Bioremediation of Chlorate and Chromate in Cold Temperature Using Manitoba's Native Microorganisms

By

Mehdi Motevasselin

A thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

Department of Civil Engineering

University of Manitoba, Winnipeg, Manitoba

Author's Declaration

I declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Groundwater and soil contamination is a growing concern worldwide for public health officials. Chlorate and Chromium (VI) are two chemical contaminants with adverse health effects found separately or together from anthropogenic sources. Several remediation methods were developed to eliminate these compounds from the environment. These ions could be reduced to less toxic and less-mobile forms by physicochemical or biological treatment strategies in ex-situ or in-situ processes. Bioremediation is an approach that employs microorganisms' capability to neutralize pollutants and mostly does not leave any undesirable footprint on the environment. Bioremediation of chlorate and chromium (VI) needs electron donor materials to reduce them to harmless chemicals. The most suitable electron donor is acetate due to its commercial availability and non-fermentable specification.

In this study, synthetic groundwater was prepared based on the in-situ mineral groundwater composition and inoculated with native microbiota derived from a site that experienced a high concentration of the ions because of historical activities in the past. Acetate was chosen as an electron donor, and the effect of adding macro-nutrients (phosphorus and nitrogen source), micro-nutrients (ATCC 1191 minerals solution), and temperature drop were investigated. Chlorate and hexavalent chromium, with initial concentrations of 1000 mg/L and 3 mg/L, respectively, were successfully removed from the environment when the electron donor and macro-nutrient additives were present in the media. Adding minerals solution did not change the performance of the reaction. However, the removal rate in samples without added phosphorus and nitrogen sources was insignificant. Complete removal of the chlorate and chromium ions was observed at room temperature (21±1 ºC) after 50 days (35 days for chlorate), while at the reduced temperature (10±1 ºC), the removal occurred after 80 days. ATP (adenosine triphosphate) analysis revealed that the

microbial activity under cold conditions could reach similar higher temperature levels with some delays.

Acknowledgments

I would like to thank Dr. Beata Gorczyca for her consistent support and encouragement as a supervisor throughout my study and research. I also appreciate my advisory committee members: Dr. Richard Sparling, Dr. Indra Kathryn Kalinovich, and Dr. Sri Ranjan, for their support and valuable comment. The appreciation is extended to Dr. Victor Wei for his time and efforts that keep the environmental lab running, Dr. Gary Anderson for letting us use his lab and instruments, and Dr. Tom Ward.

Finally, I need to appreciate Saeideh's patience and guidance in this journey.

Mehdi

Structure of the Thesis

This is a manuscript (sandwich) style thesis with two manuscripts that consists of contributions from Mehdi Motevasselin (author), Dr. Beata Gorczyca Dr. Richard Sparling, and Dr. Indra Kalinovich, Dr Ramanathan Sri Ranjan (co-authors).

Chapter 1 states the problem and necessity of the research, motivations, background, and objectives.

Chapter 2 is a literature review on chlorate and hexavalent chromium as environmental contaminants, their chemistry, and remediation strategies. The section looks at biological treatment methods and research involved in bioremediation to remove pollutants at the end.

Chapter 3 provides information on the materials and methods used in this research, including descriptions of the media preparation, chemical and biological microcosm study, sampling procedure, analyzing instruments, and acid digestion process.

Chapter 4 contains the results and discussions of the microcosm study included in a manuscript to be submitted to the Groundwater Monitoring & Remediation journal.

Chapter 5 contains the results and discussion of bioremediation studies in continuous column experiments.

Chapters 6, 7, and 8 focus on the conclusion, engineering significance of this research, and recommendations for future work.

Contribution of Authors

Experiments described in Chater 4, the microcosm study in warm and cold conditions, were entirely done by the primary author. The committee members, Dr. Beata Gorczyca, Dr. Richard Sparling, and Dr. Indra Kalinovich, provided experimental design input and commented on and corrected the manuscript.

The second manuscript (Chapter 5) contains the batch tests and column study results written based on collaborative work with Kieran Milner, who did the batch tests and GC measurement, and Emiley Kiely-Smith, who collected samples from the columns and analyzed the data.

The principal author used his data for the chemical study batch test and the results of other tests to write the manuscripts under the supervision of Dr. Beata Gorczyca, Dr. Ramanathan Sri Ranjan, Dr. Richard Sparling, and Dr. Indra Kalinovich.

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Chapter 1: Introduction

1.1 Research Motivation

Historically, dumping chemicals in waste lagoons was common in chemical production industries in the 20th century. In an industrial area in Manitoba, lagoons that contained brine sludge from a chlorate spill were covered by soil in the 1990s. Remedial excavations were conducted in 2001 and 2008 to remove the brined sludge and sludge-impacted soils for off-site disposal. The current impacts in groundwater at the site are related to the historical brine sludge disposal and historical site operations and consist of a co-mingled plume with elevated electrical conductivity, chlorate, and chromium (including hexavalent chromium) concentrations. The groundwater flows from the area to the Assiniboine River, which downgradient communities use as a water source for agricultural and/or potable purposes.

Establishing an efficient, cost-effective treatment method that reduces both co-contaminants to acceptable levels is the primary purpose of this research. Determining the necessary materials and additives, investigating the effect of temperature (as the site is considered to be in 'cold regions'), and studying the potential consequences of the treatment method are the other goals.

1.2 Background

The contaminated site is in Brandon, Manitoba, next to the Assiniboine River. It is considered a cold climate site (Stempvoort and Biggar, 2008), with an average air temperature of 2.7 ± 1.1ºC and an annual average groundwater temperature of 7.2 ± 1.2ºC (Province of Manitoba, 2017). The average annual precipitation received at the site is estimated to be 461.7 mm of precipitation. Based on the data for the snow depth at month-end, the ground of the area is covered by snow for seven months of the year (Government of Canada 2021).

The site's surficial sediments consist of alluvial and glaciofluvial deposits (coarse sand and gravel). The area's ground consists of a layer of sand and gravel (ranging in thickness between 3 and 7 m) on top, followed by a clay till (2 to 4 m in thickness), a thick gravel layer (1 to 2 m), and an undetermined depth of a till layer at the bottom.

Groundwater flows towards the Assiniboine River with an average horizontal hydraulic gradient of 0.031 m/m and an average hydraulic conductivity of 1.8 x 10^{-5} m/s. Depending on the distance from the river and the location of the monitoring well aquifer zone, the groundwater depth ranges from 2.2 m to 6 m. Surface water contamination is likely to happen since the river's level rises and falls in different seasons, exposing the shore to groundwater, directly discharging into the river.

1.3 Problem Statement

The importance of preserving subsurface aquifers from contaminants is undeniable while they maintain one-third of the world’s freshwater, forty percent of agricultural demand and a fourth of industrial need (IAH 2020). In Manitoba, thirty percent of the population relies on well-water for their consumption and 21 out of 48 municipalities in the province combine groundwater with surface water or deliver it alone in their distribution systems (Rutherford 2004).

The current impacts in groundwater at the site are related to the historical brine sludge disposal and historical site operations and consist of a co-mingled plume with elevated electrical conductivity, chlorate, and chromium (including hexavalent chromium) concentrations. Maximum and average chlorate concentrations (4,200 mg/L and 1,400 mg/L, respectively) and chromium concentrations (3.02 mg/L and 0.75 mg/L, respectively) have been observed in the downgradient plume.

Although research is available for perchlorate remediation, minimal research is present for the bioremediation of chlorate in soil and groundwater. There are limited studies on the viability of chlorate bioremediation under cold conditions. Several studies have examined the bioremediation of chlorate and perchlorate at ambient temperatures (Hunter, 2002; Jin, 2012). Based on a review of the available literature, the effect of low temperatures has not been assessed in terms of chlorate bioremediation. Therefore, it is unknown if the low groundwater temperatures at the site (<10°C) will support chlorate bioremediation.

1.4 Research Hypothesis and Objectives

The main hypothesis is that presence of electron donors and available nutrients in in-situ bioremediation are limiting factors to reduce chlorate and chromium (IV) simultaneously. The following objectives were determined to examine this hypothesis:

- Determining the ability of indigenous microorganisms to detoxify chlorate and chromium (VI) to identify the necessity of bioaugmentation or ex-situ bioremediation as an alternative option for decontaminating the site
- Analyzing the effects of acetate and nutrients as the limiting factors of biological stabilization of chlorate and chromate
- Investigating the impact of lower soil temperate on the in-situ bioremediation during Manitoba's long winters

Chapter 2: Literature Review

This literature review introduces chlorate and hexavalent chromium, their chemistry and application in the industry, and their abundance in the environment. After that, the health concerns of these two ions and regulated values in the world and Canada are explained. Remediation methods and physicochemical techniques used to remove the ions in industrial waste streams and contaminated sites are described in the next section. Biological treatment technologies, including phytoremediation and bioremediation, are elaborated as the processes with minimum environmental concerns. The latter part consists of a literature review on bioremediation, required conditions and materials, affecting parameters, and the efficiency of using microorganisms to eliminate chlorate and chromium (VI).

2.1 Chemistry of Chlorate and Chromate

2.1.1 Chlorate

Chlorine is the seventeenth element in the periodic table with seven valence electrons and the capability to obtain oxidation states ranging from -1 to +7. Chloride anion (Cl^-) has the lowest free energy change ($\Delta_f G^0 = -131$), which makes it persistent in the environment, while chlorite (ClO_2) and hypochlorite (ClO^-) are strong, active oxidizers tend to react quickly and reduce to chloride (EnviroGulf Consulting 2007).

Perchlorate and Chlorate. Perchlorate is an oxyanion of Cl^{+7} surrounded by four oxygen atoms; an arrangement makes it reluctant to oxidize despite its high redox potential. As a result, the ion is very stable and does not break down spontaneously unless in the presence of active microorganisms and their desired condition. Perchlorate salts are highly soluble in water, and they can reach 2000 g/l of concentration in the form of sodium perchlorate. The second stable oxyanion, chlorate, occurs when perchlorate loses an oxygen atom, and chlorine's oxidation state reduces to

+5 (Figure 1). It is also manufactured in various salts like sodium chlorate and calcium chlorate in industry and agriculture. Reports show an inventory of 1 million tons in the U.S from domestic production and importation in 2011(US EPA 2016).

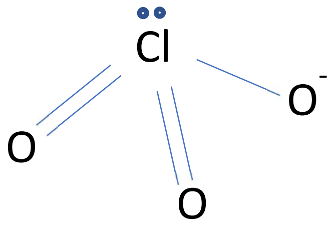

Figure 1 - Chlorate ion chemical structure

Although this ion is used in oxidizing chemicals and bleaching agents, it remains in the environment unchanged unless certain reducing conditions are provided(EnviroGulf Consulting 2007). The salts are non-volatile with negligible vapour pressure and highly soluble in water, making them mobile in the environment unless in the presence of redox reactions. (US EPA 2016).

Perchlorate and chlorate are two chlorine oxyanions known for their large oxidation capacity, making them suitable for various applications in the industry (Taraszki 2009). Perchlorate is the most stable form of chlorine oxyanions that may occur naturally in some arid regions (Srinivasan and Viraraghavan 2009). Chlorate is a semi-stable ion that can form from the dissociation of perchlorate in the reduction process. Still, it is rare to find in the environment, and elevated concentrations in groundwater indicate anthropogenic activity. The other two chlorine oxyanions, chlorite and hypochlorite, are not stable and decompose to oxygen and chloride ions (Coates and Achenbach 2004; EnviroGulf Consulting 2007)

Potential Risk to Environmental and Human Health. Chlorate has been identified as posing a potential risk to human health. The applicable Canadian federal criteria to chlorate concentrations are for the drinking water pathway at 1 mg/L (Health Canada 2016). There are no federal criteria for surface water or groundwater. However, the Province of British Columbia has published water quality guidelines that are protective of both agricultural (3 mg/L) and freshwater aquatic receptors (30 mg/L) (B.C. Ministry of Environment and Climate Change Strategy, 2020). The European Union decided to decrease chlorate's concentration in drinking water to 0.25 mg/L instead of 0.7 mg/L in 2020, and the higher value is only acceptable if "a disinfection method that generates chlorate, in particular chlorine dioxide, is used for disinfection of water" (E.P. and the Council 2020).

Uses of Chlorate in Industry. Sodium chlorate, calcium chlorate, and magnesium chlorate are common salts applied as bleaching agents or herbicides in the pulp and paper industry and agriculture (US EPA 2016).

2.1.2 Chromium (VI)

Chromium has an atomic number of 24 and, as the first element in group 6b of the periodic table, is a member of the transition metals family (IUPAC 2018). At ambient temperatures (20° C to 25° C), Cr has a silvery colour, and it is very hard, brittle, corrosion-resistant, and with high polishing capacity (Winter 2002).

Hexavalent and Trivalent Chromium. The electron configuration of the metal, having six electrons at the outer layer, allows it to take part in chemical bonding with six oxidation states, Cr^{+1} to Cr^{+6} (Schwarz 2010). The species ionized into cations with a positive 3 charges, Cr(III) and Cr (VI), are the most stable forms. Cr (IV) and Cr (V) occasionally appear as intermediate cations in the chemical reaction, and Cr(II) only exists if there is not any oxidant material (including oxygen) present (Kotas and Stasicka 2000). The element is the twenty-first most abundant one in the earth's crust. A high concentration naturally occurs in trivalent oxide form combined with iron (Brandhuber et al. 2005). Chromium (Cr) is a transition metal with six valence electrons that theoretically lets it take part in chemical bonding with different oxidation states of Cr(I) to Cr(VI) (Schwarz 2010). The metal and its ions have extensive applications in various industries such as metallurgical, refractories, and chemicals. Regardless of the many ions, it can form, only two cations, trivalent and hexavalent chromium are stable and may be found in the natural environment (Kotas and Stasicka 2000).

The hexavalent chromium species are highly soluble and transformable in the water, while trivalent chromium is only soluble below a pH of 4. By decreasing water acidity, $Cr(OH)_3$ becomes the dominant Cr(III) species in the aqueous phase. This transformation happens until pH 10 and at $pH \approx 8$ precipitates the most chromium out of the solution with the most negligible solubility (Brandhuber et al. 2005).

Thermodynamically the most stable ion of chromium is Cr(III). Almost all natural occurrence of the metal is in this form, and the presence of other states is a sign of human activities (Santonen, Zitting, and Riihimäki 2013). Due to the readiness for Cr(VI) to reduce to Cr(III) in the presence of an appropriate electron donor, there is not any natural occurrence of it on the earth's crust. Cr(VI) reduction by soil fulvic acid has been demonstrated to happen slowly at a high pH range. Any abiotic reductant with rich electron layers, including Fe(II) ions and H_2S, can accelerate the reduction reaction (Brose 2008). As a result, most hexavalent chromium sources are new in the environment and of anthropogenic origins (Barnhart 1997; Guertin, Jacques, Jacobs, and Avakian 2005). Hexavalent chromium can mostly be found in the systems at equilibrium with the air and occurs as both anionic species of $HCrO^{-4}$ and $CrO_4{}^{-2}$ that are soluble in water and considered highly mobile ions. Conversely, trivalent chromium forms chromium (III) hydroxides that are solid in neutral pH and precipitate (Świetlik 1998).

