VI). Inhalation of Cr(VI) has been demonstrated as a cause of lung cancer and ingestion of a Cr(VI) containing compound has been shown to be carcinogenic in laboratory animals (U.S. Department of Health and Human Services 2018). Shallow groundwater can potentially contain perchlorates and chlorates, because perchlorate is possibly present as fertilizer impurity (Mastrocicco et al. 2017). The aerospace and military industries as well as pyrotechnic applications and herbicides are among the areas of chlorate and perchlorate use. Perchlorate and chlorate can reach drinking water. As a result of their very high solubilities, they undergo ready transport in water systems. Thyroid hormone production is affected by perchlorate ingestion, since it could affect the thyroid's iodine uptake (Harris-hellal et al. 2013) and a link has also been found between drinking water chlorate exposure and different congenital anomalies (Righi

et al. 2012). In the US, perchlorate contamination threatens the Colorado River, the water source for over 30 million people.

Nitrate, chromate (CrO_4^{-2}) (occurring as hexavalent chromium), perchlorate and chlorate are all oxidized compounds. All four of these compounds have been the subject of biological reductive treatment (Garbisu et al. 1998; Hunter 2002; Wang, Baltzis, and Lewandowski 1995; Zhu et al. 2016a). The products of chlorate and perchlorate bio-reduction can be chloride (Hunter 2002), nitrate bio-reduction, N_2 and N_2O gasses (Warneke et al. 2011) and Cr(VI) bio reduction, Cr(III) (Garbisu et al. 1998). Chloride is an innocuous product (Hunter 2002), nitrogen gas is harmless (Los Alamos National Laboratory 1999) and trivalent chromium is less toxic than hexavalent chromium (Garbisu et al. 1998). As water matrices become more complex, so too can treatment process design and operation. For example, biological chlorate reduction can be competitively inhibited by nitrate (Hunter 2002). In other research it was shown that although multiple oxidized contaminants accepted electrons, there was found to be an electron accepting order, such that nitrate accepted electrons prior to other compounds, including hexavalent chromium (Xia et al. 2013). Such interactions can inhibit or delay the treatment of target compounds in water sources.

Understanding these interferences and interactions is key to both the successful treatment of multiple co-occurring oxyanions, as well as the appropriate use of added substrates, since in some cases an initial non-target oxyanion may require reduction prior to the treatment of a subsequent target oxyanion. Research involving bio-reduction has indicated that multiple oxidized contaminants can be simultaneously reduced successfully, for example nitrate, nitrite,

chlorate and perchlorate (Chung et al. 2007). To the knowledge of the authors of this work, however, the current combination of oxidized compounds has not been concurrently well investigated. The goal is to investigate the biological reduction of nitrate, chromate, perchlorate and chlorate and to evaluate the interactions and sequence of the reductions.

5.3 Experimental

5.3.1 Biological Reduction Tests

Testing was performed to determine whether the contaminants of the groundwater, (i.e. chromate, perchlorate, nitrate, chlorate), all of which are electron acceptors to bacteria, could be reduced biologically, if an electron acceptor were provided. The electron donors used in the microcosm testing were: EOS-PRO, industrial sugar wastewater, and molasses, (with COD values of 2,000,000, 99,440, and 1,053,000 mg/L, respectively). Microcosms were initially tested and the information from that stage was used in the column testing, to simulate in-situ groundwater treatment. In the columns, only EOS-PRO and industrial sugar wastewater were used as substrates.

5.3.1.1 Microcosm Tests Using Multiple Organic Substrates

Microcosms

A total of 128 microcosms were prepared in this experiment. The microcosms included the microcosms of the selected substrate and each one's controls (blanks and no phosphate nutrient). The carbon substrates were: EOS-PRO, Industrial sugar wastewater (ISW), EOS-PRO + ISW, and Molasses. Borosilicate glass bottles (125 mL), sterilized by autoclave, were used for all the microcosm tests. For all tests, wet soil (30 grams) was placed in each bottle. Then added

were the desired substrate amount, groundwater and nutrients, with a total combined volume of 100 mL. Considering the concentrations of chlorate, chromium, nitrate and perchlorate, a volume of substrate to achieve one hundred times the stoichiometric demand was added. To provide conditions that were anaerobic/anoxic, aluminum rings and butyl rubber caps were used to crimp close the microcosm bottles.

Sixteen microcosms/replicates were prepared with 60mL EOS-PRO/L of GW, 16 were prepared with 50mL ISW + 40mL EOS-PRO/L of GW, 16 were prepared with 60mL ISW/L of GW, 8 were prepared with 40mL (molasses) + phosphate/L of GW, 4 blanks with no substrate were prepared, 4 were prepared which had molasses without phosphate, and 4 ISW without phosphate. Phosphate was added as a nutrient to molasses and ISW microcosms, increasing dilution, so EOS-PRO and mix microcosms received 15% and 10% by volume additions of DI water.

All microcosms were mixed continuously at 30 rpm on a rotary shaker, at room temperature. The tests were all done in duplicate. At certain time intervals, bottles and duplicates were sacrificed and desired analyses were performed, unless specified otherwise. For a minimum of 6-8 hours after microcosm removal from the rotor, solids were left to settle. It was essential to allow for settling since the soil was very fine in nature. Membrane filters, 0.2 µm (Paul Laboratories), were used to filter the decanted liquid. Chlorate, COD (a surrogate test for the organic substrate content of the microcosms), Cr(VI), nitrate and perchlorate were analyzed in the filtered samples. Some sample microcosms were sampled and placed back on

the rotors for later re-sampling. Generally, however, the contents of the sacrificed bottles were centrifuged, then filtered, and analyzed for the concerning contaminants.

On days 7 and 11, the day 3 bottle was resampled, so the COD of the microcosm was not measured. From day 19, each bottle was not sacrificed as before; after that, previously sampled microcosms were tested for COD. Twenty mL of liquid would be removed, and the bottle would be returned to the shaker for later sampling. When there was no observed significant degradation, resampling was done. This was to maintain enough samples for longer incubation. With continuous sampling, EOS-PRO as well as possibly other organics are lost and there were lower COD levels in second re-samplings of microcosms. To sample, bottles were left to sit 8 hours to allow fines to settle prior to removing the top clear liquid. The loss of oil resulted from the removal (with the liquid) of the oil film which formed at the top during settling. Resampling of the same microcosms was done on days: 3, 7, 14, 19, 26, 44, 50, 71, 82, 92, 99.

Addition of Nutrients

Since the phosphorous content of the molasses and ISW microcosms was not adequate to support microbial growth, phosphorous (phosphate buffer) was added to these microcosms. EOS-PRO already contains phosphate, so none was added. Since there was nitrate in significant amounts in the contaminated groundwater and soils used (a potential bacterial nitrogen source), no nitrogen source was added initially. Upon degradation of nitrate, however, in some microcosms, nitrogen and phosphorous were supplied by adding di-ammonium phosphate.

Control Microcosms

The three control microcosm types introduced to investigate growth vs. non-growth conditions were: (1) Blank: no carbon substrate addition, (2) ISW with no phosphate, (3) Molasses with no phosphate. The blank microcosms were to test for the biodegradation rates without external carbon substrate/electron donor, while the introduction of microcosm controls without phosphate were to investigate phosphate's impact on oxyanion biodegradation.

Microbial Characterization

Microcosm content, roughly 30 mL, was shipped overnight to Research and Testing Laboratories (Lubbock, TX), using autoclaved containers for analysis of bacterial communities. The total numbers of archaea, bacteria and Cr(VI) reducing bacteria were evaluated.

The process used to determine the total numbers of microorganisms was as follows: DNA was extracted from the present microorganisms utilizing Illumina next-technology (Anon 2015), which uses clonal amplification as well as sequencing via synthesis. Specific for archaea and bacteria, 515F-806R was the initial primer chosen for the study. Based on Somenahally et al. (2013), 27YMF and 534 R were the primers for the chromium reducing bacteria. After the generation of sequences, data examination was done for short, singleton, noisy and bad read sequences removal. A 4% divergence was used to cluster the quality-checked sequences utilizing USEARCH clustering algorithm (Anon 2015). A National Center of Biotechnology Information (NCBI) derived in-house-maintained database was used to identify the obtained

sequences. Each organism's percentages, up to species level identification, were included in the final results that were obtained.

Analyses

Chlorate, COD, Cr(VI), nitrate, perchlorate as well as phosphate were measured in microcosms, using various methods, discussed below. Since the ion-chromatograph method used was found to have poor chlorate detection, some samples were sent to Silver State Labs (Las Vegas, NV) for chlorate degradation checks. Nitric acid (pH = 2) was used to preserve TDC ICP analysis samples. Ion chromatography was used to measure nitrate concentrations in molasses microcosms, since the molasses color would interfere with the colorimetric method.

5.3.1.2 Column Investigations

Column Layout

Four columns were built to investigate oxyanion bio-reduction at varying flowrates (Figure 15). Two columns (UMCf-A and UMCf-B) were packed with dried deeper clay-like UMCf soil and two (QAL-A and QAL-B) were packed with shallower alluvial soil. The columns were 1.27 m long and 5.08 cm in diameter. Table 33 shows the columns' soil characteristics.

Table 33: Soil Parameters Used in the Columns Packed for Biological Reduction Investigations

Parameter, Units	Soil Type	
	QAL	UMCf
Volume of column occupied by soil (excluding the top cover), cm^3	2329	2329
Weight of soil used in column, g	2213	3026
Bulk density of dry QAL and UMCf, g/cm^3	1.7	1.55
Volume of solids only in the soil (estimated based on bulk density), cm^3	1301	1952
Estimated porosity of the column (%)	44	16

Field porosity values varied from: 35-61% (QAL) and 51-66.8% (UMCf).

The soils which were dried, were saturated with electron donor/nutrient free groundwater prior to the biodegradation testing. The columns were fed actual groundwater from a contaminated site in downflow mode. Given the fine nature of the UMCf material, pressure was needed to achieve reasonable flowrates. A pressurization of 15 psi (103.4 kPa) was used during saturation (UMCf columns) and was then lowered to 10 psi (68.9 kPa) for the start of column operation. Pressurization was achieved by using a booster pump (Aquatec CDP6800) fitted with an in-house built pressure regulator. The QAL columns were initially gravity fed. Due to significant flow decrease, QAL columns were then pressurized after day 28. When QAL columns were also pump pressurized, the gravity influent tank was replaced with an analogous pump configuration. Although the pressures were intended to be 10 psi (68.9 kPa) and 5 psi (34.5 kPa) for the UMCf and QAL columns, respectively, when regulators were in use, there were sometimes variations due to system clogging and regulator adjustments, etc. so cleaning and adjustment were needed.

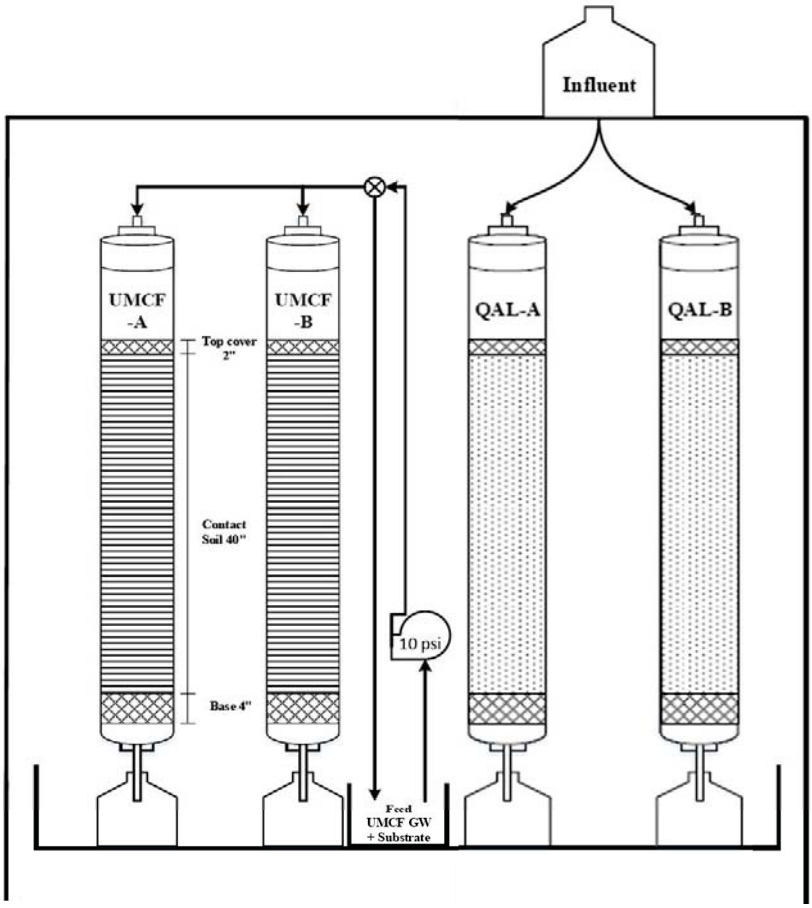


Figure 15: Initial Column Layout for Biological Reduction Tests, QAL, Columns are Gravity fed (day 1-28). After day 28, QAL Gravity Feed was Replaced by an Analogous Pump Pressure System.

Testing Procedures

Table 34 shows column operation details. Of note are the changing feed compositions over time.

Table 34: Biological Reduction Column Operation Information

QAL		UMCf	
Days	**Feed Variation**	**Days**	**Feed Variation**
1-2	7% ISW and 2% EOS-PRO in 91% GW (45880 mg/L COD equivalent)	1-7	7% ISW and 2% EOS-PRO in 91% GW 45880 mg/L COD equivalent)
3-5	Dilution of the previous feed by GW	8-10	Dilution of the previous feed by GW
6-8	GW only	11-13	GW only
9-14	7% ISW and 2% EOS-PRO in 91% GW (45880 mg/L COD equivalent)	14-19	7% ISW and 2% EOS-PRO in 91% GW 45880 mg/L COD equivalent)
15-17	0.2% EOS-PRO in 99.8% GW (4000 mg/L COD equivalent)	20-31	0.2% EOS-PRO in 99.8% GW (4000 mg/L COD equivalent)
18-29	GW only		
30-36	0.4% EOS-PRO (8000 mg/L COD equivalent)	32-40	0.4% EOS-PRO in 99.6% GW (8000 mg/L COD equivalent)
37-160	1.5% ISW and 0.4% EOS-PRO and 1.9% Phosphate in 96.2% GW (9260 mg/L COD equivalent)	41-165	1.5% ISW and 0.4% EOS-PRO and 1.9% Phosphate in 96.2% GW (9260 mg/L COD equivalent)

One QAL column had issues with clogging. Effluent valve cleaning and five days of operation with only groundwater were used to resolve the issue. Therefore, there was a five-day offset between UMCf and QAL column initial substrate addition. Shallower groundwater (Well C-S) was used for the alluvial soil and deeper groundwater (Well C-D) was used for the clay-like soil. Groundwater was used as collected. The substrates used for both soil types were either ISW, EOS-PRO, both or neither. They were mixed with the groundwater in the influent feed tanks and fed downflow through the column tops. Initially the feed was not prepared daily, but was later, to minimize degradation in the influent tanks. Ice packs were generally used and changed daily, to minimize degradation in the influent tanks and sample collection vessels. Initially, there was no addition of extra phosphate; later, 120 mg/L as PO_4 was added.

Analyses of Column Influent and Effluent/Data Collection

Sampling was conducted daily. Parameters tested included, chlorate, Cr(VI), nitrate, perchlorate, phosphate. Data was also collected on flowrate, and throughput volume. Where identified, data from total collection volumes below 80 mL were not included. Composite samples were used for the total dissolved chromium (TDC) measurements and Cr(VI) measurements utilized grab samples. Filtered samples were used for Cr(VI) measurements, and settled (but not filtered) effluent samples were used for TDC measurements. Although duplicates of each column were run, data were plotted for each column as single point results. However, error bars were included for replicate analyses.

5.3.1.3 Analytical Methods

COD, phosphate and Cr(VI) were measured using Hach Methods 8000, 8048 and 8023, respectively. Nitrate was measured with Hach Method 10020 (Test 'N Tube™ Vials). Chlorate/perchlorate were analyzed using ion chromatography (Dionex ICS-2000) and total dissolved chromium was measured using inductively coupled plasma (Thermo ICP 6300).

5.4 Results and Discussion

5.4.1 Microcosm Study

5.4.1.1 Chromium

Figure 16 shows the concentrations of Cr(VI) over time in the microcosms.