Potential Risk to Environmental and Human Health. The United States Environmental Protection Agency and World Health Organization consider hexavalent chromium as a carcinogenic compound that directly causes lung cancer in case of inhalation. It is also toxic if swallowed or absorbed through the skin and may cause allergic breathing and skin reactions. Some organs like blood, kidneys, eyes, skin, respiratory system, gastrointestinal system, and mucous membranes can be targeted by entering the human body (EPA 1998; WHO 2013, 2009). In Canada, the water quality guidelines for protecting freshwater aquatic life for hexavalent chromium and trivalent chromium are 1 and 8.9 µg/L, respectively (CCME, 1997).

Uses of Chromium in Industry. Cr(VI), as the second stable form of the chromium, has a wide range of applications in pigments production, anti-corrosion coatings (chrome plating, spray coatings), wood preservatives and fungicides, stainless steel and other high chromium alloys, leather tanning, and in chemical synthesis as an ingredient or catalyst. The apparent consumption of chromium in 2018 exceeded 500 thousand tons, and its worth was 1.2 billion dollars (U.S. Geological Survey 2020). Most of the chromium consumption (80 percent) in the world relates to the metallurgy industry, specifically producing stainless steel. The rest goes for wood preservation, leather tanning, paint and pigments, refractory and metal finishing (Barnhart 1997). Canada is estimated to own 20 million tons of chromite ore in different provinces, including Manitoba, but mainly relies on importation for its needs(Government of Canada 1994).

Hexavalent chromium may introduce to the body by ingestion, skin penetration or inhalation. Ingestion of 29 mg/kg body mass impacts the lung and blood system and might be fatal. Lower amounts may also cause bleeding in the gastrointestinal system, liver and kidney damage with diarrhea, vomiting, and indigestion (Guertin, Jacques, Jacobs, and Avakian 2005). Receiving low doses of Cr(VI) in drinking water increases the risk of stomach cancer around industrial areas in the long term (Beaumont et al. 2008). Comparing two cities in the same watershed in Taiwan indicates that living in areas with contaminated water and soil raises the risk of gastric cancer by 60 times greater (Tseng, Lei, and Chen 2018).

The skin reactions to chromium (VI) contacts could be differentiated into allergic contact dermatitis and skin ulcers. Dermal effects of exposure to concentrations in the range of 4–25 are primarily allergic reactions, while by increasing the concentration over 20 ppm, skin ulcers appear more frequently in non-sensitized people (Shelnutt, Goad, and Belsito 2007). The effects like rashes, ulcers, and sores may occur by contacting the solutions and fumes, mists, and vapours (WHO 2013).

The primary route of a carcinogenic effect of chromium compounds is inhalation. Although the trivalent ion is considered inert if it enters the body by breathing, chromate and di-chromates are classified in group A of inhalation cancer factors by USEPA (Guertin, Jacques, Jacobs, and Avakian 2005).

Recent studies revealed the mechanism of Cr(VI) in the respiratory tract and lung cells that result in cancer. Chronic inflammation and injury in the lung cause genomic instability after repetitive exposures and impact genes involved in DNA repairs that end up in cancer(Rager et al. 2019).

Due to Cr's toxic and carcinogenic nature (VI), it is regulated by authorized organizations. Table 1 contains the different regulated values for chromium in drinking water.

Table 1 - Regulated chromium concentrations in different areas(Bryjak et al. 2016)

Agency	Contaminant	Drinking water limit (µg/l)	Type of limit
USEPA	Total Cr	100	MCL
EU Drinking Standards	Total Cr	50	MCL
Canada	Total Cr	50	MCL
India	Cr (VI)	50	MCL
State of California	Total Cr	50	MCL
	Cr (VI)	10	MCL
	Cr (VI)	0.02	PHG
∗MCL=Maximum Contaminant Level; PHG=Public Health Goal			

Chromium (III) is considered a micronutrient necessary to metabolize sugar and fat in humankind, with a safe daily intake of 50 to 200 µg (Anderson 1997). Even though a regular diet may not contain the minimum level of that range, it is not recommended to receive extra doses anymore (Panchal, Wanyonyi, and Brown 2017). Trivalent chromium did not show any toxic effect by ingestion (when lower than 5 g/kg of body weight), skin allergic reaction, or asthma (WHO 2009). Although trivalent chromium can be found in living tissues in trace amounts, exposure to high concentrations for a long time might be harmful (Santonen, Zitting, and Riihimäki 2013)(WHO 2014).

2.2 Remediation Methods

2.2.1 Chlorate

Chlorate and perchlorate are non-volatile compounds resistant to photodecomposition (Couture 1998). Therefore, remediation techniques should be applied to remove them from the contaminated environment. Suggested processes should consider the final product's contamination scale, environmental circumstances, and characterization. Using ex-situ methods is more common to treat drinking waters, while in-situ practices are preferred for site treatment purposes (Taraszki 2009). Chlorate formation is one of the steps in the perchlorate reduction pathway, and most research on perchlorate reduction applies to chlorate. Several treatment methods studied the remediation of contaminated sites either with abiotic or biologic processes. Ion exchange by selective or non-selective resins, adsorption by activated carbon, membrane filtration, catalytic reactors, and chemical and electrochemical reduction are some non-biological ex-situ technologies proposed to remediate perchlorate(/chlorate). The biotic process using PRB (perchlorate reducing bacteria) currently involves more research due to its flexibility to be done in-situ or ex-situ in bioreactors (Taraszki 2009; Srinivasan and Viraraghavan 2009).

2.2.2 Hexavalent chromium

Hexavalent chromium forms highly soluble anionic species in water, hardly adsorbed by inorganic surfaces in the soil, making it a highly movable ion in groundwater(Gheju 2011). The abiotic treatment methods are based on two different attributes, physical removal and chemical reduction. The first method primarily removes the ion from the contaminated environment by adsorption in ion exchange units, filtration, or electrocoagulation processes and may need further treatments.

The chemical approach first targets the ion species to reduce them to less toxic (i.e., hexavalent chromium to trivalent chromium) (Bryjak et al. 2016). A variety of reductants was studied to reduce the contaminant chemically or biologically, and the reduction process is preferred since the reverse oxidation reaction hardly may occur(Lofù et al. 2016).

Kinetics experiments, simulating natural conditions, investigated chromium transformation in the aquatic and solid phases. Using batch microcosm and column experiments showed that the oxidation reaction from Cr(III) to Cr(VI) is relatively slower than the reverse reaction in the environment. The reduction reaction occurs with a half-life time of 15 minutes to 53 days, depending on aerobic or anaerobic conditions in the lake or river sediments. Yet, the value for oxidation reaction, which forms hexavalent chromium, has been reported in a range of 0.58 years to 37.2 years for similar media (Salem et al. 1989).

2.2.3 Physiochemical Remediation Methods

2.2.3.1 Chlorate

Ion exchange is a well-known method to remove oxyanions from water. Positively charged surfaces of resins, made from synthetic or natural polymeric materials, are coupled with an anion (usually chloride) with the ability to exchange with chlorate or perchlorate. (ITRC 2008). Using adsorbents like granular activated carbon is another standard method to remove unwanted materials from drinking water. Coating the adsorbent with a surface-active material can boost the capacity and increase the breakthrough time when perchlorate is targeted (Parette and Cannon 2005).

Electrochemical cells are another abiotic option to reduce perchlorate and chlorate. A nickel cathode coupled with a platinum anode considerably reduces perchlorate to chloride ions in a highly concentrated perchloric acid solution. The mechanism is controlled by chlorate formation, the first and slowest reduction step; the chlorate reduction occurs rapidly (Rusanova et al. 2006). The reaction rate constant was measured for chlorate and perchlorate at active titanium electrode in perchloric acid solution, and the result for chlorate was 10^5 greater than perchlorate's (Brown 1986).

Metal-based material like **zero-valent iron** is capable reduce oxyanions. The removal rate of perchlorate by elemental iron at neutral pH is relatively slower than the other methods and minimizes the ion by 66% in 2 weeks (Moore, De Leon, and Young 2003). The approximate pseudo-first-order rate constant for chlorate reduction at room temperature (20 ^{o}C) is $0.0262 \pm 0.003\ \text{min}^{-1}$, which is 50-fold of the perchlorate removal rate of $0.032 \pm 0.001\ \text{h}^{-1}$. Though the removal rate and yield with elemental iron are temperature-sensitive, heating the media up to 200 ^{o}C gives similar rates for perchlorate (Oh et al. 2006).

Some studies indicated the removal of chlorine oxyanions in **chemical reactors using catalytic beds and hydrogen gas**. Adding a rhenium-based complex to a combination of 5% Pd-carbon powder under a hydrogen atmosphere yields a bimetallic heterogeneous catalyst that decomposes perchlorate in batch tests at 20 ^{0}C (Keith D. Hurley, Zhang, and Shapley 2009). Efficient transformation of chlorate to chloride ion was reported based on a study using MoOx–Pd/C catalyst and 1 atm H_2 at room temperature in less than an hour (Ren et al. 2020). Cellulose-based catalysts coated with palladium, platinum, and iron oxide showed promising results in batch and continuous column tests for the chlorate hydrogenation process by reducing the concentration to 0 from 200 mg/L in 160 minutes (Sikora et al. 2021). With a similar initial chlorate concentration, catalysts prepared by spreading palladium nanoparticles on a commercial activated carbon base could reach 90% of removal in 3 hours. Since these catalysts are used in chemical reactors, it is essential to maintain their conversion rates for numerous cycles of reuse and regeneration (Sikora et al. 2020).

Another emerging method is **ultraviolet photolysis** of chlorate with commercially available U.V. lamps. The process depends on the U.V. wavelength, pH, irradiation time, coexisting salts, and reducing agents that might be added to the media(Kishimoto, Yamamoto, and Nishimura 2015) (Jung et al. 2017).

Both ion exchange and membrane systems are ex-situ treatment options and generate concentrated perchlorate and chlorate brine which need further treatment prior to disposal (Bardiya and Bae 2004). The remainder of the above techniques is costly for groundwater treatment and are more suitable for source control than removing dissolved contaminants in a downgradient plume adjacent to a river.

2.2.3.2 Chromium (VI)

Activated carbon is one of the oldest known methods to adsorb hexavalent chromium from aqueous solutions. The isotherms and effects of parameters like pH, temperature, particle size and other ions in the solution have been investigated since the 1970s. (Zheng, Kemeny, and Cook 1977)(Huang and Bowers 1979). Modifying activated carbon with zero-valent iron and silver bimetallic nanoparticles is a newer suggested approach to improve the removal rate and reduce contact time in the system(Kakavandi et al. 2014).

Some natural inorganic **zeolite or synthetic base anion exchanger resins** can adsorb Cr(VI) at low pH values. When the resin sites are saturated, they should be regenerated, which can be done by sodium hydroxide alkaline brine. The regeneration effluent is highly concentrated with hexavalent chromium that needs secondary processes to remove the ion. The technology is commercially available and mainly used to recover chromium from industrial wastewater streams(Guertin, Jacques, Jacobs, and Avakian 2005).

Despite the adsorption, chemical processes do not leave any hexavalent chromium for further stages and transform the ion completely to the innocuous trivalent form. These methods use electron donor materials to maintain the charge demand of Cr(VI) reduction. A reducing agent chemical could do them in a reactor or electrochemical cells.

The chemical method uses a reactor with a reducing solution like sodium metabisulphite in a low pH range to first reduce Cr (VI) to Cr (III). Then in the next step, the solution enters a neutralization tank. Sodium hydroxide (lonely or in combination with calcium hydroxide) is introduced to the tank to adjust the pH to a basic range, precipitating the trivalent chromium ions out of the solution. Finally, the slurry flows to a settlement tank, and the solid phase is removed (Ramakrishnaiah. C and Prathima.B 2012).

In electrochemical removal of Cr(VI), zero-valent iron oxidation occurs at the anode that produces Fe^{2+} and releases two free electrons in an acidic solution. The electrons at the cathode side reduce the chromium ion, which is easy to precipitate by changing the pH of the solution. Applying the technique to wastewater samples containing 1 g/l of chromium (VI) leads to 90 % removal when the media concentration increases to 100 g/l of sulfuric acid (Peng, Leng, and Guo 2019). A study on rinsing waters from a plating industry with concentrations up to 1700 mg/L of Cr^{6+} found the reduction process depends on ferrous iron release at the anode since iron salts decrease the electrode's efficiency. The process could reduce the concentration to less than 0.5 mg/L in case of eliminating the salt formation and raising the voltage (Martínez, Rodríguez, and Barrera 2000).

Photocatalytic degradation of harmful contaminants is becoming increasingly attractive for wastewater treatment of dilute streams, particularly with ultraviolet (U.V.) light-emitting diodes (LEDs), which are seen as reliable, continuous, and low-cost light sources

Photocatalysis is another chemical method that uses metal oxide catalysts to complete the reaction at a faster pace. The technique relies on photocatalysts' ability to generate electrons from water or other sacrificial reagents (used as an electron donor) on semiconductor metal oxide particles under U.V. light irradiation. Titanium dioxide, zinc oxide, sodium tantalate, and Tungsten trioxide are some materials with this ability. Titanium dioxide and zinc oxide showed the best performance in lab experiments by reducing hexavalent chromium from 1ppm to zero in 20 minutes at pH 7. Sodium tantalate is a better option when the pH is not concerned, and it is possible to adjust it to an acidic range as low as 3.5 (Ramakrishnaiah. C and Prathima.B 2012).

Zero valent iron (ZVI) was successfully tested as an electron donor, reducing chromate contaminations to chromium (III). Commercial ZVI produced from scrap iron, ductile cast iron, or steel is a less expensive material used in permeable barriers to decontaminate groundwater. Although the reactions are slower than other chemical methods, their capacity for in-situ treatments is worthwhile (Gheju 2011).

2.2.4 Biological Remediation

Although the physicochemical methods are effective in many areas, they mostly need expensive materials and equipment. Furthermore, undesirable by-products or waste might require further measures to be safely disposed of in the environment and eliminate the risk of transmigration of pollutants. These side effects and costs raise the necessity of developing sustainable methods at lower costs and risks. Biological treatments are the procedures that apply living organisms to detoxify chemicals in the environment with the least co-contamination and maintenance cost. These methods mostly fall under two categories based on the type of the organism: phytoremediation and bioremediation (Edwin-Wosu and Kinako 2007).

2.2.4.1 Phytoremediation

When the living plants are used to clean up the soil or groundwater, the method is called phytoremediation. Plants are resistant to harsh weather and only need sunlight as their energy source, which reduces the maintenance cost to a minimum. Their roots provide an excellent filtration system with an extensive environment for microorganisms to grow. (Edwin-Wosu and Kinako 2007).