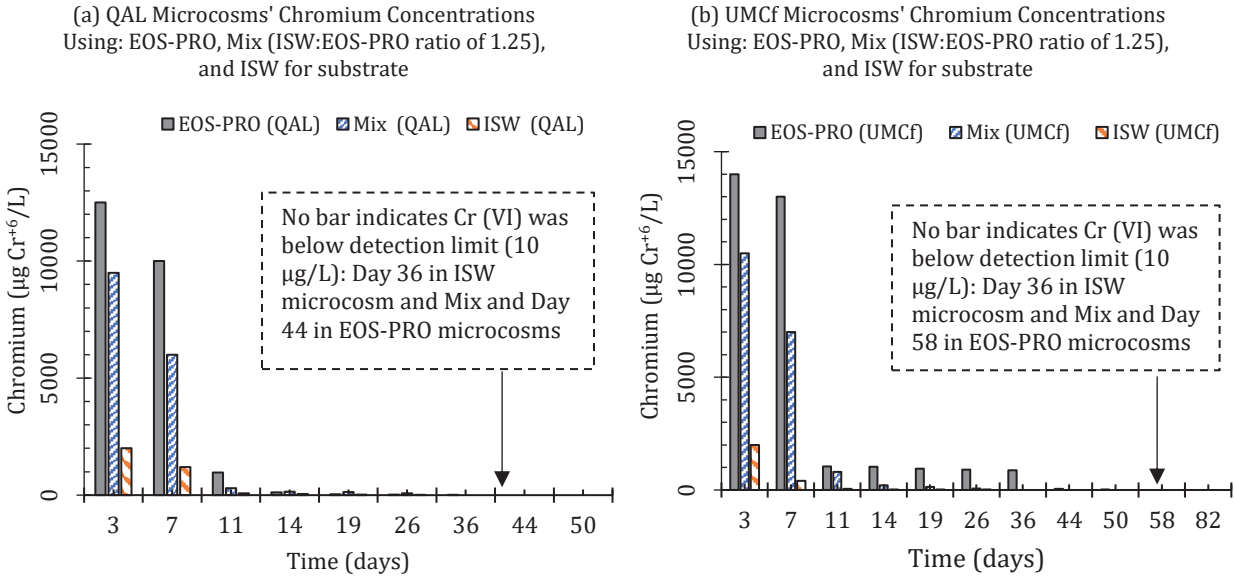


Figure 16: Cr(VI) Concentrations with Different Substrates (a) QAL (b) UMCf

Figure 16 shows that there is a dramatic decrease in Cr(VI) for all substrates, although ISW, followed by "Mix" are shown to have the most immediate declines. This study did not investigate the possibility that the Cr(VI) reduction was abiotic rather than, or in addition to, biotic. A later investigation (not presented here) showed that ISW could abiotically reduce Cr(VI) in groundwaters by 52% (UMCf) and 59% (QAL) after 4 days. Abiotic reduction of Cr(VI) to Cr(III) has previously been indicated, using sugarcane molasses (Chen et al. 2015) as well. It is expected, therefore, that a significant portion of ISW related Cr(VI) reduction was abiotic. A further discussion on abiotic reduction also follows. The Cr(VI) reduction results for microcosms containing a substrate of molasses are shown in Figure 17. Again, Cr(VI) reduction with time was evident.

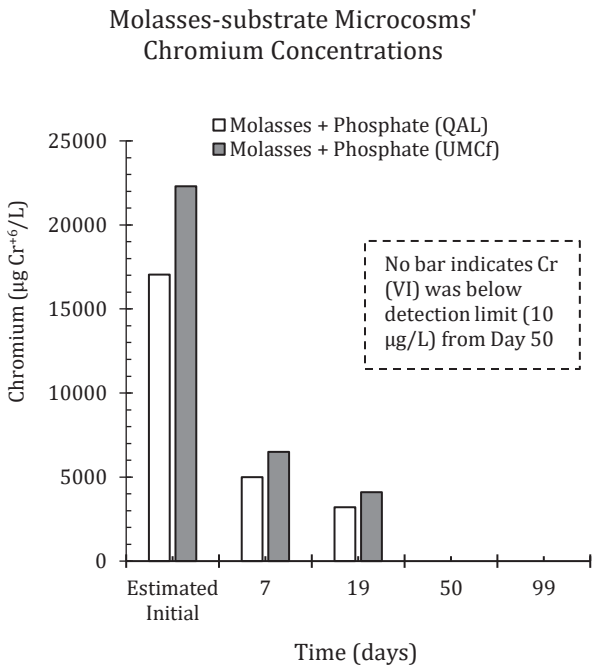


Figure 17: Cr(VI) Concentrations (Molasses with Phosphate)

With these microcosms, significant gas was generated indicating biological reduction, however, there was also no evaluation done on abiotic reduction. For blanks (data not shown), when no substrate was added, there was little change in chromium concentration from day 7 to day 99 and much chromium remained at day 99. For the ISW microcosms with no added phosphate, degradation over time was observed but chromium remained after 99 days; this was also true for the molasses phosphate blanks. Therefore, even if chemical chromium reduction were occurring, total reduction was not taking place without added phosphate, which again implies a biological mechanism, at least in part. As shown later, the COD results for those two phosphate free mixes

showed that organics were present to allow for further chemical reduction, which evidently had not taken place.

5.4.1.2 Nitrate

Figure 18 shows nitrate results for the various added substrates with the two groundwaters/soils.

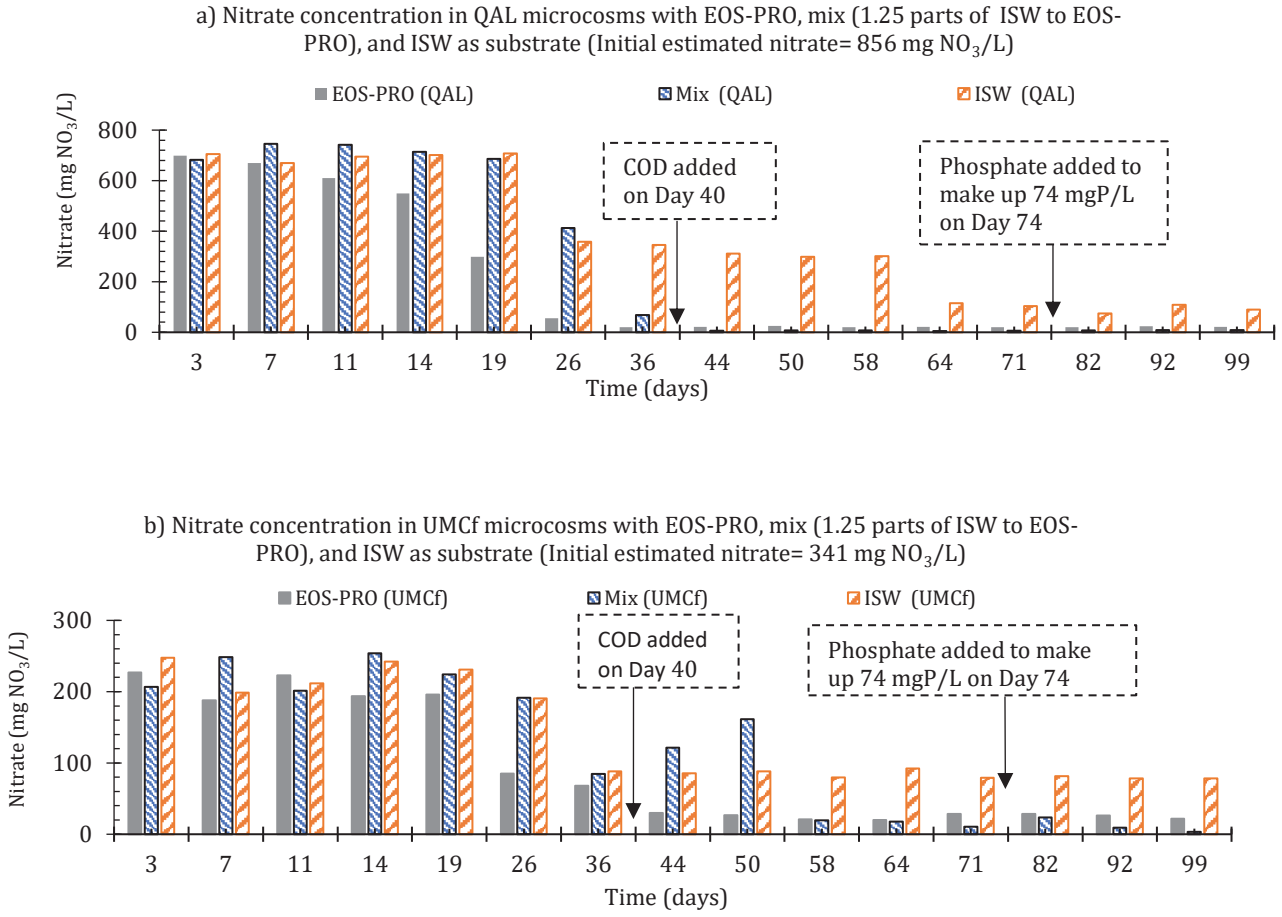


Figure 18: Nitrate Concentrations with Different Substrates (a) QAL (b) UMCf

For QAL groundwater, Figure 18(a) shows that EOS-PRO microcosms had very little nitrate after 36 days. Mix microcosms took slightly longer to degrade and ISW microcosms still had significant nitrate remaining after even 99 days. ISW microcosms also showed the poorest reduction results over time for UMCf groundwater, however, in this case after even 82 days, all mixes still had noticeable nitrate levels. The main nitrate degradation appeared to start after day 14 or 19 in QAL (depending on the substrate) and day 19 or 26 in UMCf. Chromium was mostly gone by day 14 (except for EOS-PRO UMCf samples). That shows that Cr(VI) seems to degrade prior to nitrate, in apparent contrast to findings which placed nitrate as a preceding electron acceptor to Cr(VI) (Xia et al. 2013). There was also a noticeable spike in nitrate after day 36 for the mix microcosms (Figure 18b) which could be due to substrate addition, although no such increase was present in the other microcosms at that time.

Figure 19 shows nitrate results for the molasses microcosms.

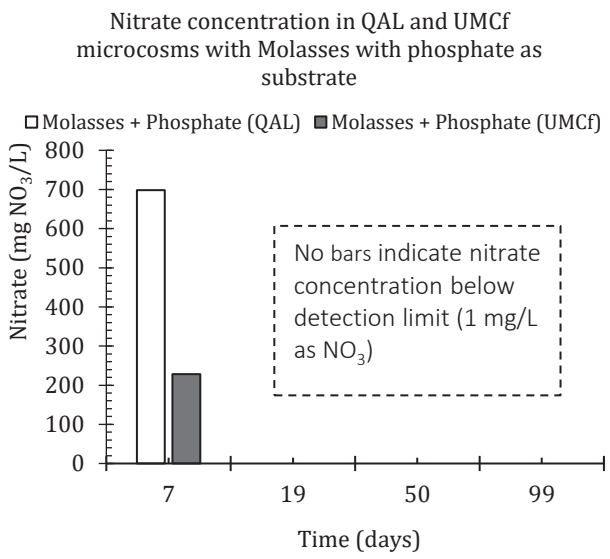


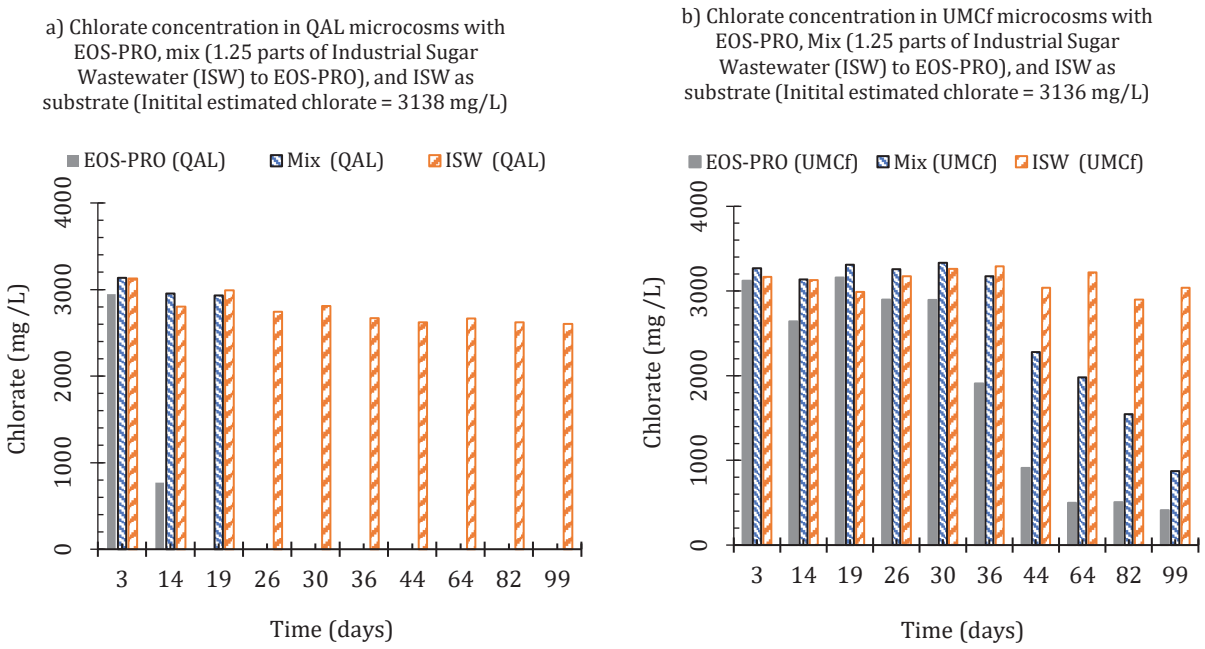
Figure 19: Nitrate Concentration (Molasses with Phosphate) Measured by IC

Clearly after 19 days, no nitrate was detected for either groundwater, indicating effective treatment and concurrent or, in this case, even preferential treatment to chromium. This indicates that the reaction order depends also on the type of organic substrate provided.

The blanks (data not shown) indicated no significant/real nitrate degradation without added organics, since there were relatively minor concentration changes over time. The molasses and ISW blanks with no added phosphate were also found to have no real degradation of nitrate by day 99, again supporting a biological reduction mechanism for those two substrates.

5.4.1.3 Chlorate

Figure 20 shows the results of chlorate concentration for the microcosms over time.



Note: Detection limit = 5 mg/L

Figure 20: Chlorate Concentrations with Different Substrates (a) QAL (b) UMCf

As shown in Figure 20, EOS-PRO QAL samples showed complete chlorate degradation by day 19 followed next by a complete and precipitous reduction by day 26 for Mix samples. ISW microcosms were highly ineffective at reducing chlorate. In UMCf microcosms, the order of substrate efficiency was analogous to QAL samples, but none of the microcosms performed very well, with all still containing significant chlorate after 99 days. Interestingly, in the case of EOS-PRO microcosms, for example, the degradation of chromium, nitrate and chlorate was concurrent for QAL samples, but the degradation of chlorate took longer to begin than the other

two contaminants in UMCf samples. This indicated that the water matrix can have unforeseen effects on degradation sequence, as well.

Figure 21 shows the chlorate results for the molasses microcosms.

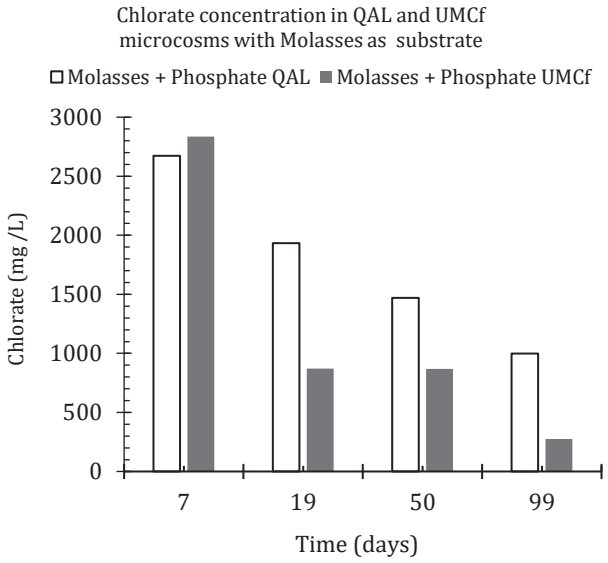


Figure 21: Chlorate Concentrations (Molasses with Phosphate)

In stark contrast to the effective nitrate treatment, very slow degradation was seen of chlorate in both groundwater types, but in this case the UMCf microcosms were apparently treated more effectively. Even after 99 days, however, chlorate was still present in samples.