Chlorate

Phytoremediation of perchlorate and chlorate has been vastly studied, and two effective mechanisms have been proposed to elaborate on how it happens: (1) uptake and phytodegradation; and (2) rhizodegradation. Uptake and phytodegradation adsorb perchlorate unchanged through the roots and send it to the leaves or body of the plant. If the plant contains reducing compounds, it starts to degrade the perchlorate by using solar energy. Otherwise, the contaminant accumulates in the body of the plant, and there will be a risk of entering the food chain by herbivorous animals. In the second mechanism, called rhizodegradation, the plant does not reduce the ion directly but stimulates microorganisms in the habitat soil by releasing some compounds. This increases the soil bioactivity and accelerates the bioremediation process in the root area. The availability of organic carbon or other electron donors in the soil is a limiting factor for the plants. Under appropriate conditions, perchlorate half-life could drop to less than an hour in the rhizosphere(Taraszki 2009).

A study in hydroponics bioreactors measuring perchlorate, chlorate and chloride concentrations in growth solutions indicated that the removal rate is relatively slow when only plant uptake is the dominant mechanism. Nevertheless, after forming a microbial community in the rhizosphere, the removal rate enhanced considerably and reduced perchlorate and chlorate concentration to zero without oxygen (Yifru 2006).

Chromium (VI)

Studies on chromium detoxification by phytoremediation suggest three main routes for the removal process, phytoaccumulation, phytostabilization, and phytoextraction.

Similar to perchlorate reduction, some plants are capable of uptake and accumulating large quantities of heavy metals like chromium. *Leptospermum scopari*um can phytoaccumulate hexavalent chromium and transform it to less toxic chromium-organic acid material, trioxalatochromium(III), in the tissue.

Plant roots decrease the chance of erosion and subsequent migration of the contaminants. Phytostabilization relies on the specific environment in the root zone for microorganisms to reduce Cr(VI) to Cr(III). These microorganisms may directly lead to reduction reactions or change the pH to reduce solubility and stabilize chromium as a solid.

Phytoextraction refers to the membrane capacity of the plant root. Some plant roots, which are long fibrous with high surface areas, adsorb chromium 10 to 100 times greater than stems, leaves or flowers(Guertin, Jacques, Jacobs, and Avakian 2005). Sorghum plant (Revathi, Haribabu, and Sudha 2011) and Pistia stratiotes (Prajapati, Meravi, and Singh 2012) are the plants were used in the studies and successfully removed chromium content in lab experiments from contaminated wastewater.

Other laboratory tests also revealed Raya (Dheri, Brar, and Malhi 2007) and Tuberose (Ramana et al. 2012) have the sportive capacity to remove chromium from contaminated soil. The studies on different plants with similar capability is still ongoing and researchers examine different plants in contaminated sites to find other species. Yet, the solution is not developed enough to ensure decontamination occurs by using the in polluted sites.

2.2.4.2 Bioremediation

Bioremediation uses microorganisms' abilities to reduce chemical contaminants. Oxyanions reduction results from an energy-yielding process under anaerobic conditions and the presence of electron donors (Suthersan and Payne 2005). Major group of the bacteria with the capability of reducing chlorate are called Perchlorate Reducing Bacteria (PRB) (He et al. 2019). Despite the toxicity of hexavalent chromium for living cells, the reduction can occur in the cytoplasm area of some gram-negative microorganisms, known to be chromium resistant(Dhal et al. 2013).

Chlorate

The presence of chlorate and perchlorate reducing microorganisms in different media like soils, sediments, groundwater aquifers, sludges, wastewater, animal waste, and other environments was investigated vastly and proven. This provides a variety of inoculum sources for ex-situ and in-situ bioremediation (Hatzinger et al. 2002). These microorganisms utilize electron-donating substrates under anaerobic conditions to remove oxygen from the oxyanions to conserve energy allowing them to produce biomass. If the electron donor is an organic molecule, carbon dioxide (if H_2 is not used as an electron donor) is also released. The existence of adequate electron donor materials in the environment is crucial for the remediation process. Several types of these materials were successfully tested in the experiments and field sites. Simple, pure chemicals like acetate, ethanol, glucose or more complex commercial products like vegetable oil, whey, molasses, and yeast extract are the most tried electron donors (ITRC 2005). Dissolved organic carbon present in water can also be used by reducing bacteria, and increasing the DOC levels causes a lower concentration of chlorate (Suthersan and Payne 2005).

PRB strains and the reduction conditions were studied in both lab experiments and deriving from contaminated sites. Wu et al. confirmed the capability of acetate, citric acid, lactate, and molasses as electron donors for perchlorate reduction. Complete removal of over 200 mg/L of perchlorate occurred in 4 to 7 days for wastewater samples and 8 to 29 days in creek water samples. They suggested the removal rate is dependent on cell concentration, and lower initial cell counts in creek water delay the completion of the process (Wu et al. 2001).

In a study, acetate was used and some other commercially available organic materials, including starch, yeast extract, chicken litter and biosolids. In this case, in less than a week, chicken litter and yeast extract removed perchlorate in the contaminated soil from over 150 mg/L to below detection limits. Their high organic material content, accompanied by other nutrients for microorganisms, can boost microbial growth at the initial stages and enhance the reaction rate (Kannepalli and Farrish 2016).

Still, the electron donor should be chosen considering some affecting parameters. The primary considerations are the capacity to remove oxyanions with the lowest possible ratio of reductant to oxyanion, the material's cost, and the process's type. For example, using commercial organic materials lowers the material cost but increases biomass residue in the environment. Although this is not a concerning matter in in-situ remediation, it increases the capital cost to design further treatments in ex-situ processes. Using hydrogen gas as an electron donor might be more suitable despite the gas production cost (He et al. 2019).

Effects of Temperature on Bioremediation. Studies showed that the temperature directly affects the reaction rates, and microorganisms faster reduce the perchlorate at a higher temperature. The final perchlorate concentration in flasks on a rotating shaker with increased turbulency was 80 percent of the initial at 15 ^{o}C after 30 hours, while increasing temperature improved the yield to complete removal at 40 ^{o}C.

The fast pace of industrialization and population growth increased environmental pollution dramatically. The vital need for a sustainable method to eliminate pollutants urged researchers and industries to substitute traditional methods like dumping the contaminated material or treating it with physicochemical processes. Employing microorganisms or plants to neutralize toxic materials, called bioremediation, is a cost-effective technique with the least environmental consequences, received significant interest in the past decades (Verma and Kuila 2019)(Megharaj, Venkateswarlu, and Naidu 2014).

The process could be done in-situ or ex-situ based on differences in cost, pollutant type, site condition, degrading process requirements, etc. In ex-situ treatment, the material is moved from the primary location to be treated in biopile, composting, land farming, or bioreactor. The in-situ approach includes the degradation of materials without removing them from their current system, using engineered processes like bioaugmentation and biostimulation. Bioaugmentation refers to transferring a specific type of microorganism to the polluted site when the native culture cannot detoxify chemicals. However, in biostimulation, the indigenous microorganisms could reduce the hazardous material if they receive the proper amount of nutrients(Azubuike, Chikere, and Okpokwasili 2016; Sharma 2020).

Most of the available literature regarding cold climate bioremediation is in terms of petroleum contamination which has shown success, albeit slower degradation rates, especially in anaerobic groundwater conditions (Yeung et al., 2013; Walworth et al., 2001; Jeong et al., 2015; Gomez and Sartay, 2013). There are limited publications on the application of bioremediation for chlorate remediation in cold climate scenarios. One study that evaluated the temperature effects on anaerobic perchlorate degradation using wastewater indicated that significant perchlorate biodegradation did occur at temperatures near 10°C. However, degradation rates decreased significantly under this temperature; confounding factors such as elevated dissolved oxygen and nitrate concentrations also minimized biodegradation (Dugan, 2009). Similarly, chlorate soil bioremediation using activated sludge occurred at 10°C; with minimal degradation at 5°C; however, this study was conducted in aerobic conditions in which chlorate biodegradation would be minimized in comparison to anaerobic conditions (Jiang et al., 2009). A study conducted at 15°C showed perchlorate concentrations of 260 ug/L decreasing to near non-detectable in 12 days using acetate, lactate, citrate, or ethanol as an electron donor (Taraszki, 2009).

Effects of pH on Bioremediation. The reduction process is sensitive to pH, and it stalls when the pH is a value of 9. Decreasing pH to lower than 8 accelerates the process and reaches its maximum at 6.85, and it slows down lower than 6 (Zhu et al. 2016).

Chromium (VI)

Chromium reducing bacteria is ubiquitous in the environment, and several strains were recognized to detoxify contaminated areas. The process mainly occurs when an electron donor is present in the environment, and cell density is high enough to start (Suthersan and Payne 2005). The bacteria can use environmental organic matter as an electron donor. If it is not available in the contaminated area, molasses, lactate, glycerol, acetate, yeast extract, gluconate, or fructose could be introduced to the environment. Some of these materials are capable of reducing hexavalent chromium chemically as well. Electrons are released in an exothermic process during cellular respiration, which facilitates the biological treatment of chromium. Also, the donors could mostly be used as carbon sources by microorganisms to grow in the environment and increase the cell density (Rahman and Thomas 2021).

Effect of organic carbon supplementation of different kinds on Cr(VI) reduction were studied in numerous lab tests. A recent study using molasses as an electron donor in groundwater samples containing 50 mg/L and 100 mg/L of chromium (VI) showed a significant removal after 40 hours (Yang et al. 2021).

Microcosms study on contaminated soil was done by Lara. et al. used acetate as an electron donor. The unsterilized soil samples containing 1.8 g/kg of Cr(VI) were entirely cleaned by adding acetate in 30 days at 30 ºC while sterilized soil with additional acetate and unsterilized soil without acetate did not show any significant removal. The test proves that coexistence of microorganisms and electron donor results in chromium reduction in soil (Lara, Morett, and Juárez 2017).

The effect of pH on chromium reduction using sugarcane molasses was investigated in a solution containing 20 mg/L of Cr(VI) at a constant room temperature 20 °C. The research concludes at pH levels lower than 2.6, chemical reduction of hexavalent chromium with molasses is the dominant reaction since the results for sterilized and unsterilized mediums are similar. By increasing the pH to higher than 3, the removal rate for the sterilized solution drops drastically, and bioremediation controls the reduction process (Yan and Chen 2019).

Temperature is another affecting factor in both chemical and biological processes. Laxman and More studied the effect of temperature on the removal rate in mixing reactors with 20 ppm Cr(VI) solutions and glucose as an electron donor. The results for hexavalent chromium measurements after 48 hrs for three temperatures, 28 °C, 37 °C, and 50 °C showed 60, 62, and 88 percent removal, respectively (Laxman and More 2002).

Chapter 3: Materials and Methods

3.1 Initial Solution

Several samples were collected from the site to find the composition and the worst case of chlorate and chromium concentration. Given the limited availability of site groundwater for the laboratory experiments, a synthetic, simulated initial solution was prepared to mimic the major and minor ion compositions. The initial solution was prepared by dissolving laboratory-grade chemicals and DI water (pure deionized water produced by the Millipore Elix-10 system with TOC<10 ppb and resistivity of 10 - 15 MΩ·cm.) based on *Table 2*.

Table 2- Simulated Initial Solution Composition

	Well data, groundwater		**Initial Solution in DI water**	
	(mg/L)	(mMol)		(mMol)
Chlorate	3900	46.71	Sodium chlorate (1000 mg/L chlorate)	11.97
Chromium(VI)	3.03	0.058	K_2CrO_4 (3 mg/L as Cr(VI))	0.058
Sulfate	672	9.73	0.1 g of $MgSO_4 . 7H_2O$	0.405
pH	7.25- 8.07		7.3-7.7	

Synthetic media in the text refers to the initial solution with added nutrients. Sodium acetate as electron donor, ATCC 1191 minerals solution as micronutrients, and phosphorus and nitrogen source. The additives are represented in *Table 3*.

Table 3- Nutrients added to the initial solution to prepare synthetic media

	Well data, groundwater		**Synthetic media**	
	(mg/L)	(mMol)		(mMol)
Phosphorus and Nitrogen	4.16 (P) 15.9 (N as nitrate and nitrite	0.134 (P) 0.3 (N)	0.5 g of $(NH_4)_2HPO_4$	3.786 (P) 7.57 (N)
Mineral mix			1ml/100ml	
Sodium acetate	-	-	2200 mg/L NaAcetate	37.263

3.2 Microcosm Study

The experiments were accomplished in two steps; first, they investigated the possibility of chemical reduction in sterile conditions and then studied the effect of bioremediation. All the glassware and DI water were sterilized before preparing solutions using an autoclave for 30 minutes at 121 °C. The biosafety cabinet was disinfected with a DNA-away solution and a U.V. lamp to prevent bacterial contamination, and finally, the solutions were filtered using 0.2 µm sterilized filters.

3.2.1 Chemical Reaction test

Three sets of triplicate bottles were filled with the solutions under anaerobic conditions and were left at room temperature (20 °C) for 30 days. The 30 days period is extended time based on a study that was done in 20 days and described in chapter five, the second journal manuscript. The first group contained the initial solution without any additional nutrients as the control set to verify the stability of chlorate and chromate in the aqueous phase. The second series was the solutions prepared with DI water, sodium acetate and chromate to observe the possibility of any chemical reaction at the mentioned temperature. The last series held synthetic media (initial solution + nutrients) to see any chemical reaction in the solution. 2ml samples were withdrawn from each bottle and tested for chromium(VI) content after 1, 3, 7, 15 and 30 days. The chlorate concentration of synthetic media was measured to check the possible chemical reaction of chlorate.

3.2.2 Biological Reduction test

The second step of the experiments was designed to investigate the bioremediation possibility by native microorganisms (present in soil and groundwater collected from the site), the effect of adding nutrients (acetate as electron donor and carbon source substrate, diammonium hydrogen phosphate as phosphorus and nitrogen source, and ATCC 1191 minerals mix), and consequences of a temperature drop on the process.

Five series of microcosms were prepared in triplicate groups to determine the affecting parameters on the bioremediation. The first group contained the initial solution and acetate labelled "C" (carbon source). The second series was made by adding acetate and the minerals solution to the synthetic water marked "C+M" (carbon source + minerals). Instead of the mineral's solution, diammonium hydrogen phosphate was added to the third group and labelled "C+NP" (carbon source + nitrogen and phosphorus source). The fourth series contained the synthetic media and was shown by "C+NP+M" (carbon source + nitrogen and phosphorus source + minerals mix). After inoculation, all the 12 bottles described above were placed in a dark cupboard at room temperature (20 ºC). The last three bottles were identical to group four, but they were stored in a cold chamber at 10 ºC after adding inoculum.

All the bottles at this stage were 500 ml volume filled with 400 ml of the solutions.