5.4.1.4 Perchlorate

In perchlorate data (not shown), very modest degradation was observed. There was a 17-20% decrease in EOS-PRO microcosms after day 82 with both groundwaters. Although roughly complete perchlorate reduction was found in previous research during *Dechlorospirillum* sp. DB growth, probable inhibition by chlorate was noted, since perchlorate reduction did not take place until much of the chlorate was reduced (Prata 2007). Again, additional discussion on this topic follows. In the current study, perchlorate reduction was clearly even less favorable since, for example, in the case of QAL and EOS-PRO, chlorate was absent from the effluent after only 19 days.

5.4.1.5 Nutrient and Carbon Source/Electron Donor Evaluation

Chemical Oxygen Demand

COD is an indirect measurement of the organic substrate of the feed and effluent groundwater. The three organic substrates were used to promote heterotrophic bacterial growth. They served as both a source of carbon and a source of energy (i.e. electron donor) to the biological reduction process. The COD was monitored to ensure that the biodegradation of the oxyanions of concern was not limited by the lack of carbon source/electron donor. In the case of microcosms containing EOS-PRO, adsorption of oil to the soil phase is suggested (Sarria Cortes 2016) while the other two carbon substrates were expected to stay in the liquid phase. The initial COD incorporated in all microcosms was estimated as 12,000 mg/L (EOS-PRO, ISW, or a mixture). COD values over time are shown in Figure 22. The day 3 values above 12,000 mg/L are proposed to have resulted from the dissolution of biodegradable solids in the ISW.

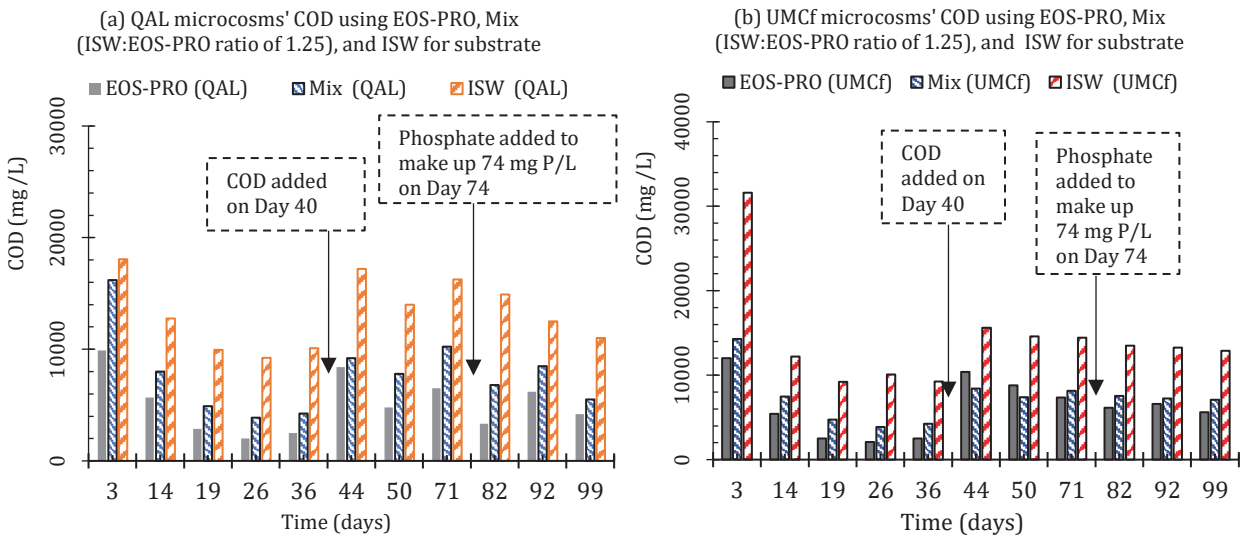


Figure 22: COD Concentrations Remaining in Microcosms with Different Substrates (Filtered Samples), (a) QAL (b) UMCf

COD concentrations decreased with time in the microcosms indicating carbon substrate use. After a noticeable trend of decrease of COD readings in the samples by day 36, additional COD was injected on day 40. The EOS-PRO fed microcosms had smaller COD concentrations in solution than the other substrates, probably due to sorption of oil to the soil. The other substrates are not expected to sorb much to soils. All the microcosms contained more than enough COD to promote biodegradation.

Considering the degradation of COD in QAL over time (day 3 to day 36), the following values were determined: 224 mg L^{-1} day^{-1}, 362 mg L^{-1} day^{-1}, and 241 mg L^{-1} day^{-1}, for EOS-PRO, Mix and ISW, respectively, indicating the steepest degradation for Mix microcosms. During that time period in the QAL EOS-PRO microcosms, roughly 0.38 mg L^{-1} day^{-1} of Cr(VI),

20.6 mg L^{-1} day^{-1} of NO_3^- and 28.7 mg L^{-1} day^{-1} of ClO_3^- were degraded. Prata (2007) reported a roughly 17 mM decrease in acetate over the 9 hours (2677 mg acetate L^{-1} day^{-1} or 2539 mg COD L^{-1} day^{-1}) that it took for reductions of about 5 mM ClO_3^- (1113 mg L^{-1} day^{-1}) and 5 mM ClO_4^- (1326 mg L^{-1} day^{-1}), with *Dechlorospirillum* sp. DB (Prata 2007). The complexity of the water matrix made comparisons difficult, but that was an approximately 10-fold increase in substrate utilization rate, compared to the current work, but far higher reduction rates were also taking place.

Figure 23 displays the COD in microcosms with molasses.

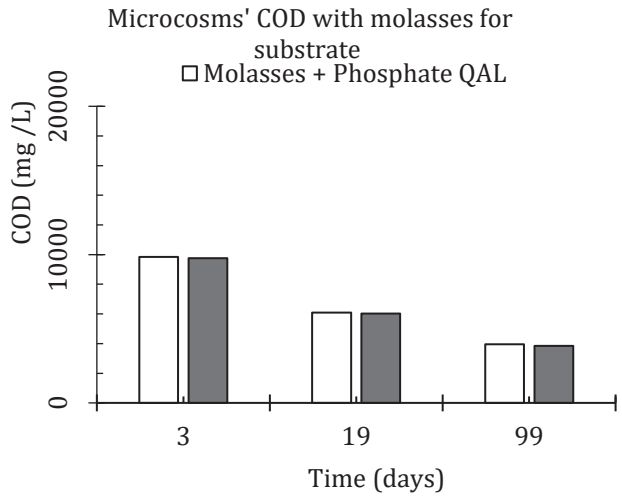


Figure 23: COD Concentrations of Filtered Samples (Molasses with Phosphate)

Again, it is clear from Figure 23 that COD decreased with time, as was expected.

In data from the blanks that are not shown, those with no added substrate had COD values below 180 mg/L, and there was no obvious decrease between 3 and 99 days. The COD in blanks, with ISW but no added phosphate decreased, over that period, from approximately 10,000 mg/L, but remained above 8600 mg/L. Those in molasses microcosms without phosphate, however, dropped from over 11,000 mg/L, yet remained over 10,400 mg/L. Figure 23, which shows that molasses microcosms with added phosphate had larger losses in COD content, lends support to a biodegradation mechanism in the case of molasses microcosms' COD loss, since at least there was biological growth occurring and some electron acceptors would be required.

Phosphate

Notably, ISW and EOS-PRO contain 51.2 and 72 mg P/L, respectively, therefore phosphate was added to ISW and molasses microcosms. Figure 24 shows microcosm phosphate results. Since the day 3 phosphate was higher than anticipated, and similar to the COD value trend, it was proposed that dissolution of ISW juice pulp increased those concentrations. As can be seen in Figure 24, there was, in general, a decline in phosphate concentrations over 68 days, with some unexplained increases. More phosphate was added on day 74 to prevent phosphate limiting conditions. The goal was a concentration of 70 mg P/L. However, it was detected that a higher dose was mistakenly added in the EOS-PRO UMCf, but phosphate limiting conditions were nonetheless averted.

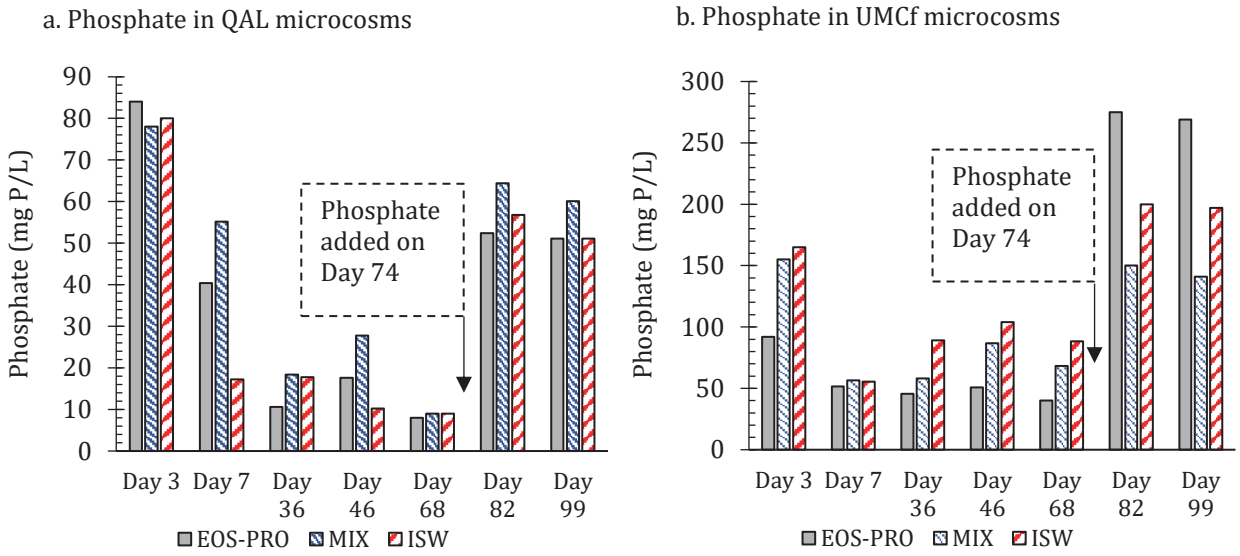


Figure 24: Phosphate Concentrations with Different Substrates (a) QAL (b) UMCf

5.4.1.6 Microbial Genera

The most predominant genera of bacteria were found to be *Pseudomonas* sp., which were also the largest component of the chromium reducing communities. This was not surprising since the site soil has long been contaminated with Cr(VI) and previous research reported that *Pseudomonas* strains showed both tolerance and reduction capacity for Cr(VI) (Wani and Ayoola 2015). More data on the microorganisms present and their percent distribution may be found in Appendix B.

5.4.2 Biodegradation Using Columns

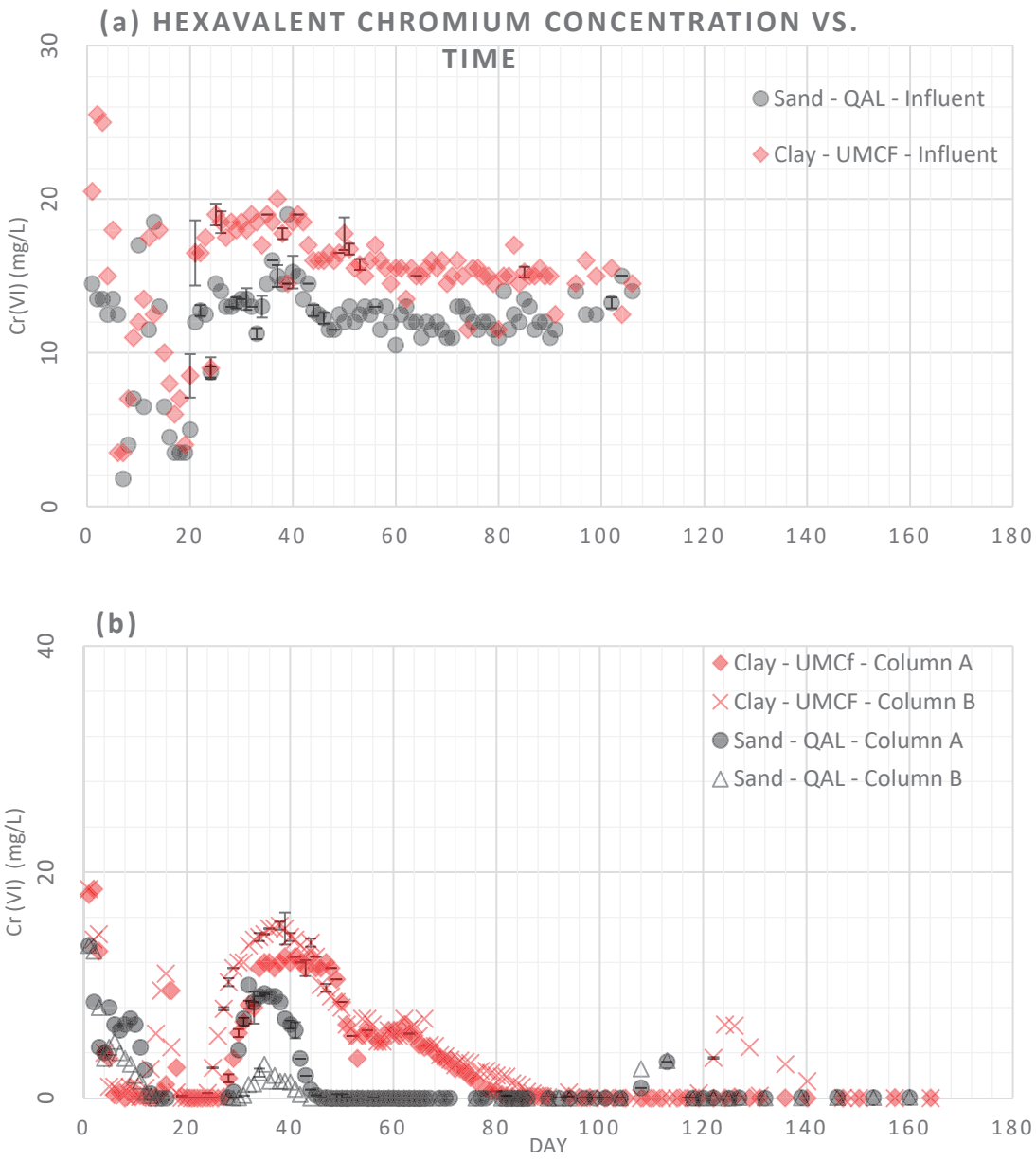
During the biodegradation column testing, days 105 and 110 both experienced two-hour column disturbances resulting from power outages. High effluent flow resulted after some cracks were observed in both QAL column packed materials, on day 106. There was no maintenance of constant flow until day 113. The UMCf-B column material was observed to have small cracks on it on day 120. Although the soil was very fine, the UMCf columns ran more smoothly compared to the QAL columns.

5.4.2.1 Hexavalent Chromium

It is important to note that there was variation in influent chromium concentration, due to varied collection times and wells. Figure 25 shows the Cr(VI) concentrations for the columns. The initial concentration fluctuations seen in the influent are somewhat reflected in the effluent. Once the influent was stabilized, close to day 20, very low UMCf effluent values were observed. From day 18-29, the QAL columns were receiving no substrate and a subsequent peak in Cr(VI) was observed. A low substrate dose in the UMCf columns also preceded effluent peaks. With a steady, mid-range, COD dose, all columns began to show steady decreases in effluent Cr(VI), with QAL showing a more rapid decrease. They stayed very low, until QAL spikes on days 108 and 113, which were presumed to be due to the power outage and crack formation in the columns. On day 106, subsequent to the power failure, no cracks were evident in UMCf A, but on day 116 a small crack was observed in UMCf B. There was a spike in chromium concentration on day 120 and after, in UMCf B, which may be related to the cracking. Due to

column pressurization and the clayey soil, small cracks slowly closed following re-pressurization.

In other column study research, Cr(VI) reduction to Cr(III) was accomplished very effectively by *Shewanella oneidensis* MR-1 in the 0.31 to 2.85 mg/L range. Cr(VI) was proposed to have inhibitory effects on microbial activity, however (Alam et al. 2006). These effects did not seem present in the current research at the given Cr(VI) concentrations, where reductions from roughly 12 to 15 mg/L of Cr(VI) to below detection limits (10 µg/L) were successful.



Notes:

- The average influent Cr(VI) concentration in the columns was: 14261 ± 1987 µg/L (Days 1-36) and 12377 ±997 µg/L (days 37-160) for QAL and 17385± 1829 µg/L (days 1-40) and 15360 ±1325 µg/L (days 41-165) for UMCf
- Influent Cr(VI) concentrations were lower than actual groundwater concentrations, probably due to chemical/biological reduction in the feed tank. In the influent that contained ISW, abiotic reduction may have occurred since ISW has such potential. Those influent readings are not considered for statistical analysis. During the first two weeks of operation, the influent feeds were surrounded with ice packs, but they were insufficient to maintain the desired 4^0C temperature. The ice was later increased, and the influent was changed more regularly to maintain consistent concentrations.

Figure 25: Column Cr(VI) Concentrations (a) Influent (b) Effluent

The Cr(VI) data were also plotted vs. Empty Bed Contact Time (EBCT) for stabilized data (day 40 onwards) and are shown in Figure 33 of Appendix A. It was shown that Cr(VI) reduction was generally improved with increased contact time. For the QAL columns, 2-5 days EBCT were adequate for total Cr(VI) removal. For the UMCf columns, EBCTs greater than 15 days resulted in very high Cr(VI) removals.

5.4.2.2 Total Dissolved Chromium (TDC)

Figure 26 shows TDC for the columns. There were two interesting observations. First, the trends were similar to the Cr(VI) data, but the peak concentrations were slightly lower. This could indicate either some interference with the Cr(VI) results causing slightly higher readings, or that chromium continued to be reduced and precipitate after sampling, in spite of the low pH preservation effort. Otherwise, the differences could have just resulted from differences between grab and composite samples. Second, the TDC influent readings did not fluctuate early on, as the Cr(VI) measurements, but that could be due to the smaller number of included data points missing those fluctuations.

After influent stabilization just after day 20, for TDC, and consistent mid-level organic substrate feed concentration starting around day 40, consistent TDC reduction was seen in QAL effluent by day 49 followed by a steady decrease in UMCf columns from just after day 40 until day 83.

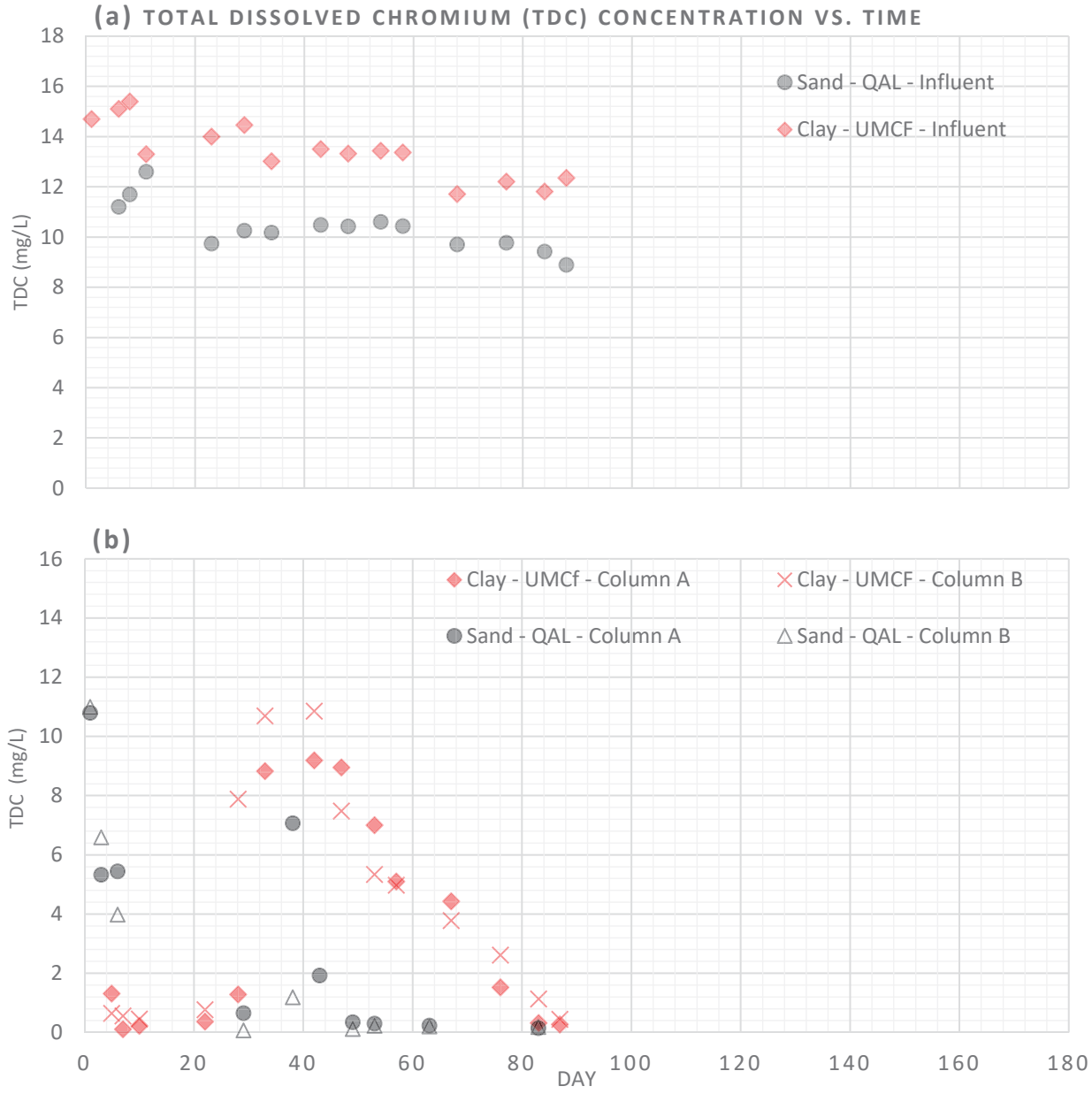
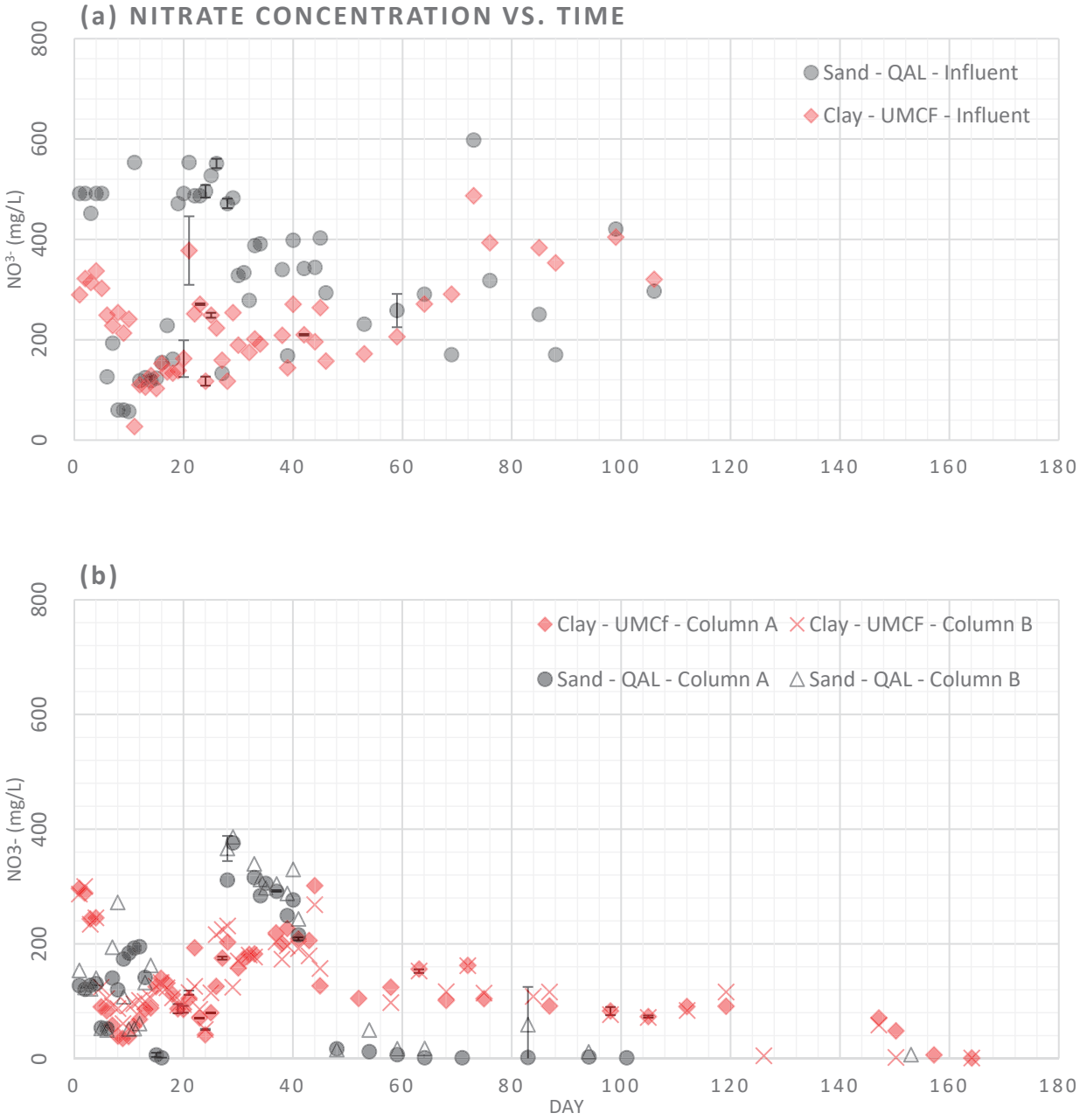


Figure 26: Column TDC concentrations (a) Influent (b) Effluent

5.4.2.3 Nitrate

The column nitrate concentrations are shown in Figure 27. As previously mentioned, the 150 mg/L as NO_3 initial QAL concentrations of influent and effluent were less than the 400 mg/L as NO_3 observed in groundwater. The lower values for the first days were probably due to feed bottle degradation. After initial instability, a peak was seen in the QAL effluent nitrate values (first data point on day 28) that occurred when the Cr(VI) values were just beginning to increase, which would indicate Cr(VI) preference. However, nitrate values began to decrease slightly before Cr(VI) values, in contradiction of Cr(VI) suppression. Starting on day 48, there was approximately complete nitrate reduction in the QAL data. The UMCf columns also had a peak of nitrate which coincided with the Cr(VI) peak but the nitrate took far longer to be removed, even once Cr(VI) was being reduced effectively.

Assuming some stabilization of influent took place just after day 20 and consistent organic feed stared at around day 40, full nitrate reduction followed quickly in the QAL columns and took more than 60 extra days in the UMCf columns, as per the reported data.



Note: Three data points excluded due to high standard deviation between duplicates

Figure 27: Column Nitrate Concentrations (a) Influent (b) Effluent

The nitrate concentrations were also plotted vs. EBCT for stabilized data (Figure 33, Appendix C). Nitrate treatment was also improved with increasing contact times. For the QAL columns, above 11 days EBCT resulted in high nitrate removal. For the UMCf columns even contact times up to 30 days did not reliably remove all of the nitrate.

5.4.2.4 Chlorate/Perchlorate

Chlorate and perchlorate column concentrations are shown in Figures 28 and 29. Since chlorate initially did not degrade, not all samples were measured. It took a long time to see chlorate degradation in both soil types, particularly UMCf. QAL appeared to have adequate chlorate treatment ability later in the column run. Again, the peak seen for the QAL columns on day 108 is thought to be due to the power outage on day 105 and the ensuing crack formation.

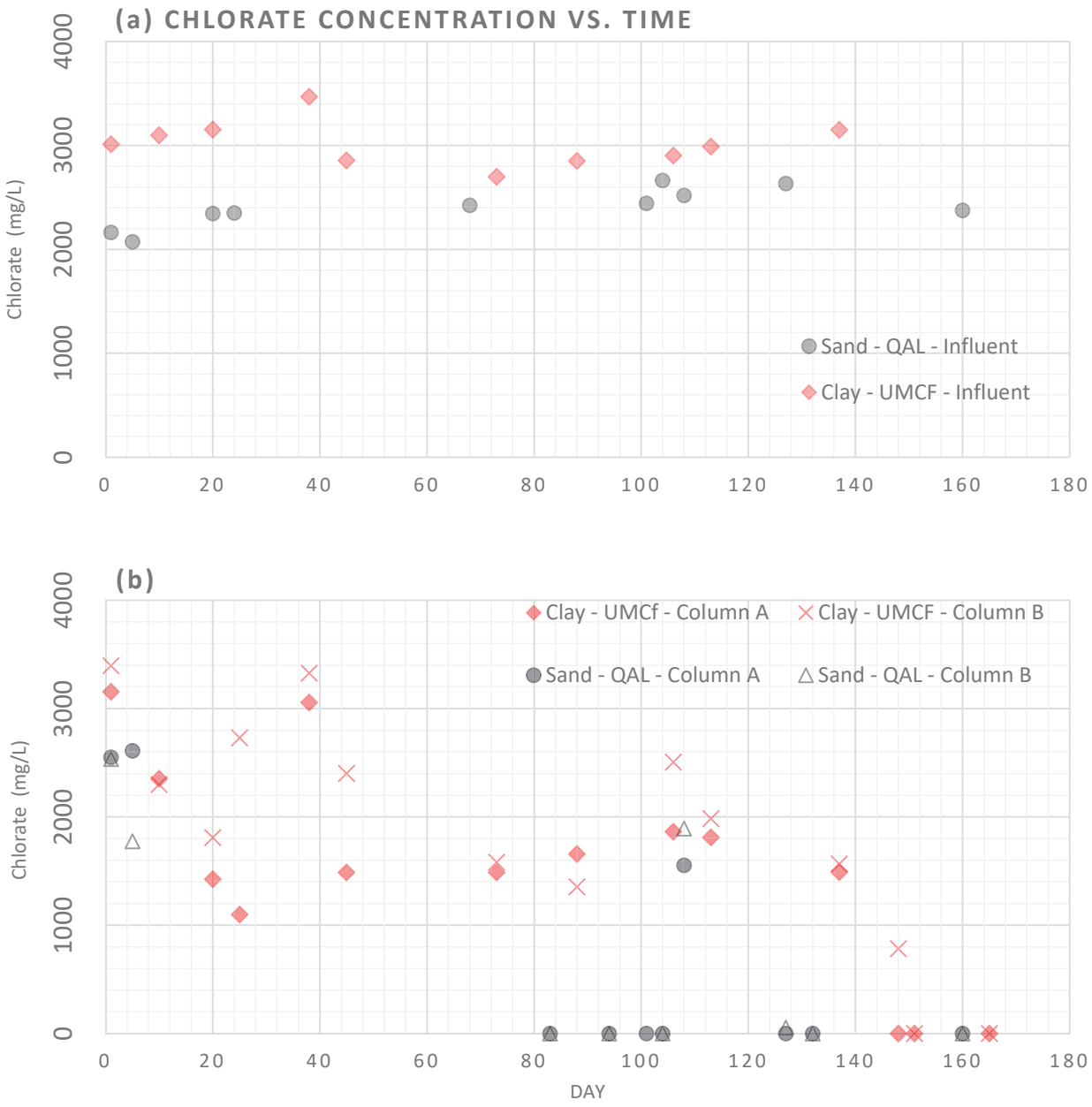


Figure 28: Column Chlorate Concentrations (a) Influent and (b) Effluent

For the QAL columns on day 17 (not shown in the graph), the lower perchlorate value was related to the prior influent feed with lower perchlorate (roughly 300 mg/L). The influent

concentration was reduced by approximately 50% on day 115 in both QAL columns. For the UMCf columns, the lower initial feed concentration explains the lower effluent initially. There was no obvious perchlorate degradation throughout the experiment in the UMCf columns, but the QAL columns showed degradation beginning around day 100 and improving to roughly complete degradation by about day 130 in column A.