3.2.3 Inoculum

The inoculum should contain native microorganisms from the contaminated site and carry the least chemicals and minerals to the microcosms. Three sources of possible microorganisms were available from the area's: dry soil, wet soil (19% moist) and groundwater samples. Adam and Duncan's method (Adam and Duncan 2001) was used to verify the media's microbial activity. The groundwater's ATP activity was tested to confirm whether it contains any microorganisms. Finally, 4 g of the dry soil, 4 g of the wet soil, and 2 g of groundwater provided the inoculum for each bottle which was 2.5% wt of each microcosm.

Figure 2- Samples prepared for Fluorescein Diacetate Assay test

3.2.4 De-gassing and Sampling

After the inoculation, all bottles were closed with rubber caps that allowed syringe needle penetration and self-sealed after taking out the needle. They were all degassed for 5 minutes with a vacuum device and then filled with nitrogen for 2 minutes through a clean, sterile syringe needle (as shown below, in Figure 3). This cycle was repeated four times to ensure that the reactions proceeded under anaerobic conditions.

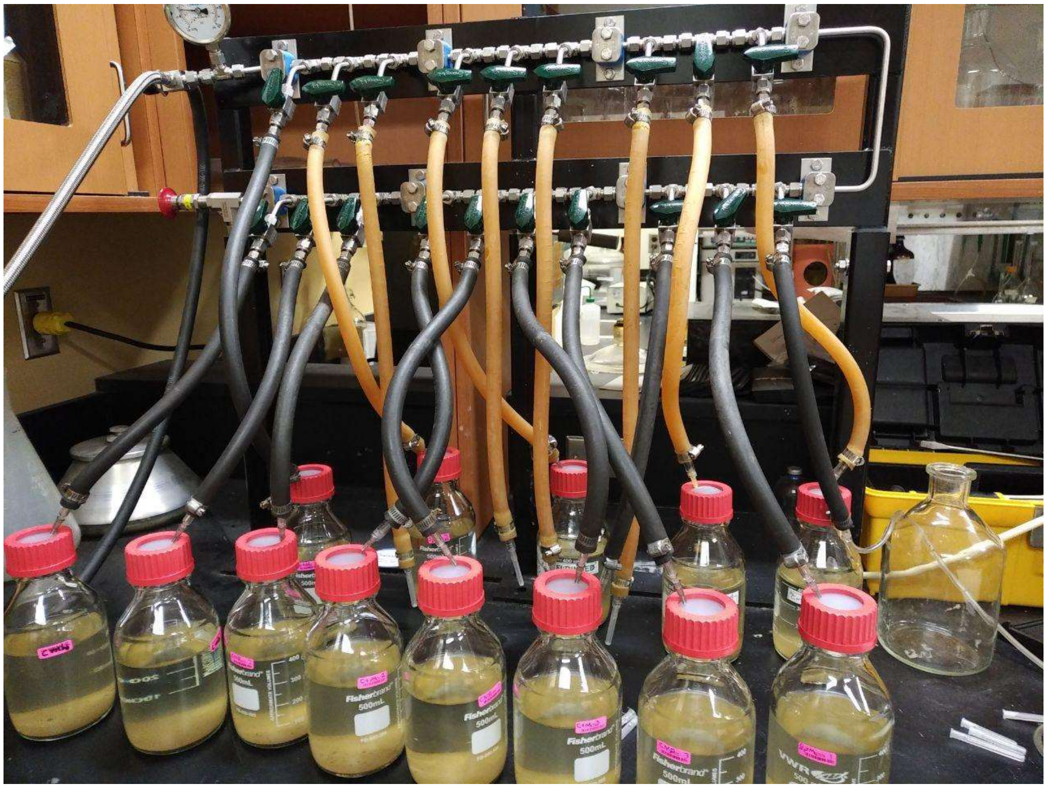

Figure 3 - Degassing process

On days 3,7, 14, 21, 28,35, 50, 56, and 80, 10 mL sample aliquots were withdrawn using a new sterile syringe and filtered through 0.45 µm filters after starting the biological reduction test. The intervals were selected based on the test results with different media, described in chapter five. Since they were taken just in 20 days, it was decided to have some median points to assure that the experiment is on track. The large gap occurred for the last test because it was interrupted by Covid lockdown.The degassing cycles were repeated after each sampling to avoid any aerobic reaction.

3.2.5 Analytical Methods

The solutions for the FDA (Fluorescein Diacetate Assay) method were prepared based on Adam and Duncan's procedure (Adam and Duncan 2001), and the final samples were put in Biochorm Ultraspec 2100 spectrophotometer to measure the fluorescein absorbance.

Hach method 8023 uses a HACH DR-3900 spectrophotometer to measure chromium (VI) of aqueous solutions directly in the range of 0-0.6 mg/L. By diluting 2ml of each sample in 8 ml of DI water, the concentration of hexavalent chromium would be in the instrument's detection limit between 0-0.6 mg/L. The result shown by the machine must be multiplied by 5 to remove the dilution effect. The test was repeated without dilution for the samples below 1 mg/L to ensure correct results.

Chlorate, chloride, and acetate ions were analyzed using a Metroohm 930 IC after diluting 100 times and 50 times lower concentrations, and the results were corrected based on the dilution factors.

The technique used to analyze total chromium was inductively coupled plasma optical emission spectrometry (ICP-OES) with the instrument model of Varian 725-ES made by Agilent.

3.2.6 Digestion of Solid Precipitate

Amongst the samples that showed the biological reduction capability, three bottles (one from each triplicate) were chosen as representatives to analyze solid precipitate that formed during in the microcosms. The solutions were shaken well to have thoroughly mixed slurry suspensions, and then 50 ml of each was transferred to a beaker. Each sample was filtered using a 0.45 µm (similar pore size to syringe filters) paper filter using a vacuum pump to remove all solid particles that might remain in the syringe filters. The sample filters and another clean paper filter as control were digested using sulfuric and nitric acids based on the EPA-3050B method. The final solutions completely dissolved papers and ions in the solid phase, making them proper for the ICP test. The final results obtained from the ICP test represented the amount of chromium in the solid phase of 50 ml of solutions.

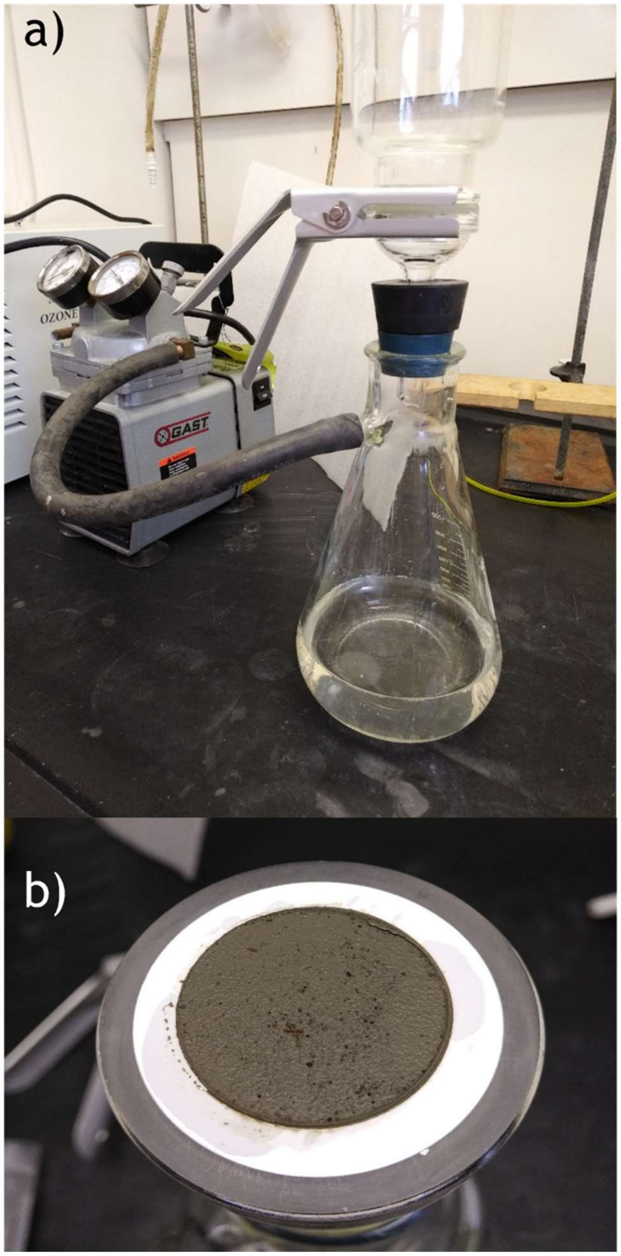

Figure 4 - Filtering the suspension, a) vacuum pump and the funnel b) a filter digested later

Figure 5 – Solid precipitate digestion equipment

Chapter 4: Journal Manuscript 1

Bioremediation of Chlorate and Chromium Contamination with Manitoban Native Culture in Cold Conditions

Mehdi Motevasselin[a], Indra Kalinovich[b], Beata Gorczyca[a], Richard Sparling[c]

[a]Department of Civil Engineering, [c]Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2 [b]Dillon Consulting Limited (email: motevasm@myumanitoba.ca, ikalinovich@dillon.ca, beata.gorczyca@umanitoba.ca, Richard.Sparling@umanitoba.ca

4.1 Abstract

Groundwater is an essential source of drinking water globally, therefore, needs to be protected from any contamination. Chlorate and hexavalent chromium are two chemicals with adverse health effects and may cause contamination in industrial areas. The objective of this study was to determine if the native microorganisms collected from site contaminated with chlorate and chromate can lessen the concentration of these chemicals to acceptable levels. Several anaerobic microcosms experiments were conducted with synthetic groundwater (media), native microorganisms, acetate as an electron donor, nitrogen and phosporous and minerals. The microorganisms utilized 2200 mg/L acetate to remove 1000 mg/L of chlorate and 3 mg/L of chromium (VI) entirely from the environment, provided that the groundwater is supplemented with additional nitrogen and phosphorous (with the CNP molar ratio of 100:10:5). The added trace minerals solution prepared based on ATCC 1191 did not improve the remediation process . A native microbial culture derived from a Manitoban contaminated site removed the chlorate and chromate from the supplemented groundwater at 20 °C in about 40 days. The same removal was

achieved at 10 °C, but in a longer timespan of 80 days.

4.2 Introduction

Groundwater is a vital drinking water resource that is sensitive to anthropogenic pollutants as it is a migration pathway to ecosystems. With increasing industrial development in North America, the quantity of released chemicals into the environment increases. Oxyanions with the generic formula of $A_xO_y^{z-}$ (where A represents a chemical element and O represents an oxygen atom) are compounds with various applications in the industry. There are several sites with increased contamination associated with past/historical operational practices and a lack of controls which are now mandatory. The balance between the negatively charged oxyanions and typical groundwater physicochemical properties provides oxyanions with the ability to be transported effortlessly in groundwater systems. Their release in groundwater (e.g., through agricultural herbicides and defoliants, or industrial effluent, waste, and/or discharge) may result in potential risk to human and/or environmental health, requiring effective and efficient in-situ treatments (Adegoke et al. 2013; Weidner and Ciesielczyk 2019; Sarria et al. 2019). Chlorate and chromium are common co-contaminants in groundwater at pulp and paper mills, sodium chlorate facilities, and electroplating industries. Given the scale of some of the contamination plumes, ex-situ methods may be cost-prohibitive. Stimulating bioremediation in groundwater can provide a cost-effective, sustainable process, with minimal inputs required for optimizing conditions for decontamination. The efficacy of bioremediation of oxyanions has not been evaluated for cold region conditions.

A sodium chlorate production facility located in Canada's prairie provinces is one of the world's largest and highest-producing sodium chlorate facilities. Since the 1960s, the facility has manufactured sodium chlorate by processing a brine solution (NaCl) through electrolysis to produce a purified crystalline powder readily soluble in water. The end product, sodium chlorate, is transported off-site in rail cars for use as an industrial bleaching agent in the pulp and paper industry. Due to historical waste storage practices and ongoing operations, groundwater at the site has been impacted by both chlorate and hexavalent chromium, used in the electrolysis production process.

In-situ remediation approaches for chlorate may not be applicable for other co-contaminants (e.g., hexavalent chromium) and may result in the undesirable mobilization of other metals (e.g., arsenic, barium) from native soils and/or associated with historical brine sludge waste. These sites often go unremediated (e.g., brownfields), or use costly, ex-situ techniques to control groundwater contamination (e.g., pump and treat).

Sample collection site. The site location is considered a cold climate site (Stempvoort and Biggar, 2008), with an annual average air temperature of 2.2 ± 1.1 °C (Government of Canada, 2017) and a yearly average groundwater temperature of 7.2 ± 1.2 °C (Province of Manitoba, 2017).

Investigations at the site have determined that surficial sediments consist of till and alluvial, and glaciofluvial deposits (coarse sand and gravel). Distinct soil types have been encountered at the site, including a top layer of sand and gravel (ranging in thickness between 3 and 7 m) followed by a clay till (2 to 4 m in thickness), a 1 to 2 m thick gravel layer, and finally followed by a till layer of undetermined thickness.

Groundwater flow direction has consistently been northeast towards the Nearby Unnamed River with an average horizontal hydraulic gradient of 0.031 m/m and an average hydraulic conductivity of 1.8×10^{-5} m/s. Depth to groundwater has ranged from 2.2 m to 6 m depending on the proximity to the nearby river and the monitoring well aquifer zone. As the Nearby Unnamed River water levels fluctuate with seasonal flooding events and regional precipitation, it is possible that near-shore groundwater sampling locations could represent a situation of direct discharge with little to no attenuation between the well locations and the river.

The current impacts in groundwater at the site are related to the historical brine sludge disposal and historical site operations and consist of a co-mingled plume with elevated electrical conductivity, chlorate, and chromium (including hexavalent chromium) concentrations. Maximum and average chlorate concentrations (4,200 mg/L and 1,400 mg/L, respectively) and chromium concentrations (3.02 mg/L and 0.75 mg/L, respectively) have been observed in the downgradient plume. Analytical results have shown that the majority of the chromium observed is in the hexavalent chromium form.

Although there is no particular value for chemical concentration in Canada's recreational water criteria due to insufficient data, chlorate is regulated to be less than 30 mg/L for freshwater aquatic life in British Columbia(Health Canada 2012) (British Columbia Ministry of Environment 2002). The maximum allowable concentration for chlorate in drinking water is restricted to 1 mg/L and for total chromium is 0.05 mg/L. In the Canadian Drinking Water Guidelines, the concentration of chromium is protective of health effects from chromium (VI) (Health Canada 2016).

Objectives

The main hypothesis is that presence of electron donors and available nutrients in in-situ bioremediation are limiting factors to reduce chlorate and chromium (IV) simultaneously. The following objectives were determined to examine this hypothesis:

- Determining the ability of indigenous microorganisms to detoxify chlorate and chromium (VI) to identify the necessity of bioaugmentation or ex-situ bioremediation as an alternative option for decontaminating the site
- Analyzing the effects of acetate and nutrients as the limiting factors of biological stabilization of chlorate and chromate

- Investigating the impact of lower soil temperate on the in-situ bioremediation during Manitoba's long winters

4.2.1 Chlorate

Chlorate (ClO_3^-) as an oxyanion is known for its strong ability to oxidize materials. This ion exists in various compounds like sodium chlorate, calcium chlorate, potassium chlorate, and magnesium chlorate, produced for different purposes in chemical manufacturing. Sodium chlorate is one of the most common salts used in agriculture as an herbicide and in the industry for bleaching papers or generating antiseptic water products (US EPA 2016).