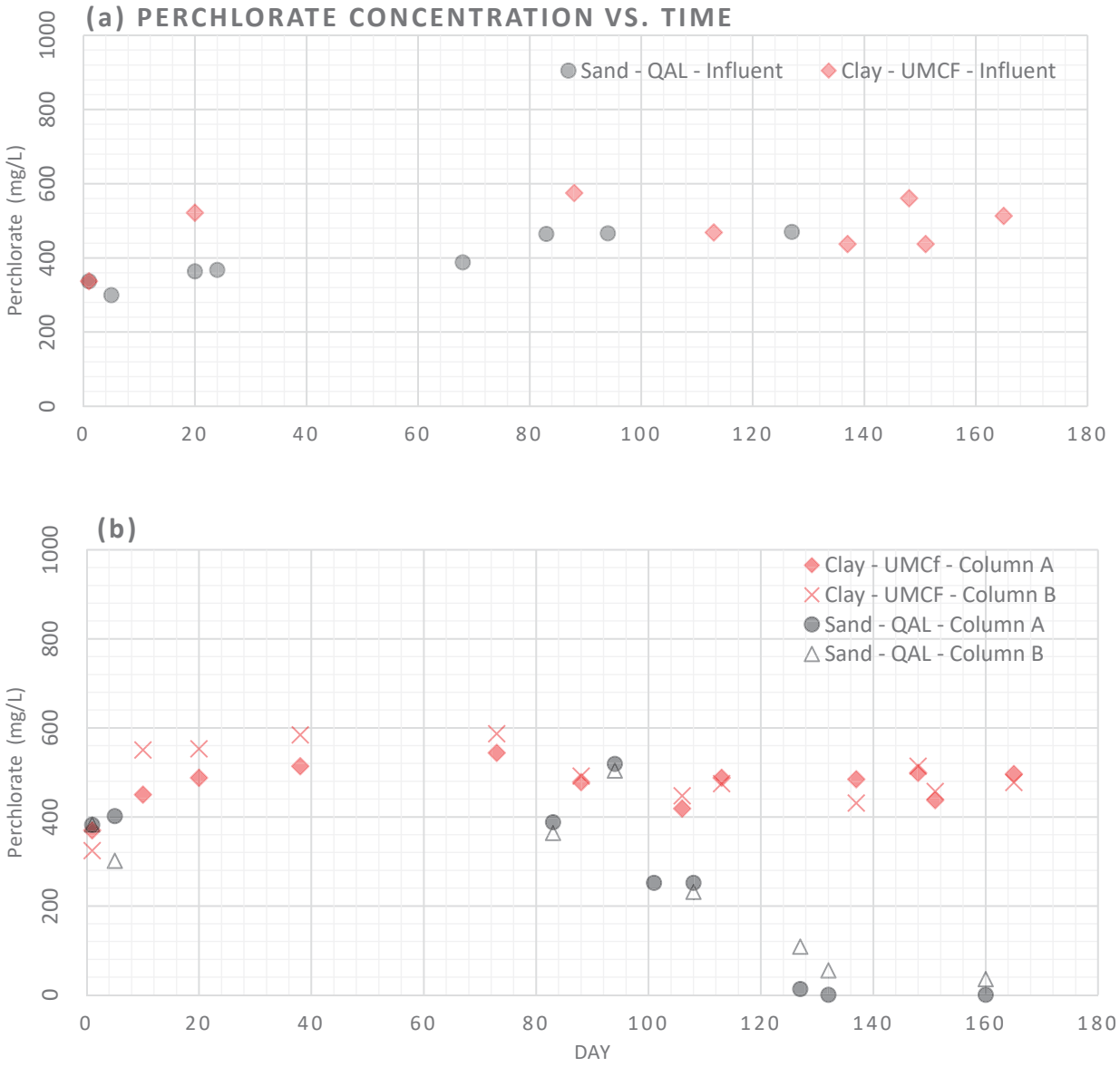


Figure 29: Column Perchlorate Concentrations (a) Influent and (b) Effluent

The graph of chlorate concentration vs. EBCT (Figure 33, Appendix C) shows significant chlorate removals for EBCTs as low as 2.5 and 7.75 days for the QAL and UMCf columns,

respectively. In the case of perchlorate (same Figure), EBCTs between 20 and 30 were still inadequate for perchlorate removal.

5.4.2.5 Nutrient and Carbon Source/Electron Donor Evaluation

Chemical Oxygen Demand

Figure 30 shows the influent and effluent COD concentrations for all columns. The early COD effluent readings seemed to correspond relatively well, where available, to the changing influent doses. Upon stabilization of influent, around day 40, the effluent was also stabilized relatively, although visibly at lower concentrations. Presumably that could be accounted for by biodegradation processes. The nearly doubling of COD values on day 108 was expected, since the columns had crack formations resulting from a power outage.

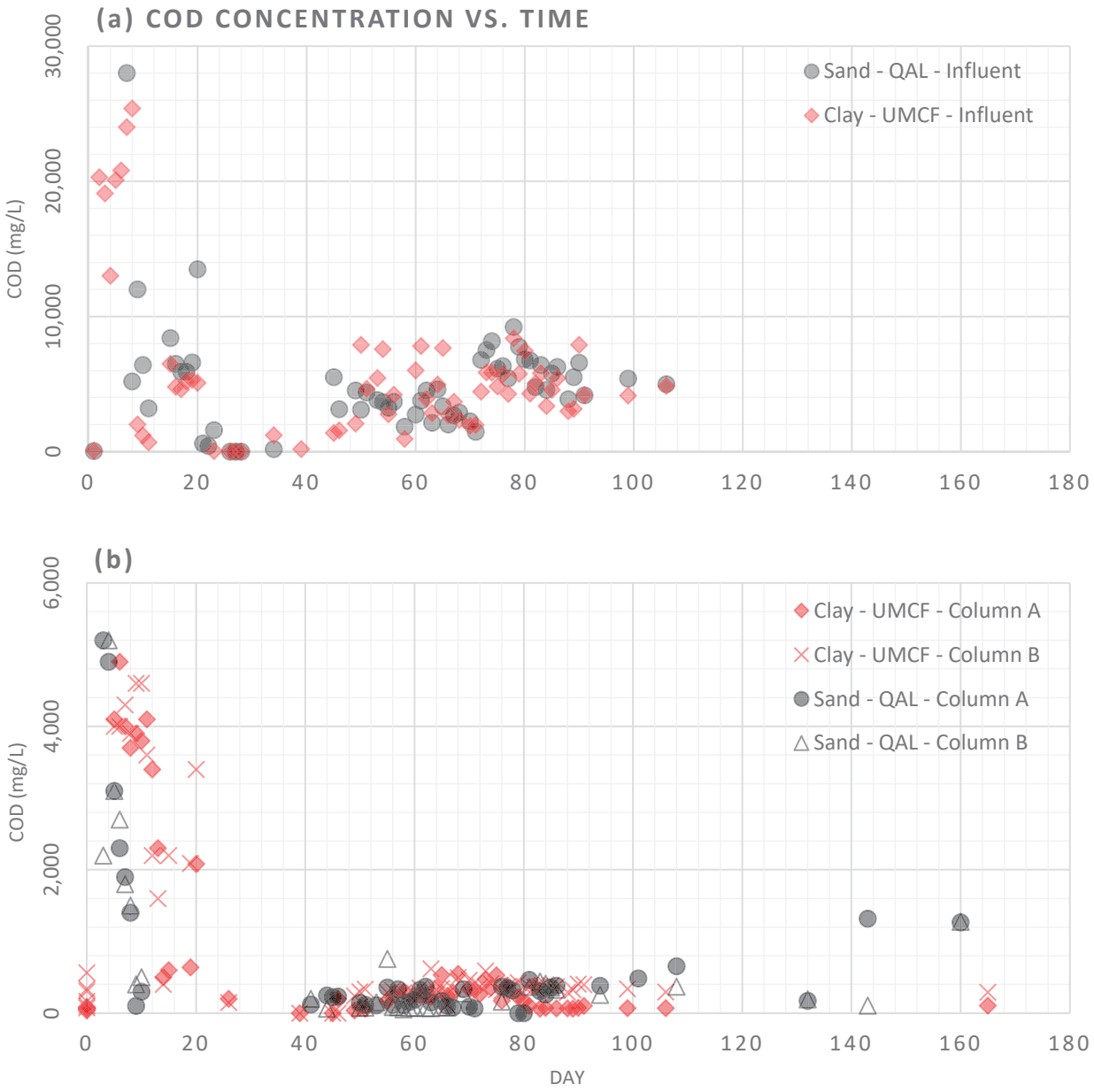


Figure 30: Column COD Concentrations (a) Influent and (b) Effluent

5.5 Analysis of Results

5.5.1 Reduction Rates and Sequence

Overall and maximum reduction rates were computed. The overall reduction rates were calculated as the change in concentration of each contaminant, divided by the time taken to reach either the minimum detection limit, or until testing was stopped. The maximum reduction rates were the largest rate changes between any of the tested time steps for the given oxyanions. The rate values are presented in Table 35.

Table 35: Chromium Reduction Rates Throughout Biological Treatment Process

Compound	Reduction Rate	QAL								UMCf							
		EOS-PRO		Mix		ISW		Molasses		EOS-PRO		Mix		ISW		Molasses	
		(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})	(mM d^{-1})	(mg L^{-1} d^{-1})
Cr	Overall Average	0.007	0.39	0.009	0.47	0.009	0.47	0.007	0.34	0.007	0.38	0.012	0.62	0.012	0.62	0.009	0.45
	Highest between Tests	0.043	2.3	0.048	2.5	0.10	5.0	0.033	1.7	0.058	3.0	0.076	3.9	0.13	6.8	0.043	2.26
Nitrate	Overall Average	0.37	23	0.35	22	0.23	14	0.73	45	0.077	4.8	0.070	4.3	0.068	4.2	0.31	19
	Highest between Tests	0.84	52	0.93	58	0.81	50	0.94	58	0.26	16	0.19	12	0.20	12	0.31	19
Chlorate	Overall Average	2.0	165	1.4	121	0.06	5.4	0.26	22	0.33	27	0.27	23	0.012	0.99	0.35	29
	Highest between Tests	2.4	198	5.0	419	0.43	36	0.79	66	2.0	165	1.3	112	0.38	32	2.0	164
Perchlorate	Overall Average	0.008	0.77	0.012	1.2	0.008	0.79	0.008	0.76	0.02	2.1	0.012	1.2	0.01	1.3	0.014	1.4
	Highest between Tests	0.10	9.8	0.25	25	0.20	20	0.41	40	0.31	30	0.34	34	0.31	30	0.46	46

Chromium overall reduction rates were in the 0.007 to 0.012 mM day^{-1} (0.39 to 0.62 mg L^{-1} day^{-1}) range (Table 35). The highest reduction rates ranged from 0.033 to 0.130 mM day^{-1} (1.7 to 6.8 mg L^{-1} day^{-1}). For both groundwaters, the highest reduction rates were found when

ISW was the organic source. The rates were at least twice as fast as for most of the other organic sources used. In general, the smallest chromium reduction rates were found with molasses as the organic source. It is proposed that two factors might be responsible for these observations, the solubility of the carbon source during biological reduction, and some abiotic reduction processes. One factor contributing to the high ISW rates is the high solubility of the ISW, compared to EOS-PRO, for example. Solubility increases the accessibility of organics to microorganisms. In fact, prior research found that an increase in the solubility of soil humic acid was considered partially responsible for its increased capacity to mediate the bioreduction of Cr(VI) (Gu and Chen 2003). Molasses, which is highly soluble, had lower reduction rates indicating that another reduction process was taking place with the ISW samples. It is also thought that some abiotic (e.g. chemical) reduction of Cr(VI) took place with ISW, increasing the reduction rates. This will be addressed below.

Another variable that may have increased the reduction of Cr(VI) by ISW was pH. In comparing the current rates to those of other studies, previous research investigated species of *Pseudomonas* spp. for Cr(VI) bioreduction. For one strain and an initial chromium concentration of 1.9 mM (100 mg/L) and pH of 6, a reduction rate of 0.23 mM day^{-1} (12 mg L^{-1} day^{-1}) was calculated and is precisely in the range of the current results (Wani and Ayoola 2015). Lower reduction rates (0.19 mM day^{-1} or 10 mg L^{-1} day^{-1}) were found at a pH value of 5, and even lower reductions at pH values of 8 and 9, respectively (Wani and Ayoola 2015). The pH in the ISW microcosms ranged between 6.32 – 6.69, while in the mix microcosms and EOS-PRO microcosms the pH was higher, in the 7.37 – 8.04 and 7.35 – 8.10 ranges, respectively. It seems that there was a more optimal pH in the ISW microcosms for reduction to take place and that is

proposed to have contributed to the higher initial reduction rates. At Cr(VI) concentrations of 0.96 mM (50 mg/L), a bioreduction rate of about 0.23 mM day^{-1} (12 mg L^{-1} day^{-1}) was found (Mangaiyarkarasi et al. 2011). Meanwhile, at lower Cr(VI) concentrations of 0.038 mM (2 mg/L), a reduction rate of only 0.030 mM day^{-1} (1.54 mg L^{-1} day^{-1}) was observed (Jin et al. 2017). All these literature values are quite similar to those of the current work. Half saturation constants for the reduction of Cr(VI) (summarized in Table 36) by two cytochromes of *Shewanella oneidensis* MR-1 were found to be 34.1 ± 4.5 and 41.3 ± 7.9 μM (1.77 ± 0.23 and 2.1 ± 0.41) mg/L (Belchik et al. 2011), indicating that the Cr(VI) reduction rate is not expected to slow significantly until relatively low concentrations are reached.

Table 36: Literature Half Saturation Constants for Oxyanions of Concern

Contaminant	Half Saturation Constant, Ks (mg/L)	References
Chlorate	60 – 75	Logan et al., 2001
Cr(VI)	1.8 – 2.1	Belchik et al., 2011
Nitrate	0.02 – 1.9	Hareendran et al., 2009
Perchlorate	< 0.1	C. Wang, Lippincott, & Meng, 2008

In terms of reduction sequence, Cr(VI) was overwhelmingly the first of the oxyanions to degrade. As shown in other research, the apparent lack of inhibitory effect due to the other oxyanions is not surprising. Microbes have been shown suitable to treat Cr(VI), even in the presence of other electron acceptors such as oxygen and nitrate (Tseng and Bielefeldt 2002). Although some research has shown that nitrate had an inhibitory effect on the bio-reduction rate

of Cr(VI), Cr(VI) reduction nonetheless preceded nitrate reduction (Vatsouria et al. 2005). Other studies have demonstrated the preferential reduction of hexavalent chromium by bacteria as compared to other oxyanions (Chovanec et al. 2012; Smith and Gadd 2000). Meanwhile in other work, nitrate was found to not affect the Cr(VI) reduction rate by E. *coli* in anaerobic cultures (Wang and Xiao 1995).

The overall average rates for nitrate (as NO_3) reduction were 0.068 – 0.73 mM day^{-1} (4.2 – 45 mg $L^{-1}d^{-1}$) for the different electron donor organics. The highest rates ranged between 0.19 – 0.94 mM day^{-1} (12 – 58 mg $L^{-1}d^{-1}$). Reduction rates with molasses were the highest in both cases for both groundwaters. Previous research was conducted on nitrate reduction (i.e. biological denitrification) using *Azospira* sp. OGA 24 bacteria and acetate as the organic substrate. For an initial 5.6 mM (350 mg/L) concentration, a degradation rate of 3.25 mM day^{-1} (200 mg L^{-1} day^{-1}) was found (Rossi et al. 2015). In other work, for nitrate at 3.6 mM (221 mg/L), there was found to be a 1.2 mM day^{-1} (73.8 mg $L^{-1}day^{-1}$) reduction rate (Sahinkaya and Kilic 2014). These values are also similar to the current results. In the current research, there was a clear difference between the reduction rates of QAL samples and UMCf samples. QAL samples exhibited far higher reduction rates overall. That difference was at least partially due to the higher initial concentrations of nitrate in the QAL groundwater (856 mg/L vs. 241mg/L). In support of this, previous research showed that higher nitrate concentrations, 8 and 16 mM (500 and 1000 mg/L), had higher reduction rates of about 2.3 and 4.7 mM day-1 (140 and 290 mg L^{-1} day^{-1}), respectively (Chitra and Lakshmanaperumalsamy 2006). Denitrification half saturation constants (Table 36) have been estimated at between 0.02 and 1.9 mg/L (Hareendran et al. 2009), much lower than the current concentrations, which explains the relatively rapid reduction rates.

In terms of reduction sequence, nitrate was in general the second of the contaminants to degrade, overall. The results indicate that Cr(VI) was a preferred electron acceptor and previous work supports that observation. Nitrate reduction was shown to be inhibited by both the toxicity of Cr(VI) and its ability to compete for electrons (Jin et al. 2017). Elsewhere, it was shown that nitrate reduction was strongly inhibited by Cr(VI) and nitrate reduction did not occur until after the soluble Cr(VI) stopped being detected (Kourtev, Nakatsu, and Konopka 2009). Other research has also shown the negative effects of Cr(VI) presence on denitrification. Mechanistically, these effects were due to restriction of gene activities, altering community composition and regulating functional genes expression. Interestingly, 3.21 mg/L of Cr(VI) was found to be the critical inhibitory concentration on electron transport system activities for heterotrophic denitrifying bacteria (Hu et al. 2019). Other research indicated that while there could be nitrate reduction in the presence of Cr(VI), the rates were significantly lower. It has been shown that for nitrate at 3.6 mM (221 mg/L), in the presence of 0.04 mM (2 mg/L) Cr(VI), there was a nitrate reduction rate of about 0.6 mM day^{-1} (36 mg L^{-1} day^{-1}) and at a Cr(VI) concentration of 0.2 mM (10 mg/L) there was a nitrate reduction rate of only 0.18 mM day^{-1} or 11 mg $L^{-1}day^{-1}$ (Sahinkaya and Kilic 2014). The Cr(VI) concentrations in the current study were in the 10 – 20 mg/L range, so chromium bioreduction clearly preceded or at the least severely slowed that of nitrate. The results clearly show that until most of the Cr(VI) was reduced, denitrification did not fully take place.

The chlorate reduction rates (Table 35) ranged from 0.012 – 2.0 mM day^{-1} (0.99 – 165 mg $L^{-1}d^{-1}$) for the overall average rates and 0.38 and 5.0 mM day^{-1} (32 – 419 mg $L^{-1}d^{-1}$) for the highest reduction rates by interval. In terms of these rates, the poorest organic source was clearly

ISW in both cases and for both groundwaters. In previous research, 9.7 mM (809 mg/L) chlorate was reduced as strain CKB (a beta subclass member of the proteobacteria) grew on acetate. The reduction rate was roughly 15.9 mM day^{-1} or 1327 mg L^{-1} day^{-1} (Bruce, Achenbach, and Coates 1999). Another investigation of bioreduction of similar chlorate concentrations of 10 mM (834 mg/L) showed a reduction rate of 24 mM day^{-1} or 2000 mg L^{-1} day^{-1} (Prata 2007). These rates were higher than those found in the current study, but it is important to note that no other electron acceptors were present to interfere with chlorate reduction, as they were in the present work. Since there was some simultaneous degradation of nitrate and chlorate, it is proposed that the presence of nitrate decreased the chlorate reduction rates. It is known that chlorate bioreduction can be competitively inhibited by nitrate (Anon 2007). Half saturation constants for chlorate reduction (Table 36) have been found to be 0.72 ± 0.30 and 0.90 ± 0.19 mM (60 ± 25 and 75 ± 16 mg/L) (Logan et al. 2001), well below the initial chlorate concentrations currently investigated.

In terms of the sequence of reduction, chlorate was generally seen to degrade after both Cr(VI) and nitrate. Since chlorate reduction is known to be inhibited by nitrate (ECHA n.d.), it was not surprising that nitrate reduction preceded that of chlorate in the current work. Much research has investigated the concurrent reduction of chlorate and perchlorate, discussed below, but rates for the reduction of chlorate in the presence of nitrate and Cr(VI) are largely absent from the literature and this work has provided new insights on the matter.

Table 35 shows that for the current research, the overall average perchlorate reduction rates ranged between 0.008 and 0.02 mM day^{-1} (0.76 – 2.1 mg $L^{-1}d^{-1}$). The highest rates ranged

from 0.1 – 0.46 mM day^{-1} (9.8 – 46 mg $L^{-1}d^{-1}$). Numerous studies have addressed the degradation rate of perchlorate in the presence of various carbon substrate as electron donors/carbon sources (Gal et al. 2008; Medina et al. 2006; Sarria Cortes 2016; Yifru and Nzengung 2012). Previous research investigated the biological reduction of 580 mg/L perchlorate using the perclace strain bacterial isolate and acetate as the carbon source. It was found that the rate of that complete reduction was 1.944 mM day^{-1} (193 mg L^{-1} day^{-1}), with the reaction order not noted (Herman and Frankenberger Jr. 1999). Nam et al. (2016) demonstrated that perchlorate at 7.0 mM (700 mg/L) fully degraded at a rate of 0.5 mM day^{-1} or 50 mg L^{-1} day^{-1}. Meanwhile at a low perchlorate concentration of just 0.5 mM (50 mg/L), the degradation rate was the same, 0.5 mM day^{-1} or 50 mg L^{-1} day^{-1} (Nam et al. 2016). Other research showed that 18.5 mM (1840 mg/L) perchlorate degraded at a rate of 6.5 mM day-1 (649 mg L^{-1} day^{-1}) and 2.5 mM (250 mg/L) perchlorate degraded at a rate of roughly 0.89 mM day^{-1} or 88 mg L^{-1} day^{-1} (Bardiya and Bae 2005). These values are all higher than the current results indicate. Research has shown a half saturation constant for perchlorate bioreduction below 0.1 mg/L, Table 36 (Wang et al. 2008), which indicates that the low reduction rates observed in this work were not due to low perchlorate concentrations but rather that they were impacted by the remaining presence of chlorate, as explained below.

In terms of reduction sequence, perchlorate was the last of the oxyanions to reduce and due to insufficient reaction time, the reduction never took place to a very significant extent. It was expected that perchlorate would degrade last among the oxyanions. For example, chlorate and perchlorate can compete for reduction by the same enzyme, (per)chlorate reductase. Increasing chlorate concentration decreases the perchlorate reduction rate (Dudley, Salamone,

and Nerenberg 2008). Research has shown that perchlorate bioreduction was completely inhibited by the presence of both oxygen and chlorate but not nitrate (Nam et al. 2016). Other research demonstrated that there was no degradation of perchlorate in the presence of nitrate; only after all nitrate had been reduced did the degradation of perchlorate start (Gal et al. 2008; Wang et al. 2014). It has even been found that nitrate, when present, was used in preference to perchlorate even when cultures had been grown on perchlorate prior (Coates and Achenbach 2004). Other research also found the concentration of electron acceptors (dissolved oxygen and nitrate) to decrease prior to those of perchlorate, the reason being that microbes will use the electron donors that provide higher energy first (Krauter 2001). It was also concluded elsewhere that utilization of nitrate as an electron acceptor preceded perchlorate (Michal C. Ziv-El and Rittmann 2009). An investigation of the simultaneous bioreduction of chlorate and perchlorate, both at 5 mM (417 and 497 mg/L, respectively), demonstrated a reduction rate of 13.3 mM day^{-1} (1326 mg L^{-1} day^{-1}) for perchlorate. It was also evident that perchlorate degradation did not begin until chlorate had degraded (Prata 2007). All this previous work explains the inhibition of perchlorate reduction and the lack of significant reduction that was demonstrated here.

The presence of interfering compounds not only delayed the start of perchlorate reduction but also slowed the rate of the reduction which did occur. Research which investigated the effects of nitrate on perchlorate reduction showed that perchlorate reduction was hardly affected when nitrate and perchlorate were at roughly equal molar concentrations. When nitrate was at 10, 100, and 1000 times the perchlorate levels, (62 mg/L NO_3^- and 0.089 – 12 mg/L ClO_4^-) however, the perchlorate reduction rate was decreased. The reduction of nitrate was seen to be concurrent with that of perchlorate at 10 and 100 times and to precede that of perchlorate at 1000

times (Herman and Frankenberger Jr. 1999). In contrast, Zhu et al (2016) considered the bacterial reduction of perchlorate in the absence and presence of nitrate and found that increasing nitrate to perchlorate ratios caused increasing but recoverable lags in perchlorate reduction, even at lower ratios (Zhu et al. 2016b). This indicates the preference that bacteria have for nitrate over perchlorate. As an example, they found that 1300 mg/L of perchlorate was degraded completely in 28 hours in the absence of nitrate (11 mM day^{-1} or 1100 mg L^{-1} day^{-1}). During that same time period, at nitrate (650 mg/L or 2600 mg/L) to perchlorate (1300 mg/L) mass ratios of 0.5 and 2, the perchlorate reduction rates were only 6.7 and 4.5 mM day^{-1} (670 and 450 mg L^{-1} day^{-1}), respectively. These were similar to the nitrate to perchlorate mass ratios in the present work of about 0.4 and 1.8 for UMCf and QAL, respectively. QAL samples had 856 mg/L of nitrate and 488 mg/L of perchlorate, while UMCf had 241 mg/L of nitrate and 642 mg/L of perchlorate. Interestingly, the QAL samples, with higher nitrate interference ratios, were indeed found to have the lower perchlorate reduction rates. Other work indicated that the reduction rate of 5 mM (500 mg/L) perchlorate in the presence of 8.1 mM (500 mg/L) nitrate was roughly 0.39 mM day^{-1} (39 mg $L^{-1}d^{-1}$) and in the presence of 6 mM (500 mg/L) chlorate, the rate was 0.49 mM day^{-1} or 48.3 mg L^{-1} day^{-1} (Ghosh et al. 2011). Other research compared the perchlorate reduction alone and in the presence of other oxyanions. Alone, roughly 11 mM (1100 mg/L) perchlorate degraded at a rate of 3.67 mM day^{-1} (365 mg L^{-1} day^{-1}). When combined with chlorate, there was preferential chlorate reduction and equimolar chlorate concentrations decreased the reduction rates of perchlorate by roughly one half (Attaway and Smith 1993). It is clear that in the presence of competing oxyanions, literature values have been demonstrated to be similar to current results.

The perchlorate reduction rates in the current work compare favorably to literature rates with interferences present. It is important to note that due to incomplete reduction of chlorate, perchlorate reductions mostly remained in the 'lag' phase for the duration of the study. If additional time were provided, perchlorate reduction rates were likely to have accelerated.

5.5.2 Reaction Orders and Influences

The third and more general method of investigating the oxyanion reduction rates was to investigate the reduction order and calculate the corresponding reaction-rate coefficients. Following the method of (Tchobanoglous et al. 2014), plots were made of c vs. t, $-\log(c/c_0)$ vs. t and 1/c vs. t to determine if the reactions were zero, first, or second order with respect to the contaminant concentration, respectively. The graph with the best linear fit, based on its R^2 value, was considered the likely reaction order. The reaction order for each combination of oxyanions and substrate, as well as their R^2 values are shown in Table 37. In analyzing the data, once concentrations neared the detection limit, only one additional data point was included, if available. Attempts were also made to exclude data from the lag period of a preceding oxyanion. The method used was as follows: Since Cr(VI) was found generally to degrade first, it was plotted from the start. When 90 % of Cr(VI) was degraded, data for both nitrate and chlorate concentrations were plotted. The only exception was for the nitrate in the molasses microcosms, which was plotted like Cr(VI), from the start, due to its early observed reduction. The goal of selecting the data in this manner was to minimize the impact of the co-contaminants in the reaction rate. This approach assumes that, for example, chlorate degradation is independent of the degradation of nitrate. That is not fully the case, but the approach allows the estimation of reaction rate during the period of time the contaminant of interest showed significant

degradation. The calculated reaction orders were nonetheless thought to be affected by the presence of other oxyanions present in the water, as discussed below.

Table 37: Oxyanion Reduction Reaction Orders and Rate Coefficients

Contaminant	Groundwater	Electron Donor/ Carbon Source	Order	R^2	Reaction Rate Coefficient (k)	Units
Chromium	QAL	EOS-PRO	1	0.8812	0.104	d^{-1}
		Mix	1	0.8755	0.076	d^{-1}
		ISW	1	0.8998	0.122	d^{-1}
		Molasses	1	0.8132	0.035	d^{-1}
	UMCf	EOS-PRO	1	0.9023	0.054	d^{-1}
		Mix	1	0.9086	0.081	d^{-1}
		ISW	1	0.8879	0.159	d^{-1}
		Molasses	1	0.8133	0.036	d^{-1}
Nitrate	QAL	EOS-PRO	1	0.9703	0.064	d^{-1}
		Mix	0	0.9477	27.8	$mg\ L^{-1}\ day^{-1}$
		ISW	2	0.7775	5.98E-05	$L\ mg^{-1}\ day^{-1}$
		Molasses	0	0.6818	18.1	$mg\ L^{-1}\ day^{-1}$
	UMCf	EOS-PRO	2	0.9173	4.48E-04	$L\ mg^{-1}\ day^{-1}$
		Mix	1	0.7393	0.016	d^{-1}
		ISW	0	0.6452	3.82	$mg\ L^{-1}\ day^{-1}$
		Molasses	0	0.8968	13.3	$mg\ L^{-1}\ day^{-1}$
Chlorate	QAL	EOS-PRO	[b]	0.6628	0.171	d^{-1}
		Mix	0	0.8687	221	$mg\ L^{-1}\ day^{-1}$
		ISW	[a]			
		Molasses	[c]			
	UMCf	EOS-PRO	2	0.9263	2.81E-05	$L\ mg^{-1}\ day^{-1}$
		Mix	0	0.9283	29.8	$mg\ L^{-1}\ day^{-1}$
		ISW	[a]			
		Molasses	[c]			
Perchlorate	QAL	EOS-PRO	[a]			
		Mix	[a]			
		ISW	[a]			
		Molasses	[a]			
	UMCf	EOS-PRO	[a]			
		Mix	[a]			
		ISW	[a]			
		Molasses	[a]			

[a] No Appreciable Degradation: 3 - 32 %
[b] all R^2 were equal
[c] Insufficient Data

Some concepts are made clear in Table 37. The reduction rates for Cr(VI) were all found to be first-order and the R^2 values were generally high (0.81 – 0.91). This implies that the rate is directly proportional to the concentration of Cr(VI) in solution. Since Cr(VI) is the most preferred electron acceptor, there is no influence from other contaminants in its degradation rate. The first-order rate constants ranged from 0.036 to 0.159 day^{-1}. Similarly, previous research has shown that Cr(VI) reduction with soil natural organic matter had a half-life of several weeks and was also a first-order reaction (Bartlett 1991). Those half-life results would indicate rate constants in the range of 0.01 – 0.03 day^{-1} (Purdue n.d.), which are not very dissimilar from the current results. In other work, first-order decay constants for Cr(VI) have been shown to range from roughly 0.048 to 1.92 day^{-1} for various cell suspension concentrations (Hansen et al. 2017). These values compare quite favorably to findings in Table 37, where rate first-order Cr(VI) reduction rate constants ranged from 0.0345 to 0.159 day^{-1}. Since the rate constants were first-order and in the range of typical values, it implies, as was evident, that the Cr(VI) degraded first without too much interference from the other oxyanions.

Nitrate reduction orders were found to vary, showing inconsistent order results and R^2 values ranging from 0.65 to 0.97. The first-order rate constants ranged from 0.016 day^{-1}, for the mix UMCf sample, up to 0.064 day^{-1}, for an EOS-PRO QAL sample. In similar tests, previous research had used a pseudo-first-order rate constant of 0.845 ± 0.062 d^{-1} to describe the reduction of nitrate by *Aeromonas hydrophila* HS01 under non-growth conditions (T. Liu et al. 2014). These literature rates were higher than those found in the current work, but it is important to remember that some simultaneous reduction of nitrate and chlorate was taking place. In previous research, denitrification was also found to be first-order in the presence of Cr(VI), with rate

constants ranging from 0.0075 to 0.1050 hr^{-1} (0.18 to 2.52 day^{-1}), with increasing Cr(VI) concentrations slowing the rates. The slower rates were proposed to be based on adverse effects of Cr(VI) on the microorganisms, and not on chemical interference (Hu et al. 2019). These rate constants agree very well with the current results and indicate that even though Cr(VI) was degraded first and did not compete with nitrate, it may have slowed the reactions through its residual effects on the microorganisms present. Most of the results for the reduction order of nitrate in this work showed that it was a zero-order reaction, that is, the degradation rate was independent of the nitrate concentration, with rate constants ranging from 3.82 to 27.8 mg L^{-1} day^{-1}. Interestingly, previous work studying the bioreduction kinetics of nitrate and perchlorate found nitrate reduction to be roughly zero-order with respect to nitrate concentration (Van Ginkel et al. 2008), but the rate constants were not calculated. Meanwhile, the trendlines for the two samples that were found to react with second-order kinetics exhibited only slightly better fits than first and zero-order trendlines.

Chlorate reduction was mostly found to be zero-order. The zero-order rate constants with R^2 values above 0.7 ranged from 29.8 to 221 mg L^{-1} day^{-1}. Competition is suggested by the very low reduction rates. Previous research indicated a ~ 2225 mg L^{-1} day^{-1} reduction of chlorate (Shah 2014), far higher than the current rates.

Since it was found that very little perchlorate reduction took place over the observed time period, reaction rates were not calculated for perchlorate. Previous research reported that reaction kinetics of perchlorate reduction are first-order (Wang et al. 2014). Other work has also indicated first-order kinetics for perchlorate biological reduction, under conditions of parts per

billion perchlorate concentrations (Logan et al. 2001). It is postulated again, that the presence of other compounds delayed the onset of perchlorate reduction, and led to the insignificant reduction over the testing period.