Chloride and its inorganic oxidation states form five species ClO_4^- (perchlorate)/ ClO_3^- (chlorate)/ ClO_2^- (chlorite)/ ClO^- (hypochlorite)/ Cl^- (chloride). Chloride and perchlorate are the most stable forms, while hypochlorite and chlorite are unstable. Chlorate is also considered a chemically stable species in the environment, and it can form when a perchlorate molecule loses an oxygen atom in the degradation process(Coates and Achenbach 2004; EnviroGulf Consulting 2007) The natural occurrence of chloride oxyanions is not common, and they are mostly the result of human activities on the earth's crust (Bruce, Achenbach, and Coates 1999).

Several methods with different approaches have been used to remove perchlorate from contaminated sites and water. These techniques are categorized into three major groups: abiotic remediation technologies like ion exchange or activated carbon systems, abiotic reduction technologies such as chemical or electrochemical reduction, and biological remediation methods(Taraszki 2009; ITRC 2008)

Researchers primarily focus on perchlorate reduction, a more common challenge globally. The research is applicable for chlorate remediation since it forms in perchlorate reduction steps and is reduced under similar conditions (Carlström et al. 2015; Bruce, Achenbach, and Coates 1999;

Taraszki 2009)

Perchlorate and chlorate act as electron acceptors in a series of biological reactions in the environment to reduce to other oxyanions, and, finally, chloride as the most stable species(Malmqvist, Welander, and Gunnarsson 1991; Kotlarz et al. 2016). Several studies have shown that the microorganisms which can reduce chlorine oxyanions in the presence of suitable electron donors naturally exist in different anoxic microbial ecosystems such as soil, groundwater aquifers, sediment, sludge, animal waste and hypersaline soils (Achenbach et al. 2001) (Hatzinger et al. 2002) (Wu et al. 2001) (Bardiya and Bae 2005) (Acevedo-Barrios et al. 2019).

Many electron donors have been tested for biodegradation of (per)chlorate. It is verified that microorganisms utilize organic carbons and start the reduction route in the presence of organic carbons. Perchlorate and chlorate are used as the terminal electron acceptors for the oxidation of a wide range of defined organic carbon compounds, including methane, acetate, methanol, glucose, saccharose and succinate, as well as vegetable oil, starch, yeast extract and even chicken litter (Lian et al. 2017; Luo et al. 2015; Hunter 2002; Kannepalli and Farrish 2016) The reduction process of perchlorate starts with transferring the electron and changing the ion to chlorate, then chlorite and hypochlorite and finally ends with chloride ion(Rikken, Kroon, and Van Ginkel 1996).

“Perchlorate-reducing bacteria (PRB) are a phylogenetically diverse group of microorganisms capable of growth by respiring perchlorate as the sole electron acceptor” (Kotlarz et al. 2016)

4.2.2 Hexavalent chromium

As a transitive metal with six valence electrons, chromium can receive different oxidation states from -4 to +6. Still, there are only three stable forms in nature: Cr^0 (or metallic chromium), Cr(III) and Cr (VI). (Guertin, Jacques, Jacobs, and Avakian 2005). Because of the specific properties of chromium ions, they are being used vastly in different industries. They are used for pigments and dyes and catalysts, tanning, or wood preservation (Lunk 2015). Chromium deposits in the earth's crust are in the form of trivalent chromium, and finding high concentrations of hexavalent chromium is a footprint of human activity.

Chromium (III) is considered a micronutrient necessary to metabolize sugar and fat in humankind, with a safe daily intake of 50 to 200 µg (Anderson 1997). Even though a regular diet may not contain the minimum level of that range, it is not recommended to receive extra doses anymore (Panchal, Wanyonyi, and Brown 2017). Trivalent chromium did not show any toxic effect by ingestion (when lower than 5 g/kg of body weight), skin allergic reaction or asthma in the studies (WHO 2009).

This means reducing this ion to a lower oxidation state decreases its toxicity in contaminated environments (Guertin, Jacques, Jacobs, and Avakian 2005).

Chromium (VI) can participate in several chemical and biological reactions in nature. Studies showed that chromium-reducing bacteria are ubiquitous in soil and groundwater samples, facilitating the reduction process under aerobic and anaerobic conditions (Kamaludeen et al. 2003). These microorganisms can utilize organic carbon of the environment as electron donors and transform hexavalent chromium to the innocuous trivalent form. Molasses, lactate, glycerol, acetate, yeast extract, gluconate, or fructose are some commercially available electron donors that could be added to the environment if the organic matter is not sufficient (Rahman and Thomas 2021).

Acetate is the most prevalent electron donor for bioremediation experiments since it is not fermentable, and it selects for organisms capable of anaerobic respiration. Being directly measurable makes it easy to monitor the consumption to keep track of the environment's carbon ratio.

Besides the electron donor materials, adding other nutrients microorganisms need may benefit the reduction rate. Trace minerals are essential for the growth of all life forms, and supplementation is necessary for the growth of organisms in the laboratory in defined media (Froese and Sparling 2021). Nitrogen and phosphorous are two essential elements for making the building blocks of living organisms, and they have been shown to be biomass limiting elements at some perchlorate remediation sites (Wang et al. 2013).

Also, the effect of temperature is well-known on microbial activities and biodegradation processes (Bardiya and Bae 2011). The experiments were designed to: firstly, show the effect of adding electron donors and other nutrients, including micronutrients (mineral's solution) and macronutrients (nitrogen and phosphorus sources) in the environment containing native microorganisms from the site; and secondly, investigate whether the reduction is possible to be done at lower temperature or not.

A wide variety of bacteria have been reported for reducing/ Cr(VI) to Cr(III), under aerobic and anaerobic condition, e.g. *Escherichia coli Pseudomonas fluorescens Intrasporangium* Sp. Q5-1, *Bacillus* sp. ES29, , *Enterobacter cloacae*, LB300" (Ilias et al. 2011).

While PRBs (perchlorate reducing bacteria) are specific organisms that can reduce chlorate with their specific enzymes, chromium hexavalent reduction is unspecific and could be done by variety of microorganisms. Chromium biological reduction is facilitated by non-specific enzymes (mostly called oxidoreductases) that can transfer electrons to the outer valence layer of metallic ions. (Rahman and Thomas 2021).

4.3 Materials and methods

4.3.1 Synthetic water

A synthetic initial solution, as shown in Table 4 was developed based on site-specific groundwater analytical data. The components of the initial solution were purchased from Sigma-Aldrich chemicals, and they were dissolved in pure deionized water produced by the Millipore Elix-10 system with TOC<10 ppb and resistivity of 10 - 15 MΩ·cm.

A decreased chlorate concentration was used to observe results in a shorter timeframe while still demonstrating the indigenous microorganisms' capability to reduce the ions. Nitrate and nitrite were not added, and only an anabolic amount of sulphate was added to prevent competition from denitrifying and sulphate-reducing bacteria. Since potassium chromate is a colourful compound, it gives a pale-yellow colour to the solutions and changing the colour could be a sign of chromate reduction in the media. Using synthetic media makes it easier to control the concentration of materials since groundwater contains variety of trace materials with unknown effect on the process(shown in the appendix).

Table 4- Initial solution composition

	Well data, groundwater		**Initial solution with DI water**	
Chemical	(mg/L)	(mMol)		(mMol)
Chlorate	3900	46.71	Sodium chlorate (1000 mg/L chlorate)	11.97
Chromium(VI)	3.03	0.058	K_2CrO_4 (3 mg/L as Cr(VI))	0.058
Sulfate	672	9.73	0.1 g of $MgSO_4 . 7H_2O$	0.405
pH	7.25- 8.07		7.3-7.7	

4.3.2 Synthetic Media

Synthetic media of the microcosms by adding the electron donor and nutrients with the concentrations shown in Table 5. Based on the stoichiometry of the chlorate reduction reaction, every 3 moles of chlorate need 4 moles of acetate to complete the catabolic and biosynthetic reactions and produce one mole of chloride ion (Rikken, Kroon, and Van Ginkel 1996).

$$CH3COO^{-} + \frac{4}{3}ClO3^{-} \rightarrow 2HCO3^{-} + 2H^{+} + \frac{4}{3}Cl^{-}$$

Ammonium phosphate was used as nitrogen and phosphorus sources, and ATCC 1191 minerals solution was added to the medium to ensure sufficient trace elements that the bacteria may need. Acetate concentration was selected four times greater than the minimum required, and the C:N:P ratio was 100:10:5 to avoid any limitation by the nutrients.

Table 5 - Additives for preparing synthetic media

	Well data, groundwater		**Synthetic media with DI water**	
Chemical	(mg/L)	(mMol)		(mMol)
Phosphorus and Nitrogen	4.16 (P) 15.9 (N as nitrate and nitrite)	0.134 (P) 0.3 (N)	0.5 g of $(NH_4)_2HPO_4$	3.786
Mineral mix (ATCC 1191)			1ml/100ml	
Sodium acetate	-	-	2200 mg/L NaAcetate	37.263

All the glassware and solutions were sterilized before starting the experiments, and the final media was filtered through 0.2 µm filters.

4.3.3 Inoculum

The ability of the native microorganisms is essential to any in situ bioremediation process. In this experiment, three possible sources of native microorganisms were available, which were taken out from a depth of four meters dry soil, wet soil (19% moist), and groundwater. It is crucial to transfer the minimum material to microcosms to avoid changing the concentration of materials while native microorganisms are transferred to the samples.

Adam and Duncan's method (Adam and Duncan 2001) was used to verify the microbial activity, and then the groundwater's ATP activity was tested to confirm if it contained any microorganisms. Finally, 4 g of the dry soil, 4 g of the wet soil, and 2 mL of groundwater provided the inoculum for each bottle which together contribute to 2.5% wt of each microcosm.

4.3.4 Microcosm Design

The experiment was planned to investigate adding carbon source, minerals solution and phosphorus and nitrogen sources and the effect of temperature on the bioremediation process using native microorganisms from the contaminated site as inoculum. To observe changing each parameter, three 500 ml bottles for every situation were filled with 400 ml of media.

The materials added to synthetic water to prepare the synthetic media for each replicate are shown in Table 6. "C" stands for carbon source and electron donor, which is acetate. "C+M" represents carbon source and minerals solution added to the synthetic water, and "C+NP" shows the replicate containing acetate, nitrogen, and phosphorus source. "C+NP+M" bottles contained synthetic water, acetate, macronutrients, and micronutrients in their media. Six bottles with the last composition were prepared, three of which remained at room temperature (20°C) with the other samples and three stored in a cold chamber (10°C) to see the effect of a temperature closer to the *in situ* temperature.

All bottles were capped with rubber disc lids after adding inoculum. They became anaerobic by conducting four cycles of degassing with a vacuum device for 5 minutes and then purged with nitrogen gas for 2 minutes.

Table 6 - Microcosm media composition

No.	Sample composition	Number of bottles
1 (C)	Synthetic water + Carbon source only (Acetate)	3
2 (C+M)	Synthetic water + Carbon source + Minerals (1:100)	3
3 (C+NP)	Synthetic water + Carbon source + Nitrogen and Phosphorus	3
4 (C+NP+M) 20°C	Synthetic water + Carbon source + Nitrogen and Phosphorus + Minerals	3
5 (C+NP+M) 10°C	Synthetic water + Carbon source + Nitrogen and Phosphorus + Minerals	3

Bottles were shaken well every day for 30 seconds to prevent the development of any concentration profile in the media and increase the bioavailability of substrate for microorganisms.

Twelve bottles, including "C," "C+M," "C+NP," and one series of "C+NP+M" media, were stored in a dark place at 20 °C. Three bottles containing the media similar to "C+NP+M" were put in a cold chamber at 10 °C for 80 days, and 10 ml samples were taken from them, as explained in the previous section. The samples were filtered using 0.45 µm filters to remove suspended particles since the machines analyzed the soluble ions.

4.3.4.1 Sampling

Samples were taken out from the liquid phase by entering a clean needle through the rubber cap after 3, 7, 14, 21, 35, 50, 58, and 80 days. 10 ml of liquid each time was withdrawn, filtered, and tested for chromium (VI) right after sampling. The rest of the samples were stored in a freezer to analyze the concentration of the other ions. After each sampling process, bottles were purged with nitrogen to ensure no oxygen content remained in the headspace.

4.3.4.2 Analytical Analysis

Chromium (VI) analysis was done based on the method 8023, HACH DR-3900 spectrophotometer machine. Chlorate, chloride, and acetate concentration in the samples were measured using Metrohm 930 IC. The solutions for the FDA (Fluorescein Diacetate Assay) method were prepared based on Adam and Duncan's procedure (Adam and Duncan 2001), and the final samples were put in Biochorm Ultraspec 2100 spectrophotometer to measure the fluorescein absorbance.

The technique used to analyze total chromium was inductively coupled plasma optical emission spectrometry (ICP-OES) with the instrument model of Varian 725-ES made by Agilent.

4.4 Results and Discussion

The FDA analysis of soil samples showed that dry soil,wet soil, and groundwater have 0.015, 0.016, and 0 mg/L (as an average of triplicate with the standard deviation less than seven percent) fluorescein concentrations, respectively. This means microorganisms are present in the soil samples, but this method is unable to detect any microbial activity in the aqueous samples directly. Further ATP analysis was accomplished to test the groundwater sample and it showed the concentration of ATP is 223 pg/L (for a triplicate measurement with a deviation less than eight percent), indicating an active microflora within the groundwater.

4.4.1 Mass Balance

4.4.1.1 Chromium

Total chromium analysis for digested filter papers showed each filter could affect the result as high as 0.022 mg/L on each sample, which is lower than the ICP machine's detection limit and negligible.

The result provides an acceptable range of recovery rate -92% as an average- for the method and proves that the reduced Cr(VI) is not in soluble form and remains in the filter with the biomass and soil particles. Figure 6 compares chromium concentrations in samples filtrate, recovered chromium from the solid phase, and the total detected chromium with the ion's initial concentration in the solution. CNPM sample at room temperature has the highest recovery rate, 2.83 mg/L, and CNPM, cold, and CNP follow that by 2.74 and 2.69 mg/L, respectively.