5.5.3 Abiotic Reduction of Oxyanions

Since abiotic reduction of chromium was suspected to have taken place alongside biological reduction processes, some investigation into such reactions has been conducted. As previously mentioned, in a supplementary investigation to this research, ISW was found to reduce Cr(VI) abiotically by over 50% in 4 days, but the mechanism was not investigated. In other research, Hansen et al. (2017) found that there was almost immediate reduction of Cr(VI) in microcosms treated with molasses, prior to the appearance of biomass. The authors state that a rapid, direct reaction between Cr(VI) and a molasses constituent is suggested (Hansen et al. 2017). Other research also showed that sugarcane molasses, without bioreduction, can reductively produce Cr(III) from Cr(VI) in the pH range of 2.0 to 6.1. The mechanism consisted of Cr(VI) accepting electrons from the phenolic hydroxyl group of plant polyphenol (Chen et al. 2015). However, the pH of the molasses microcosms ranged from 7.31 to 7.98 in this research, so the abiotic process documented by Chen et al, (2015) is not expected to have played a significant role in the molasses microcosms. On the other hand, ISW microcosms were slightly acidic, and polyphenols, which are found in citrus juices (Vinson et al. 2002), were very likely present in the samples. It is postulated that a similar reduction mechanism to that demonstrated by Chen et al (2015) took place which reduced Cr(VI) in the ISW samples, with polyphenol as the electron donor.

Zhong and Yang (2012) investigated Cr(VI) reduction using malic acid and iron-rich soils. Organics are capable of reducing Cr(VI) but at low rates. It was found that the soils could accelerate the reduction rates, but the effect decreased with increasing pH. The authors proposed a pathway which started with malic acid adsorbing to the soil surface. Soil Fe(III)-oxides led to Fe(II) production through their reductive dissolution by malic acid. Finally, Fe(II) reduced the Cr(VI) in solution (Zhong and Yang 2012). At higher pH, the soil surface charge would be increasingly negative and organic acid anion adsorption would decrease, adversely affecting the first step of the pathway (Zhong and Yang 2012). This soil-catalyzed abiotic reduction mechanism may have played a role in the current research. Molasses, for example, is known to contain malic acid (Abou-Zeid et al. 1993). Malic acid is also one of the two most abundant organic acids in many fruits (Famiani et al. 2015), so it is likely to have been present in ISW. The pH of ISW was the more acidic of the two, so this reaction would have been more likely to be favored in ISW results, as well.

It is postulated that either direct reduction by the added organics, or indirect reduction, where the added organics lead to the formation of another reductant like Fe(II), are two likely abiotic reduction mechanisms which may have played roles in the current treatment results. Other research showed that abiotic reduction of nitrate in soil with organic carbon was either not possible, or very slow (Mariën et al. 2011) so this does not appear to have influenced the reduction of nitrate.

5.5.4 Contributions and Implications

The first hypothesis of this investigation was that EOS-PRO was expected to perform better than the other organic sources used, since it was designed for contaminant remediation. This was partially correct. In the case of chromium reduction, ISW and Mix (ISW + EOS-PRO) performed better than EOS-PRO alone. It is thought that this was due to chemical (abiotic) reduction processes with the ISW and molasses, as discussed previously. In the case of nitrate, EOS-PRO performed better than the ISW and mix microcosms, but not as well as the molasses microcosms. For chlorate reduction, EOS-PRO was the overall best organic substrate. For perchlorate reduction, all substrates performed quite poorly. However, reductions of perchlorate were expected to follow the reductions of all the other oxyanions, and probably would have begun, given more time. This research, due to the wide range of oxyanions and groundwater compositions, showed the complexity of the reduction interactions. Even though overall reduction sequences were evident, the specific reduction order was found to depend on the co-contaminants and the organic sources/electron donors used. Such interactions as oxyanion interference, competition, as well as chemical processes probably played roles in the treatment effects. Each unique water source will result in its own processes, but this work did much to provide insight into those processes and interactions in a very complex, high concentration, oxyanion environment. The reduction rates obtained have clear implications for the treatment of the current contaminated site, but also provide insight into potential outcomes at other sites contaminated with multiple oxyanions.

The second hypothesis proposed that all the compounds could be concurrently reduced since they are individually biodegradable. For the given testing period, this hypothesis was not completely proven. It was found that none of the substrates reduced perchlorate appreciably in the given time frame. It was expected to be the last oxyanion to degrade, and chlorate, for example, was still being reduced. If given sufficient time, perchlorate would possibly have been degraded next. All of the substrates reduced Cr(VI). Molasses removed all of the nitrate. EOS-PRO and mix removed most of the nitrate, while only EOS-PRO and mix removed all of the chlorate in one groundwater. Again, the effects of groundwater composition and organic source/electron donor used were key to the reduction efficacy. Results did suggest that more significant reductions of all the compounds could have taken place if given adequate time and proper organic source selection. ISW was clearly demonstrated as an inferior alternative substrate, since its chlorate reduction ability was very poor. Again, to the knowledge of the authors, little work has been conducted on the simultaneous reduction of these specific oxyanions, and ISW has undergone very little research overall.

These results have important implications to any of the multitude of sites containing some or all of these oxyanions, in terms of the recommended organic sources and required contact times for specific treatment objectives.

The final hypothesis examined the oxyanion reduction sequence. While there were exceptions, the general ease of reduction followed the order Cr(VI) > NO_3^- > ClO_3^- > ClO_4^-. To the knowledge of the authors, very little information on the order of those four compounds has

ever been published, since this combination of contaminant oxyanions has seldom been previously studied.

This research shows degradation results that vary based on organic sources and groundwater type. For the site in question, results from this research provide a direct roadmap for treatment strategies that should be used for particular treatment goals. For example Cr(VI) can be treated very rapidly by ISW, whereas nitrate reduction would not be accomplished with that organic source. As another example, if nitrate is the oxyanion of concern, it will be possible to target that compound, through the use of molasses, without much interference from other oxyanions. On the other hand, if perchlorate is the pollutant of concern, it will be necessary to first reduce all the other oxyanions. These results also provide a solid basis for developing an approach to oxyanion treatment at other similarly contaminated sites, although specific site conditions will doubtlessly have their effects on treatment efforts and additional investigations will be necessary.

5.6 Conclusions

This investigation was conducted on the reductive treatment of chlorate, Cr(VI), nitrate and perchlorate. Very little direct work was conducted to separate between biological and chemical reduction.

5.6.1 Major Conclusions

After investigating microcosms by using various carbon sources, the following conclusions were drawn:

1. All microcosms, regardless of substrate, demonstrated a dramatic reduction of Cr(VI) in the first 10 days and complete reduction within the test period, although substrates had very different performances. The highest chromium reduction rates ranged from 0.033 to 0.130 mM day^{-1} (1.7 to 6.8 mg L^{-1} day^{-1}) and were similar to literature values.
2. Nitrate reduction was not as complete as Cr(VI) reduction, except in the case of the molasses microcosms. EOS-PRO was also an effective substrate with the high reduction rates ranging from 0.26 to 84 mM day^{-1}.
3. Except in the case of molasses, nitrate reduction generally came after Cr(VI) reduction.
4. Using EOS-PRO with QAL microcosms, chlorate was reduced alongside and even prior to nitrate. Far less chlorate reduction took place in UMCf microcosms and ISW was very ineffective at chlorate reduction, regardless of soil and groundwater type. The high reduction rates ranged from 0.79 to 5 mM day^{-1}.
5. Only slight perchlorate degradation was observed.
6. The reaction orders and rates for Cr(VI) matched literature expectations. Nitrate rates were affected by the presence of other oxyanions, in agreement with previous research. Chlorate was found to have lower reduction rates, which again was attributed to competition with the other oxyanions.

Column testing resulted in the following conclusions:

1. After initial instability and stopping or reducing substrate addition, strong and sustained Cr(VI) reduction was attained in column effluent with steady substrate addition, and only spiked after some column disturbances.

2. In QAL columns, nitrate was reduced around the time Cr(VI) treatment was steadying but UMCf columns took longer to reach very low concentrations of nitrate, although partial treatment had begun earlier.
3. Chlorate reduction was far more pronounced in QAL than in UMCf columns.
4. In QAL columns, perchlorate reduction was achieved, but trailed that of Cr(VI) and nitrate. No clear perchlorate reduction was seen in UMCf columns.

Overall, this project's research results provide insight on the treatment of multiple co-occurring oxidized contaminants. In general, ease of treatment followed the order Cr(VI) > nitrate > chlorate > perchlorate, but both organic source and the type of groundwater/soil being used affected the treatment effectiveness and reduction order. While much information has been developed on the orders of reduction reactions and completeness of the reductions, based on the different organic substrates, it is still noted that the groundwater and soil characteristics (matrix properties, bacteria present, etc.) also have an impact that affects treatment outcomes.

CHAPTER 6

CONCLUSIONS AND PROPOSED FUTURE WORK

6.1 Conclusions

The purpose of this research was to investigate the chemical reduction of Cr(VI) and the biological reduction of chlorate, Cr(VI), nitrate and perchlorate. Groundwater and soil underwent some chemical and physical characterization, then jar tests and microcosm tests were used in the chemical and biological treatment phases, respectively. Each phase was followed by column tests. Some of the major conclusions are presented below.

6.1.1 Soil and Groundwater

It was determined that for soil samples, levels of Cr(VI), nitrate and perchlorate were contained in both their water fractions and in rinses of the soils. For example, chromium in the moisture extraction from one soil was found to be 35 µg/L. Extractions of soils from different wells and depths showed values between 38 and 5378 µg Cr(VI)/kg dry soil. These values increased with depth for wells. Nitrate was found to range from 69 to 466 mg NO_3^-/kg of dry soil; it did not necessarily increase with depth. Perchlorate, finally, ranged from 47 to 688 mg/kg dry soil and did not always increase with depth. Various groundwater well and depth samples were found to contain numerous contaminants and at various levels. Cr(VI) readings ranged from about 24 to 21,000 µg/L, nitrate from 20 to 1,125 mg/L and perchlorate from 225 to 1,370 mg/L.

6.1.2 Chemical Treatment

6.1.2.1 Jar Tests

In this part of the research, jar testing was conducted using site soils and waters. Calcium polysulfide and ferrous sulfate were the reductants that were investigated. Although the presented chemical treatment results were mixed, removal of chromium was found for both CaS_x and ferrous sulfate. With high level Cr(VI) batch treatment, total dissolved chromium (TDC) was noted to have appreciable removals at no less than three times the stoichiometric ratio for CaS_x and not less than 10 or 20 times the stoichiometric ratio (the previous increment was 5 times) for $FeSO_4$. Apparently, at low-level chromium concentrations, neither reductant seemed particularly effective, although ferrous sulfate appeared to have shown some better results in QAL, albeit at higher stoichiometric ratios. It is thought that dissolved oxygen content was responsible for the poorer treatment at low Cr(VI) concentrations because the ratios of DO to reductant were higher. Solids content was found to promote chromium removal, and the type of solids present in suspension also impacted results.

6.1.2.2 Column Tests

Column tests were conducted for two types of soil, using calcium polysulfide as the treatment chemical. Both soils were tested at influent of approximately 1 mg/L of Cr(VI), and the finer soil was also tested with roughly 10 mg/L of Cr(VI) influent. All scenarios resulted in very effective and reliable reduction of Cr(VI) and total dissolved chromium. Even after the cessation of CaS_x addition, the columns remained capable of removing chromium for many days. The probable presence of reduced iron, elemental sulfur and thiosulfate in the columns is thought

to have promoted the sustained reducing conditions that were observed. This indicates that if such a process were applied in the field, it would allow for intermittent addition of treatment chemicals.

6.1.3 Biological Treatment

6.1.3.1 Microcosm Tests

In this phase of the research, microcosms were prepared with the two soil/groundwater types. Three organic substrates were investigated for their ability to reduce the oxyanions of concern. From the results, it appeared that substrate reduction was taking place in the microcosm samples as indicated by COD decreases over time. All oxyanions were found to degrade, except perchlorate, which showed only minor reduction. There was great variation in degree of removal, however, for different substrates and groundwater/soil types. The following are some highlighted results:

1. Evidently, there was significant and fairly rapid chromium reduction taking place in the microcosms. Very little Cr(VI) remained from 11 to roughly 50 days. ISW in QAL exhibited the fastest reaction rate. Biotic treatment was not the only cause, as chemical reduction has also been shown to occur, and may be due to reduction by polyphenol at which occurs at those optimal pH values.
2. Nitrate was completely absent from molasses microcosms after 19 days but was still found at significant levels in ISW microcosms throughout the testing period. EOS-PRO also performed well for nitrate reduction, particularly in QAL samples with very little nitrate remaining after just 36 days.

3. Chlorate was absent in QAL samples after 19 and 26 days for EOS-PRO and mix, respectively. It took far longer to begin degrading in UMCf samples. This may have been due to the higher initial Cr(VI) concentrations in those samples. When ISW was used as the substrate, there was little to no degradation, regardless of groundwater/soil.
4. Well into the testing period, the largest portion of the chromium reducing bacteria was also *Pseudomonas* sp., followed by *Acinetobacter psychrotolerans* and *Aeromonas* sp., in mix and EOS-PRO, respectively.
5. The reduction of Cr(VI) was found to follow first-order kinetics and compare favorably to literature results. The rate orders of the other oxyanions were thought to be impacted by the presence of the co-contaminants.

6.1.3.2 Column Tests

Four laboratory columns were prepared and tested. Two had QAL groundwater/soil and two had UMCf groundwater/soil. The substrates used were EOS-PRO and ISW. There was some initial influent instability, which was addressed primarily by changing the influent feed to the columns more regularly. There were also varying doses of substrate supplied over the test period. The following are some of the major outcomes:

1. Initially, COD measurements in the influent varied significantly, due to changing influent doses and suspected early degradation of the feed. Around day 45, the concentrations became relatively stable.

2. At approximately day 44, the QAL columns showed very thorough Cr(VI) reduction and soon after, total dissolved chromium was measured to be very low. The UMCf columns took until about day 80-87 to treat to very low levels.
3. Nitrate treatment was almost complete by day 48 in the QAL columns, but UMCf columns took until about day 150 to effectively treat for nitrate.
4. Meaningful chlorate reduction was not documented in QAL columns until about day 83. In comparison, UMCf columns were not seen to treat chlorate significantly until about day 148.
5. QAL columns were seen to slowly reduce perchlorate until little was left by around day 130. No such reduction was ever noted with the UMCf columns.
6. Overall, the shallower groundwater and soil samples resulted in more effective contaminant treatment than the deeper ones.
7. Treatment of all oxyanions was indeed possible but the water matrix, soil and/or the bacteria in the soil played significant roles. For example, in QAL columns, chromium and nitrate reduction followed similar patterns, while in UMCf columns, chromium reduction preceded that of nitrate. In UMCf columns, the eventual reductions of nitrate and chlorate coincided quite well, while with perchlorate there was never measurable degradation in UMCf as there was in QAL.

6.2 Proposed Future Work

6.2.1 Chemical Treatment

The improvement in treatment between CaS_x jar tests and column tests is best explained by the increase in the stoichiometric dose, increased reaction times and increased contact with

the soils. The residual treatment capacity in the columns, after stopping CaS_x addition, indicated that a surplus of CaS_x was being added to the columns. In the field, this could provide the possibility of intermittent reductant injections. It would be beneficial to further investigate this phenomenon and determine either what minimum continuous dose of CaS_x would also provide adequate treatment capacity or, how best to time episodic injections for continuous treatment.

6.2.2 Biological Treatment

This research shows that reduction of oxyanions is highly dependent on such factors as carbon source, the groundwater/soil varieties, and most probably the types and quantities of the microbes present. Additional investigations into these interactions are recommended, as well as a clearer separation between the biological and chemical processes involved in the treatment. For example, molasses could be added to autoclaved groundwater to see if any chemical reduction would take place.

APPENDIX A

This appendix provides the methodology and results from soil and groundwater analysis.

A-1 Soil and Groundwater Characterization Methods

A-1.1 Measurements of Moisture in Soil (Well A)

For moisture content calculations, from each depth of soil, triplicate 20 g samples of soil were weighed and dried for 12 hours at 105° C in an oven. The dry weight basis soil contaminant concentrations were determined using this moisture content.

A-1.2 Measurements of Contaminants in Soil Water Fraction (Well A)

Roughly 200 g of blended wet soil from each depth were centrifuged at 4400 rpm for an hour, in order to extract the water. The first trial resulted in a liquid sample from only one soil depth. The amount of soil was doubled to roughly 400 g and afterwards two of the three depths yielded liquid, the two deepest. The process was repeated three times for the two deeper soils with 400 g of soil until an amount of about 25 mL of liquid for analysis of chemicals was collected.