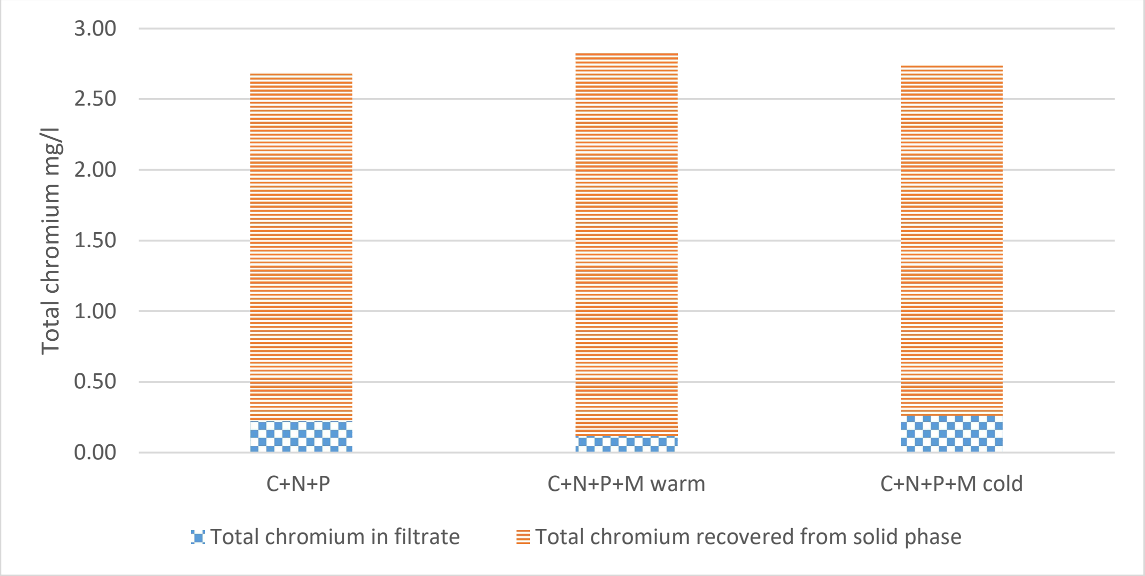

Figure 6- Total chromium in aqueous and solid phase

4.4.1.2 Chlorate

Chlorate/Chloride Mass Balance: On a molar basis, each mole of chlorate should result in a mole of chloride ion at the end stage, and the initial concentration of chlorine ions in the solution was 11.97 mMol/l, the same as the chlorate concentration. Except for the groups with the biodegradation process, ion chromatography revealed more than 90 percent of chlorine content as chlorine and chlorate ions. For the bottles that did not have enough time to reach the final stage of chlorine transformation, this ratio comes down to around 80 percent for C+N+P on day 50 and C+N+P+M on days 35 and 50 and C+N+P+M, Cold on the day 80. This value for C+N+P samples at day 35 is even lower and stands at 75 percent as an average for three bottles. The formation of chlorite and hypochlorite as intermediates before chloride is a possible reason for this deviation. After passing more time, the gap goes closer, producing more chloride. *Figure 7* illustrates the calculation results.

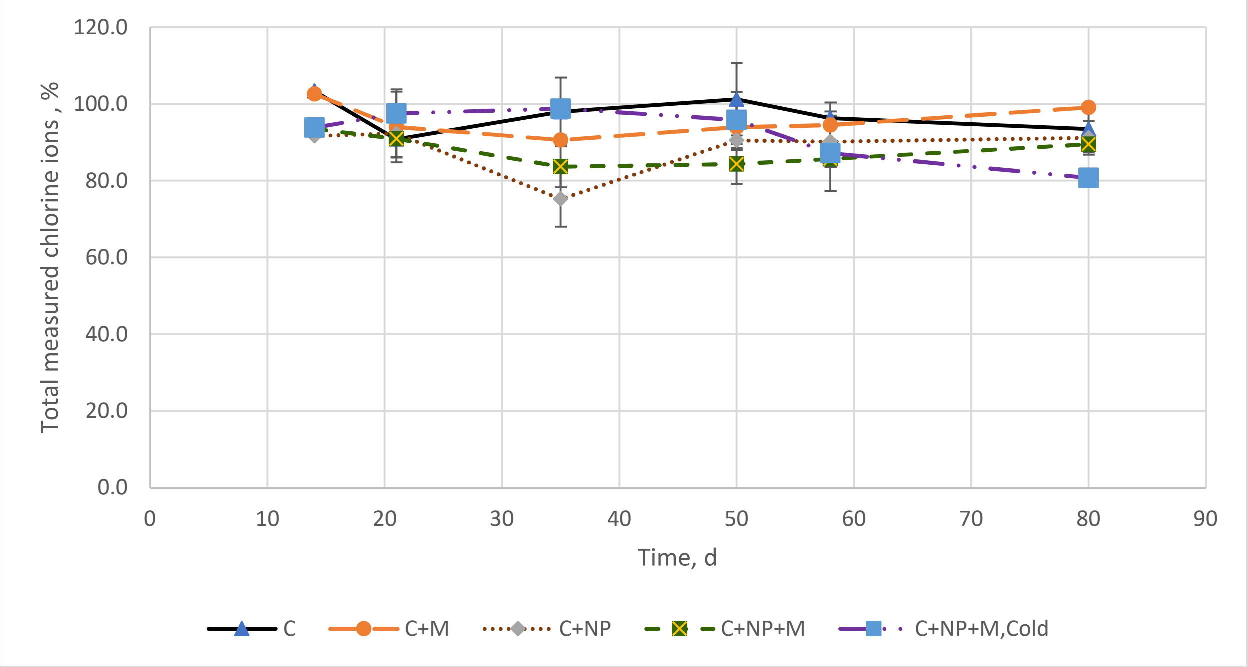

Figure 7- Overall ratio of measured chlorate and chloride to total chlorine ions

4.4.2 Microcosm Results

After 28 days, the appearance of some solutions started to change, and the bottles' solutions containing nitrogen and phosphorus source in warm conditions turned cloudy; the rest remained clear with the original colour, as depicted in *Figure 8*. This change happened for the samples stored at 10 °C after 60 days.

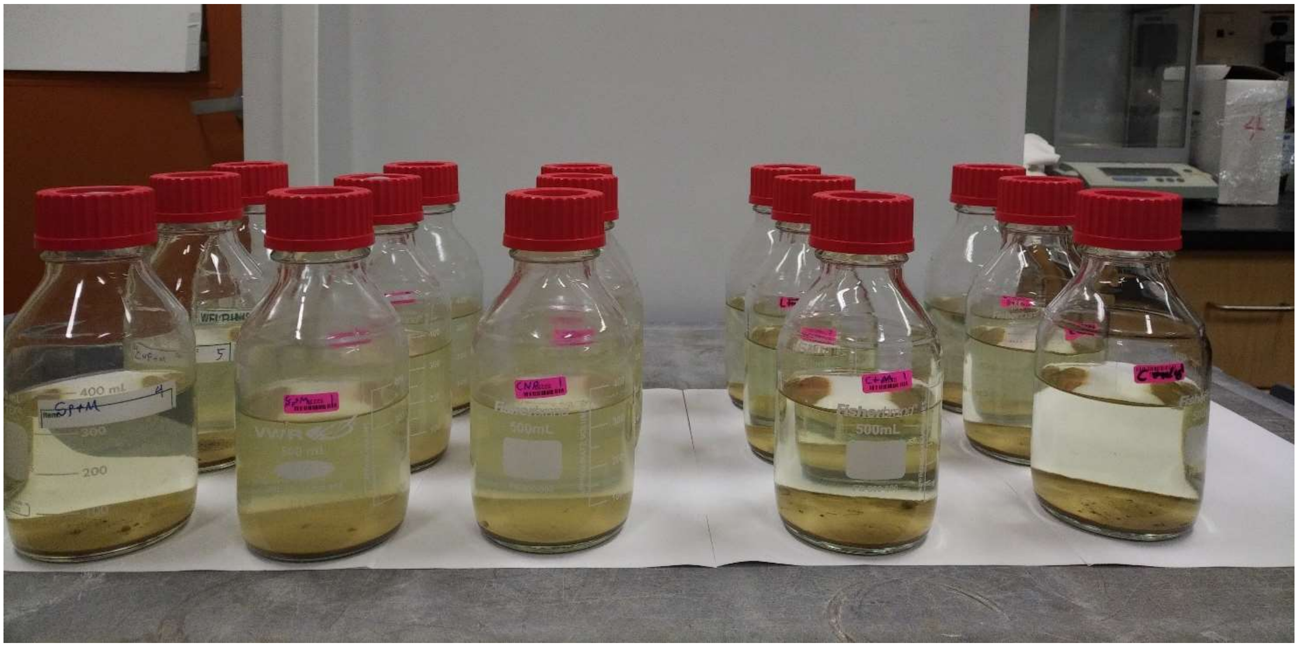

Figure 8- Appearance change after 28 days

The results from analyzing the ions in the samples showed that the main reduction process occurred simultaneously. Considering the initial concentration of both ions, which is 1000 mg/L for chlorate and 3 mg/L for chromium (VI) and their concentration at day 35 for "C+NP" and "C+NP+M," suggests the chromate reduction process is much slower than the other ion's removal. These results are displayed in Figure 9 (a-d) for all five series in the experiment period and discussed in the subsequent sections.

4.4.2.1 Chlorate

The chlorate concentration for each sample was measured using the ion chromatography technique. The initial value was 1000 mg/L as the solution was prepared with that concentration. This concentration was constant in the first 14 days of study for all samples. On day 21, the chlorate content of the samples slightly decreased to 88 and 84 percent of the initial concentration in C+NP+M and C+NP bottles, respectively, at room temperature. After day 35, there was no measurable amount of chlorate in them. In the C+NP+M microcosms at a lower temperature, the concentration change starts after 50 days, and on day 58, it drops to 770 mg/L. On the day of 80 days, the chlorate concentration of all samples with nitrogen and phosphorous sours reaches below the detection limits (1 ppm), while the chlorate content of the solutions did not receive any nitrogen and phosphorous additives continue unaffected as depicted in Figure 9b.

The results indicate that adding only acetate as an electron donor is insufficient to reduce chlorate and chromate in the isolated environment since their concentration in the "C" series is stable in the experiment period. The deficiency of macronutrients in groundwater must be compensated by appropriate bioavailable forms of nitrogen and phosphorous sources. Comparing the "C+M" and "C+NP+M" graphs Figure 9**Error! Reference source not found.**a and Figure 9b with the "C" and "C+NP" series reveal that the removal rates and yields are independent of adding the minerals solutions indicating that there were sufficient trace minerals included with the inoculum.

4.4.2.2 Chloride

Chloride is the final product of the chlorate reduction process, and it is an appropriate indicator to track the reaction chain completion. This ion's concentrations show an insignificant change in the first 21 days and then soar to 320 mg/L in the "C+NP" group and 355 mg/L in the "C+NP+M" series when no detectible chlorate was left in warm conditions. Bottles in a cold chamber at 10 °C, had not quite reached completion by the last sampling time (80 days). The other two groups, "C" and "C+M," steadily follow a constant figure for chlorate and chloride ion since microorganisms did not transform the ions, as shown in Figure 9c.

4.4.2.3 Chromium Hexavalent

The initial concentration of chromium hexavalent was 3 mg/L, measured by the spectrophotometry method based on the reaction with the Hach ChromaVer 3 Chromium reagents. This concentration remained unchanged in the first three weeks of starting the experiments. The trend continued similarly for the series without nitrogen and phosphorus sources containing only acetate or acetate plus minerals solution after 80 days. Chromate concentrations dropped after 35 days to 0.65 and 0.83 (as an average of the triplicates) for C+NP and C+NP+M solutions at 20 °C, and it was completely reduced on day 50. The concentration of Cr(VI) in the samples at 10 °C slightly changed after 58 days and then visibly decreased rapidly at day 80, as is shown in Figure 9a.

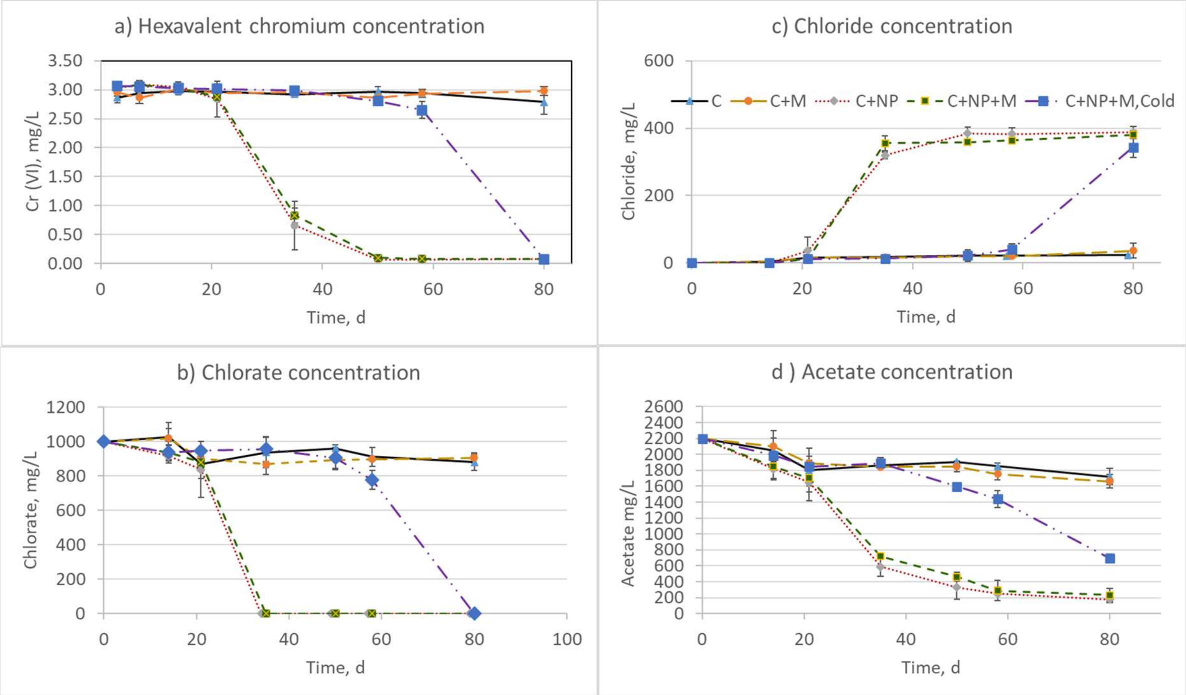

Figure 9- Ion concentration in different microcosms a) hexavalent chromium, b) chlorate, c) chloride, d) acetate

After observing Cr(VI) ion reduction in some bottles, samples were taken from all series and filtered by 0.45-micron filters to measure the total chromium content with an ICP machine suitable for measuring metal content aqueous phase. Figure 10 shows the result of the ICP test for total chromium. The graph compares the groups on days 35, 50 and 80 that indicate chromium transformed to a detectible species in the soluble form. Cr(III) may form a jelly precipitate that cannot pass the 0.45µm filter pores and get stuck in the filter while other ions remain in their soluble form.

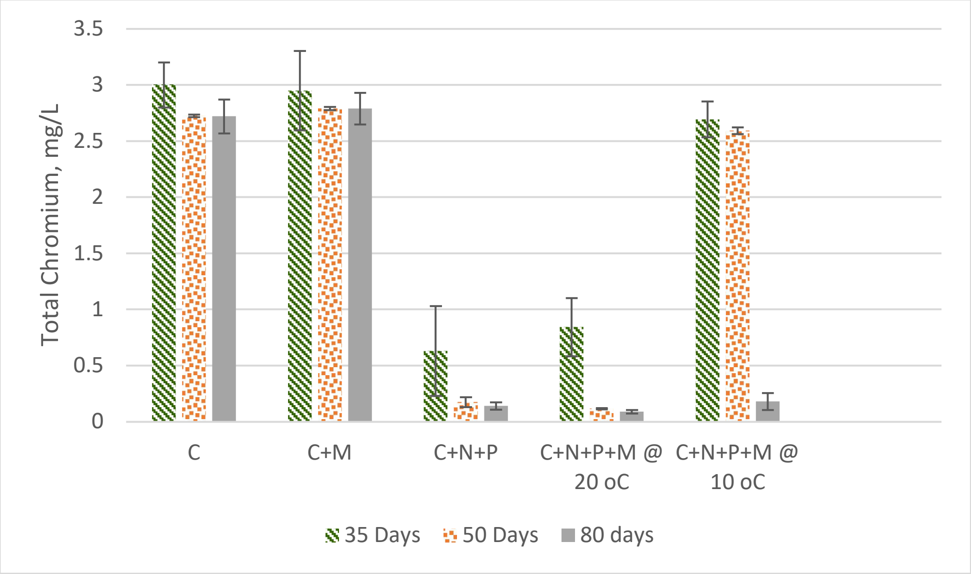

Figure 10 - Total soluble chromium concentration

4.4.3 Acetate

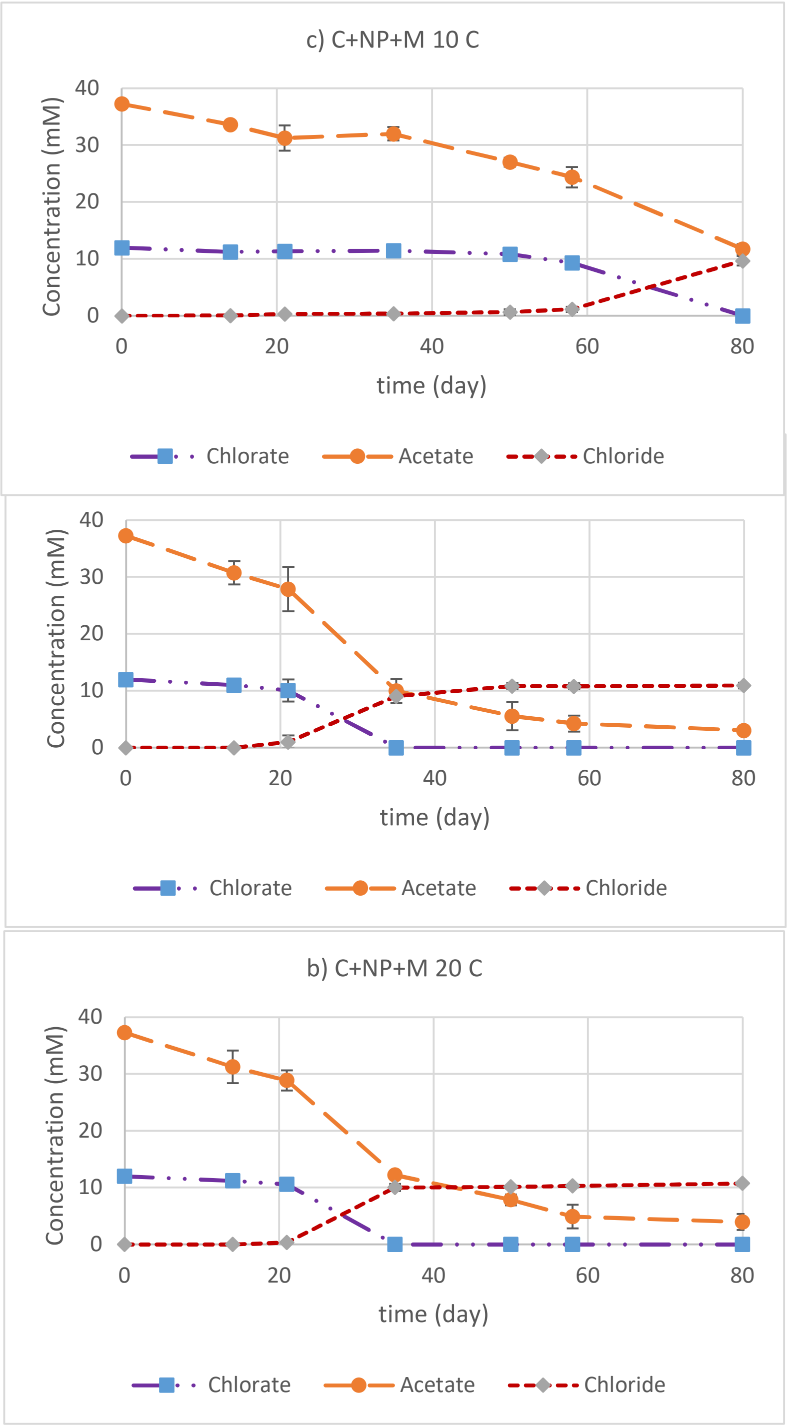

Figure 11 - Acetate, chlorate and chloride concentration in the series: a) C+NP, b)C+NP+M at 20 C, c)C+NP+M at 10 C

Microorganisms could utilize acetate, added as the primary electron donor and a carbon source to the media. The acetate concentration gradually decreased in all bottles at the beginning of the experiment at a small faster pace as the biomass increased. After day 21, by dropping the chlorate concentration in the samples containing the electron donor and nitrogen ad phosphorous source, acetate concentration declines faster. This is as expected in an initial enrichment as the population of chlorate-reducing bacteria increases exponentially during their growth phase. The acetate concentration for "C" and "C+M" groups decreases and gradually reaches 1719 mg/L and 1662 mg/L. Biomass production of the chlorate reducing organisms and the use of acetate by other microorganisms make the addition of excess amounts of the electron donor necessary.

4.4.4 Molar ratios

Comparing the molar trends for solutions where bioremediation occurred reveals substrate consumption and process completion information.

By decreasing the chlorate concentration in all three series, chloride concentration increases. change oppositely , "C+NP" and "C+NP+M" at 20 °C and 10 °C. The trends for these two ions prove that the total reduced chlorate transformed to chloride rapidly once sufficient dechlorinating biomass was reached. The graphs are available in *Figure 11* a-c.

The acetate concentration gives different values from the predicted numbers of the stoichiometric reaction, which is ¾ mole for each mole of chlorate. With the addition of 12 mM Chlorate, we would have expected to need to oxidize only 8 mM acetate. Acetate in the solution was reduced by the time and experienced a sudden drop in the periods that chlorate concentration was plunging and then decreased slowly after that. On day 35, when the whole chlorate was reduced in the solutions at room temperature, 67 percent of the acetate was consumed in "C+NP+M" samples. This ratio reaches 73 percent for the "C+NP" samples and 68 percent for the samples at 10°C after 80 days. Considering these values, the minimum amount of the electron donor that must be added to complete chlorate removal is three times higher than the stoichiometric ratios. While ¾ of the mole covers the electron demand of the remediation process, the rest is utilized as a carbon source to create biomass by other microorganisms.

4.4.5 ATP Analysis

The concentration of ATP (adenosine triphosphate) as a microbial activity indicator in the samples was measured after 50 and 80 days, and the results are depicted in Figure 12. This index has slightly changed over time for the "C" and "C+M" series compared to the injected groundwater value, 223 pg/L. ATP content of "C+NP" and "C+NP+M" bottles soared to their maximum on day 50 and slightly decreased on day 80. Although "C" and "C+M" mediums show a minor increase over time, the difference between them and samples containing nitrogen and phosphorous sources is meaningful. The elevated values of the ATP analysis result for the bottles at 10 °C compared to their initial values, confirm that reducing temperature only slows down the reaction rate without decreasing the microbial activity potential.

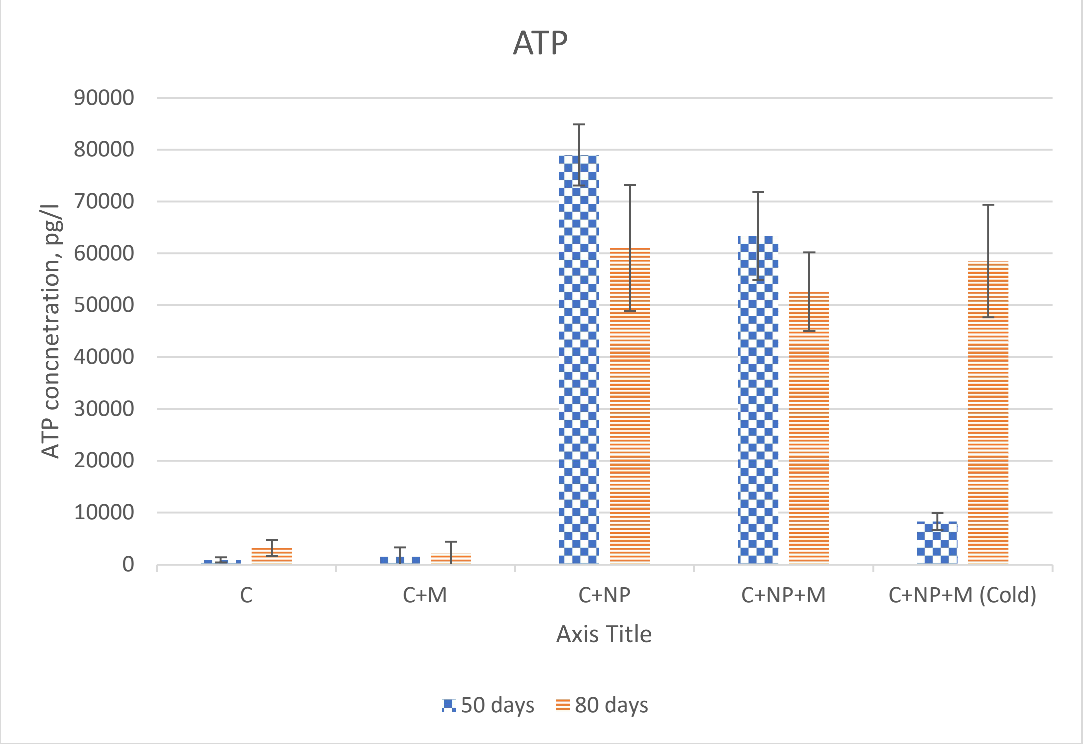

Figure 12-ATP Analysis result after 50 and 80 days

4.5 Conclusion

Bioremediation as a method with least impact on the environment was examined for two ions, chromium hexavalent and chlorate. Both ions were removed from the samples by native microorganisms transferred to the media from the site's soil and groundwater. These microorganisms utilized acetate as an electron donor and carbon source to reduce the ions below the acceptable limits under the anaerobic condition at 10 °C and 20 °C. The reduction process needs added nitrogen and phosphorous sources to complete, and the samples that contained the electron donor without macronutrients did not show any significant reduction. Micronutrient solutions prepared based on ATCC 1191 minerals recipe did not affect the remediation rate in any samples, indicating that the soil added with the inoculum provided sufficient micronutrients.

The final product of chlorate reduction, chloride, was measured and produced proportionally in the experiment, the sign of reaction completion.

As an electron donor, acetate was disproportionally consumed in the media since it could be used as a carbon source to produce biomass.

Chromate had the same fate in this environment and was reduced to chromium (III) by the microorganisms in the presence of the electron donor and macronutrients. The reaction rate for chromate is substantially slower than chlorate as it is seen that when 1000 mg/L of chlorate was removed thoroughly, only 75% of removal was observed for 3 mg/L of hexavalent chromium at 20 °C.

Doing the same experiment at a lower temperature (10 °C) impacted the reduction rate, and the removal occurred at a slower pace. This effect does not change the yield and final concentration of chlorate and chromate in cold conditions, and they reach regulated levels in a longer time with the same nutrients.

Ground water needs to be supplemented with N P and C in order to bioremediate groundwater with these concentrations of chlorate and chromate, but it can be done at relevant temperatures with respect to the in situ temperature.

4.6 Supplementary data

Table 7- Groundwater Composition

ChemName	Output Unit	Maximum	Average
Chloride (Filtered)	mg/L	46000	4472
Sulphate (Filtered)	mg/L	1690	377
Chlorate (Filtered)	µg/L	940000	45538
Arsenic (Filtered)	mg/L	0.265	0.019
Barium (Filtered)	mg/L	0.669	0.13
Calcium (Filtered)	mg/L	1480	293
Chromium (III+VI) (Filtered)	mg/L	13.2	1.2
Iron (Filtered)	mg/L	20	1.3
Magnesium (Filtered)	mg/L	429	90
Manganese (Filtered)	mg/L	7.33	0.74
Potassium (Filtered)	mg/L	404	67
Sodium (Filtered)	mg/L	29300	3143
Chromium (Hexavalent)	mg/L	14.4	1.2
pH (Lab)	pH_Units	10.3	8

Electrical conductivity (Lab)	µs/cm	102000	14191
Dissolved Hardness (Filtered)	mg/L	5470	1103
Alkalinity (total) as $CaCO_3$	mg/L	2710	450
Hydroxide	µg/L	66700	2128
Bicarbonate	mg/L	1590	427
Carbonate	mg/L	1520	57
Phosphorus	mg/L	3.8	0.39

Table 8-Minerals solution (trace elements)

Mineral	add (mg)	conc (g/l) in "1000x sol."	Final conc (g/l) in media
ferric chloride (FeCl3)	1050	2.1	0.0021
cobalt chloride	1000	2	0.002
manganese chloride	500	1	0.001
zinc chloride	500	1	0.001
nickel chloride	500	1	0.001
calcium chloride	250	0.5	0.0005
cupric chloride	250	0.5	0.0005

sodium molybdate	250	0.5	0.0005
nitriloacetic acid	10100	20.2	0.0202

Chapter 5: Journal Manuscript 2

Bioremediation of Chlorate and Chromium in Soil Packed Columns Using Native Culture from Manitoba

Mehdi Motevasselin[a], Indra Kalinovich[b], Beata Gorczyca[a], Kieran Milner[c], Richard Sparling[c], Emiley Kiely-Smith, Ramanathan Sri Ranjan[d]

[a]Department of Civil Engineering, [c]Department of Microbiology, [d] Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2 [b]Dillon Consulting Limited (email: motevasm@myumanitoba.ca, ikalinovich@dillon.ca, beata.gorczyca@umanitoba.ca, Richard.Sparling@umanitoba.ca

5.1 Abstract

Chlorate and hexavalent chromium are two chemicals with various applications in different industries. The improper waste management in the past left many sites with high concentrations of these ions, and finding a sustainable method to remove them is necessary. Bioremediation has the potential to reduce chemicals without any irreversible effect on the environment. The presence of an electron donor is a crucial factor in chlorate and chromium (VI) remediation. This study used acetate as an electron donor material with native microorganism sources from Manitoba, including manure, digestate manure, activated sludge, digested activated sludge, compost soil, and lake sediment. All the samples removed 3 mg/L of chromate in 10 days, while the bottles inoculated with fresh manure or digested activated sludge removed 1000 mg/L of chlorate simultaneously. Compost soil and digested manure could not remove chlorate from the media and sediment, and activated sludge completed the chlorate removal in 20 days. A continuous column study with the soil (collected from the contaminated site) under anaerobic conditions showed the removal yield of 80 percent for chlorate and 96 percent for chromium (VI). Precipitation and increasing solid

particles in the media gradually reduced the water flow rate in the columns.