A-1.3 Extraction of Contaminants by Rinsing with Nanopore Water (Well A, Well C-S, Well D-D)

Well A soil was tested from three depths while Well C-S, Well D-D were from one depth each, for a total of 5 soil samples. In 250 mL centrifuge bottles, additions of each wet soil (50 g) and 100 mL of nanopore water were made for each soil. Samples were placed for 24 hours on a

rotary shaker at room temperature at 45 rpm. They were then centrifuged at 4400 rpm for 30 minutes. The supernatant was measured in graduated cylinders after careful transfer, then stored in vials. The second rinse involved 100 mL of DI water addition to the bottles and their transfer back for 24 hours to the rotary shaker. The supernatant was moved carefully to another vial after 30 minutes of centrifugation of the content. This was repeated for a third rinse. The collected volumes of the rinsate were recorded for each rinse and nitrate and Cr(VI) were measured each day after filtration. Before analysis of perchlorate, the samples were all refrigerated. For those three contaminants, the reported values are in ug or mg/kg of soil. Rinsate volume was multiplied by measured concentration to calculate mass of contaminant for the extraction. With moisture content accounted for, dry soil amount was used to normalize the mass of the contaminant.

COD, hardness, phosphate, sulfate and TDS were also analyzed for the sample (first rinse) from each soil depth. This rinse was used for COD measurements even though non-polar organic molecules that were bound to the soil may not move to the polar water liquid phase.

A-1.4 Groundwater Sample Characterization

Filtered groundwater samples (0.2 um membrane filters-VWR Scientific) were measured directly for their chemical constituents.

A-2 Soil and Groundwater Characterization Results

A-2.1 Contaminants in Dry Soil, in Soil Water Fraction (Well A)

Table 38 shows the size fractions as well as the chemical contribution of the soil from Well A.

Table 38: The Contaminant Contribution and Grain Size Distribution of Soil Samples from Three Depths from Well A

Wet Soil Sample at Various Depths	Moisture Content (%)	Contaminant Concentration in Extracts		Size Fractions (%)	
		Moisture extraction	Rinsed- extraction	Sieve Size (mm)	Percent Retained
Depth:23 to 28 ft (QAL)	12 ±0.6	No liquid could be collected	Chromium= 21 ± 8 µg/L	9.5	3.19
				4.75	8.09
			Nitrate= 10.38 ± 1.7 mg NO_3/L	0.85	26.44
				0.425	11.06
			Perchlorate = 7.1215 ±1.7 mg/L	0.075	43.96
				pan	7.25
Depth:31 to 36 ft (Intermediate)	50 ±3	Chromium= 35 µg/L	Chromium= = 17±10 µg/L	9.5	6.10
				4.75	1.80
		Nitrate= 1183 ±0.42 mg-NO_3/L	Nitrate= 140.30 ± 1.0 mg NO_3/L	0.85	14.84
				0.425	11.47
		Perchlorate = 1333 ±14 mg/L	Perchlorate = 50.2101 ± 1.1 mg/L	0.075	45.51
				pan	20.24
Depth:43 to 48 ft (UMCf)	42 ±4	Chromium= 20 µg/L	Chromium= 26 ±13 µg.L	9.5	10.36
				4.75	0.00
		Nitrate= 1182 mg-NO_3/L	Nitrate= 40.10 ± 2.9 mg NO_3/L	0.85	8.00
				0.425	3.98
		Perchlorate = 1282 ±37 mg/L	Perchlorate = 45.6394 ± 1.1 mg/L	0.075	42.3
				pan	35.7

As can be seen in Table 38, the deeper the soil strata, the larger the percentage of the finest fractions of soil. The moisture extraction contaminants were fairly similar in the two deeper soil horizons. Table 39, meanwhile, shows the contaminants found in the soil.

Table 39: Contaminant Amounts in Soil from Different Depths

Soil Depth (ft)	Cr(VI)	Nitrate	Perchlorate
	(µg/kg dry soil)	(mg NO_3/kg dry soil)	(mg/kg dry soil)
QAL (23-28 feet)	150 ± 50	70.04 ±10.10	47.30 ±8
Intermediate (31-36 feet)	240 ±140	552.69 ±12.25	688.49 ±16.77
UMCf (43-48 feet)	300 ±140	466.44 ±31.63	530.85 ±15.78

Note: Extract volume was multipled by measured concentration to determine each extract's contaminant mass. The amount of dry soil (accounting for moisture content which was computed) was used to divide the previously calculated mass.

As shown in Table 39, greater amounts of the three contaminants were extracted from the two deeper soil horizons, compared to the shallowest horizon.

A-2.2 Extracted Contaminants by rinsing (Well C-S, Well D-D)

Table 40 shows the extract contaminant levels measured in the soils from Well C-S and Well D-D.

Table 40: Contaminant Amounts in Soil

Soil Depth (ft)	Cr (VI)		Nitrate		Perchlorate	
	Average± standard deviation		Average± standard deviation		Average± standard deviation	
	(µg/L)	(µg/kg dry soil)	(mg/L)	(mg NO_3/kg dry soil)	(mg/L)	(mg/kg dry soil)
Well C-S, QAL (18.3 to 23 ft)	6.08± 0.13	38.1 ± 0.9	18± 0.5	112.67±0.9	37 ±2.7	232.41 ±16.4
Well D-D, UMCf (33.5 to 38.5 ft)	1271 ± 114	5378±47	16 ± 0.5	69.38±1.5	71 ±1.4	302.41±8.5

In this case, the deeper soil had far higher Cr(VI), higher perchlorate and lower nitrate than the shallower soil from a different well.

A-2.3 Groundwater Sample Characterization

Table 41 summarizes the contaminant values from various groundwaters collected.

Table 41: UMCf And QAL Groundwater Characterization from Certain Wells. The Unshaded Region Values are Average Values from Multiple Samples or Mixed Samples. The Shaded Region Shows the Analyses Results of Total Metals

Constituent	Well B (7/22/16)				Well C-S (12/7/2017)		Well D-D (12/7/2017)	
	QAL		UMCf		QAL		UMCf	
	Conc.	St. Dev.	Conc.	St. Dev.	Conc.	St. Dev.	Conc.	St. Dev.
COD (mg/L)	38.1	0.56	10.2	1.58				
Phosphate (mg/L as PO_4)	0.73	0.26	0.753	0.148				
pH	7.33	0.139	7.30	0.075				
Nitrate (mg/L as NO_3)	1125	40.3	598	36.5	494.52		230	
Ammonia (mg/L as N)	242	2.16	291	2.08				
Perchlorate (mg/L)	1361		1370		422 (406)	11.3	501 (491)	7.07
Sulfate (mg/L)	1223	20.6	1210	42.4				
Cr(VI) (ug/L)	23.5	20.9	40	34.6	17000		21000	
Hardness (mg/L as $CaCO_3$)								
Chlorate (mg/L)					3500		3600	
Turbidity (NTU)	579	688	5.95	6.35				
Aluminum (µg/L)	51.3		13					
Arsenic (µg/L)	114.5		130					
Barium (µg/L)	51.3		26					
Cobalt (µg/L)	1.4		1.5					
Chromium (µg/L)	7.6		20.8					
Copper (µg/L)	2.9		1.1					
Iron (µg/L)	81.8		343					
Manganese (µg/L)	99.3		18.7					
Molybdenum (µg/L)	42.1		32.5					
Phosphorus (µg/L)	51.3							
Lead (µg/L)	16.8		14					
Zinc (µg/L)	10.6		12.9					
Boron (µg/L)	3.02		2.91					
Calcium (mg/L)	351		235					
Potassium (mg/L)	32.7		35.8					
Magnesium (mg/L)	157		137					
Sodium (mg/L)	779		649					
Silica (mg/L)	107		94.2					
Sulfur (mg/L)	468		476					
Strontium (mg/L)	7.05		7.24					

12/7/2017: one mixed sample was tested for each depth
Data in parenteses from Silver State Laboratory, NV

It is possible that variations, such as between measured Cr(VI) and total Cr, resulted from the different analytical methods used. It is evident from Table 41, that there can be wide variations in contaminant concentrations between wells and well depths.

Baseline field data were also provided by the client and are presented in Table 42 for well C-S. These data provide additional site information with such parameters as alkalinity, temperature, DO, etc. included.

Table 42: Select Baseline Field Data from Well C-S (4/5/17)

Parameter	Date	Alkalinity	Bicarbonate Alkalinity	TDS	Hardness	Chromium	pH	Temperature	Specific Conductivity	DO	Ferrous Iron
Value	4/5/2017	140	140	8700	1700	13000	7.34	27.82	9.35	1.88	0.00
Units	AD	mg/L as $CaCO_3$	mg/L as $CaCO_3$	mg/L	mg/L as $CaCO_3$	µg/L	—	ºC	mS/cm	mg/L	mg/L

APPENDIX B

The counts and diversity of bacteria from certain microcosms are shown in Figure 31, while Figure 32 shows those for chromium reducing bacteria in certain QAL microcosms.

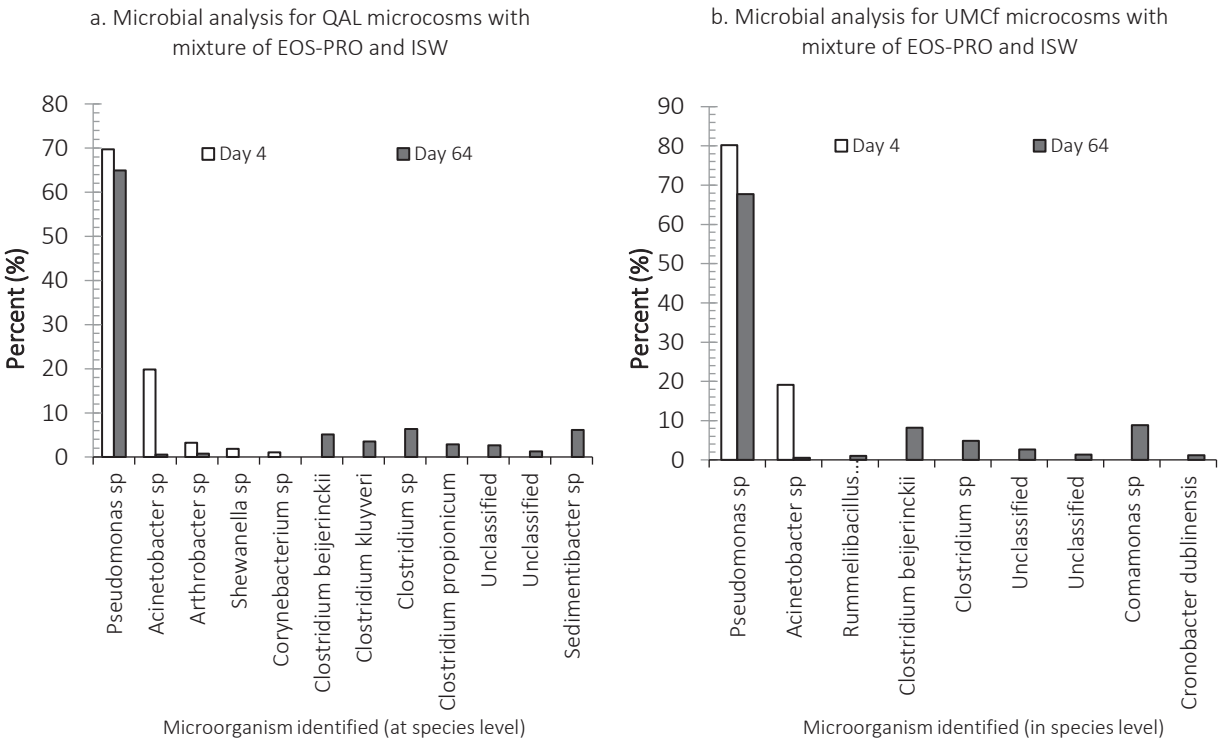


Figure 31: Microbial diversity for MIX microcosms based on DNA analysis for (a) QAL (organisms/gram of soil:4.57 x 10^8 on day 4 and 3.87 x 10^8 on day 64) and (b) UMCf (organisms/gram of soil: 2.99 x 10^8 on day 4 and 1.07 x 10^8 on day 64)

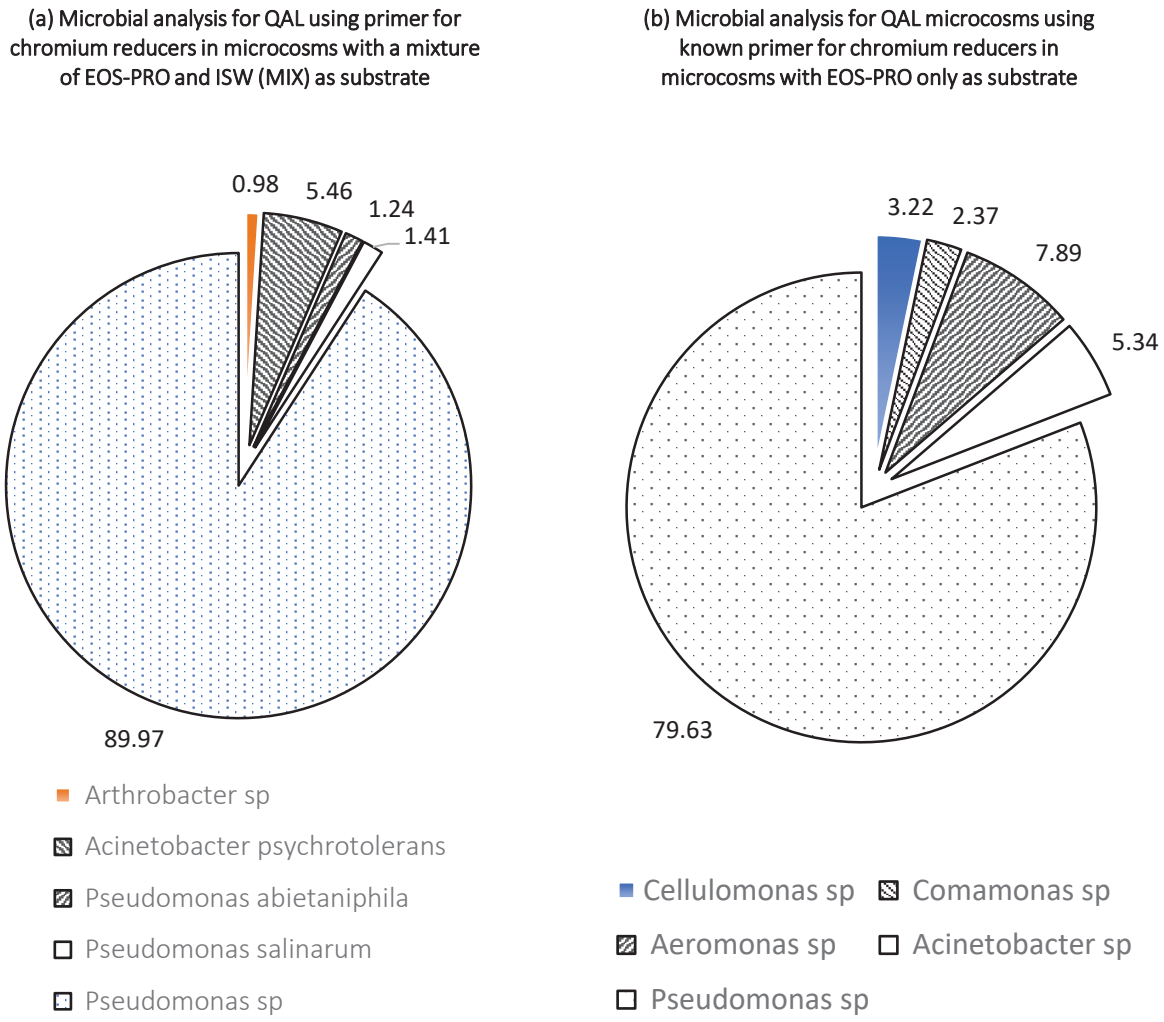


Figure 32: Microbial diversity for (a) MIX microcosms (organisms/gram of soil: 1.07 x 10^8 on day 64) and (b) EOS-PRO microcosms (organisms/gram of soil: 8.02x10^7 on day 64), in QAL using primer for chromium reducing bacteria.

APPENDIX C

The graphs of contaminant concentration versus Empty Bed Contact Time (EBCT) are shown in Figure 33.

Figure 33: Contaminant Concentrations vs. Empty Bed Contact Times

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