5.2 Introduction

Concerns about protecting groundwater and soil from increasing contamination sources are growing globally. Discarded materials may enter water sources mostly from human activities around industrial or urban areas and cause contamination (Government of Canada 2017). Lack of appropriate waste management and weak regulations in the past century left many issues in industrial sites behind. One of the most common methods to dump unwanted materials in chemical facilities was using evaporative lagoons. Developing advanced material recovery and neutralization methods steadily eliminated lagoons' application. In one of Manitoba's industrial sites, the old lagoons from the 1960s were covered by soil to prohibit the spread of chemicals in the environment. For decades, water infiltration from weather precipitations gradually dissolved some chemicals and transferred them to the ground. The groundwater depth varies from 2 to 6 m, and it flows towards a river with an average horizontal hydraulic gradient of 0.031 m/m and an average hydraulic conductivity of 1.8 x 10^{-5} m/s. To protect the surface and groundwater, preventing chemicals from moving in the area is essential. Chlorate and hexavalent chromium are two concerning ions within this site, with the highest concentration of 3900 mg/L and 3 mg/L.

5.2.1 Chlorate

Chlorate and perchlorate are two chlorine oxyanions with high oxidation capacity. (Taraszki 2009). Chlorate is a semi-stable ion that forms in the perchlorate reduction process, and it is not common to find in the environment. (EnviroGulf Consulting 2007).

Bleaching agents productions, herbicide factories, pulp and paper industry, and agriculture use chlorate in sodium chlorate, calcium chlorate, or magnesium chlorate (US EPA 2016).

Chlorate has been identified as posing a potential risk to human health. The applicable Canadian federal criteria to chlorate concentrations are for the drinking water pathway at 1 mg/L (Health Canada 2016). There are no federal criteria for surface water or groundwater. However, the Province of British Columbia has published water quality guidelines that are protective of both agricultural (3 mg/L) and freshwater aquatic receptors (30 mg/L) (B.C. Ministry of Environment and Climate Change Strategy, 2020). The European Union decided to decrease chlorate's concentration in drinking water to 0.25 mg/L instead of 0.7 mg/L in 2020, and the higher value is only acceptable if "a disinfection method that generates chlorate, in particular chlorine dioxide, is used for disinfection of water" (E.P. and the Council 2020).

Since chlorate is an intermediate ion in perchlorate reduction, and the structure and chemical properties are similar, the removal techniques apply to both. Known, effective physicochemical removal methods include: ion exchange, sorption on activated carbon, filtration, capacitive deionization, and electrodialysis. These procedures are primarily feasible for water and wastewater treatment processes while a particular stream of fluids must be preserved. The biological treatment approach that uses living organisms to remove chemicals is preferred since it reduces costs and minimizes remediation site changes. Phytoremediation uses plants to reduce perchlorate, while bioremediation relies on the microorganisms' ability to reduce the ion (ITRC 2008).

Perchlorate reducing microorganisms exist in soils, sediments, groundwater aquifers, sludges, wastewater, animal waste, and other environments. Specific enzymes produced by these microorganisms help them to switch from oxygen to chlorate under anaerobic conditions. Chlorate acts as an oxygen source substrate in an extracellular reaction transferring electrons from the electron donor to chlorine oxyanions. The electron donor could be selected from simple, pure chemicals like acetate, ethanol, glucose, or more commercial products with complex compositions like vegetable oil, whey, molasses, and yeast extract (Nilsson, Rova, and Smedja Bäcklund 2013; Hatzinger et al. 2002; ITRC 2005).

5.2.2 Chromium (VI)

A transition metal, chromium, may exist in different oxidation states with six valence electrons, but it can be found in the environment just in two forms, Cr(III) and Cr(VI). The other species are not resistant and tend to transform into stable ions. Most chromium consumption relates to the metallic form, $Cr^{0,}$ in the metallurgy industry. The ions have broad uses in other industrial facilities like pigments production, anti-corrosion coatings, wood preservatives and fungicides, stainless steel and other high chromium alloys, and leather tanning (Kotas and Stasicka 2000).

The natural deposits of chromium in the earth's crust containing high concentrations and feasible to mine occur in trivalent form. High concentrations of chromium (VI) only appear around urban or industrial areas and have anthropogenic sources (Brandhuber et al. 2005).

These two ions are different in terms of their effects on the human body. While chromium (III) is considered beneficial in trace amounts for living tissues, chromium (VI) has adverse health effects on biological systems. Asthma and respiratory systems allergies are reported in case of inhalation (Kotas and Stasicka 2000). Bleeding in the gastrointestinal system, liver and kidney damage with the symptoms of diarrhea, vomiting and indigestion are some health issues that could happen by ingestion of hexavalent chromium (Guertin, Jacques, Jacobs, and Avakian 2005). Health Canada regulated the chromium content of drinking water to be lower than 0.05 mg/L to prevent the health effects of chromium (VI) (Health Canada 2016).

Removing hexavalent chromium could be done in a physicochemical or biological path.

The physical removal first uptakes the ion from the contaminated environment by adsorption in ion exchange units, filtration, or electrocoagulation process. Adding a downstream process for the waste is the main issue with physical removal. The chemical approach first targets the ion species to reduce it to harmless materials at once (Bryjak et al. 2016).

Reducing the oxidation state of chromium (VI) to chromium (III) eliminates health concerns and stabilizes the ion in an innocuous form. Chemical reduction of Cr(VI) occurs when an appropriate electron donor for this reaction is present in the environment. The chemical electron donor could be elemental iron (Fe^0), ions like Fe^{2+} Mn^{2+}, S^{2-}, and organic materials like humic acids and fulvic acids (Guertin, Jacques, Jacobs, and Avakian 2005).

Bioremediation of chromium uses the ability of microorganisms to reduce the oxidation state of chromium in the presence of a wide range of electron donors. It is an unspecific side reaction of various enzymes (Rahman and Thomas, 2021), requiring a high density of cells (Suthersan and Payne 2005). Organic matter of soil or groundwater is the primary natural electron donor. Molasses, lactate, glycerol, acetate, yeast extract, gluconate, or fructose are other electron donors that artificially were added to the media and successfully were utilized by microorganisms. Chromium acts as an acceptor for the released electrons from an exothermic process (Rahman and Thomas 2021).

5.2.3 Objective

Chemical remediation methods of oxyanions use electron donors under certain circumstances like acidic pH or increased temperature to remove the ions quickly in fast reactions. In the first step, the experiments will answer whether it is possible to reduce chlorate and chromate chemically for a long time under sterile conditions. In the second step, different microorganism sources were added to the synthetic media to see if they could reduce the ions. And lastly, triplicate soil columns were used to observe the ability of the native microorganism to remove the oxyanions in an environment similar to a site impacted with chlorate and hexavalent chromium.

5.3 Materials and methods

Based on the groundwater composition, the synthetic water solution was prepared, as shown in Table 9.

Table 9 - Synthetic water

	Well data, groundwater		**Synthetic water solution**	
	(mg/L)	(mMol)		(mMol)
Chlorate	3900	46.71	Sodium chlorate (1000 mg/L chlorate)	11.97
Chromium(VI)	3.03	0.058	K_2CrO_4 (3 mg/L as Cr(VI))	0.058
Phosphorus and Nitrogen	4.16 (P) 15.9 (N as nitrate and nitrite)	0.134 (P) 0.3 (N)	0.5 g of $(NH_4)_2HPO_4$	3.786
Sulfate	672	9.73	0.1 g of $MgSO_4 . 7H_2O$	0.405
Mineral mix			1ml/100ml	
Sodium acetate			3000 mg/L NaAcetate	50.804
Vitamins			1ml/100ml	
pH	7.25- 8.07		7.1-7.2	

Nitrate and nitrite were not added to the solution since they compete with the other oxyanions to receive electrons. ATCC 1191 minerals solution and vitamin mixture were added to the media to give possible additional materials that microorganisms might need. Since acetate is not fermentable, it was selected as an electron donor to help select for chlorate reducing bacteria (ref.).

5.3.1 Chemical reaction Test

As the first step, chemical tests under sterile conditions were done to ensure that any reduction in the process would rely on the presence of microorganisms. The presence of a suitable electron (acetate) donor beside chromium hexavalent that is ready to reduce to the other species may interfere with the biological reduction efficiency and mislead the research from finding the actual yield of the process. Solution No.1 (Control) bottles contain the same ions as the synthetic media recipe (without acetate) and show the effect of Cr(VI) stability in the solution. Solution No. 2 (C&A) contains chromium and acetate solution as the only two ions in DI water verifies the possible chemical reaction between two chemicals, and Solution No. 3 (SW&A) represents our synthetic media. Table 10 shows the arrangement of samples for the chemical study.

Table 10 - Chemical reduction experiment

No.	**Sample composition**	**Number of bottles**
1 (Control)	Synthetic media without acetate	3
2 (C&A)	Chromium and Acetate (DI water + chromate + acetate)	3
3 (SW&A)	Synthetic media containing chromate, chlorate and acetate	3

All glassware and DI water were sterilized using an autoclave for 30 minutes at 121° C for 20 minutes. Preparation was performed in a biosafety cabinet, disinfected with DNA away from the solution and UV lamp to prevent any microorganism from entering the key. Nine 120 ml bottles were filled with 100 ml of prepared solutions and capped. After days 1, 3, 7, 15 and 30, 2ml of every bottle was taken out with a clean new syringe and diluted six times less to reach 0-0.6 mg/L of Cr(VI) concentration. This range indicates the detection limit of chromium hexavalent measurement by using Chroma Ver.3 reagent powder pillows for method 8023, HACH DR-3900 spectrophotometer machine. The results provided in the discussion section proved the experiment hypothesis, which enabled the and paved the way for the second step.

5.3.2 Biological reduction test

In the second step, different inoculum sources were tested on the media. The control solution was prepared without acetate or any other carbon source, and then five microorganism sources were tested in different bottles. Twelve 160 ml serum bottles (the six sources in duplicate) were filled with 50 ml media, and 5 ml of inoculum was added to each bottle. Inoculum sources included manure, digested manure, lake sediment, activated sludge, digested activated sludge, and soil collected from Manitoba. Due to the high carbon content of activated sludge, acetate was deleted from the medium composition for these bottles. The bottles were covered with the rubber stopper, degassed for three cycles with a vacuum pump for two minutes, and purged with nitrogen gas (N2) for 30 seconds at eight psi.

GC method was used to analyze the gas on top of the bottles to observe the carbon dioxide concentration produced by microorganisms. Chlorate concentration in the liquid was measured using the IC method, and the concentration of chromium (VI) was measured by HACH DR-3900 spectrophotometer after 10 and 20 days.

5.3.3 Continuous column studies

A bucket of soil collected from the site, at the level of the groundwater, was sieved with 2mm pore size sieves. Then six transparent columns were packed with the soil using a constant energy packer. Two 0.45 µm filters covered each column’s top and bottom to prevent microorganisms from moving out the columns. Two vessels containing the synthetic media and the synthetic media without acetate were used to feed the columns as triplicates. The feeding containers were connected to a nitrogen tank with a constant flow to prevent oxygen from entering the media, as shown in Figure 13. Upward feeding was selected to ensure that all the packing was in contact with the synthetic media. After 16, 40, 88, 112, and 136 hours samples were collected from the outlet stream on top of each column, and they were analyzed for chromium (VI) and chlorate content after weighing.

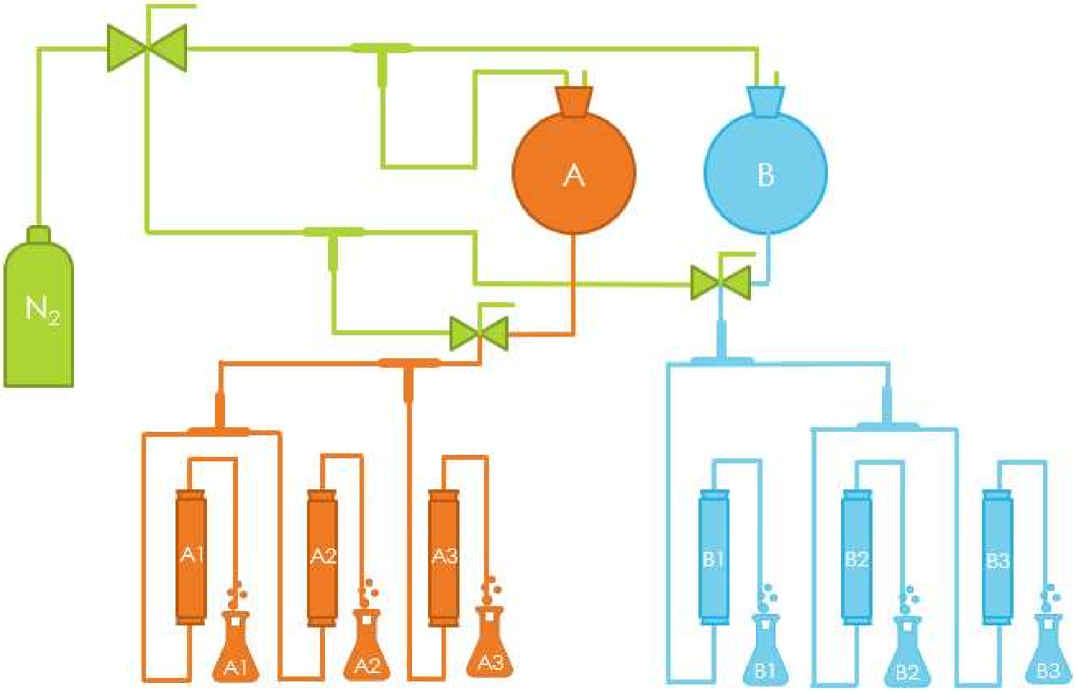

Figure 13 - Column study setup (A series feed without acetate and B series with acetate)

5.4 Results and Discussion

5.4.1 Sterile condition

Chromium concentration of the samples withdrawn from the sterile bottles showed little meaningful change. This constant concentration shows that decreasing pH and increasing temperature are essential to start the electron transferring reaction between acetate and hexavalent chromium. The chlorate concentration was only measured at the end of the period, and similar to chromium, it remained unchanged in the media. Figure 14 shows the result of the sterile condition test.

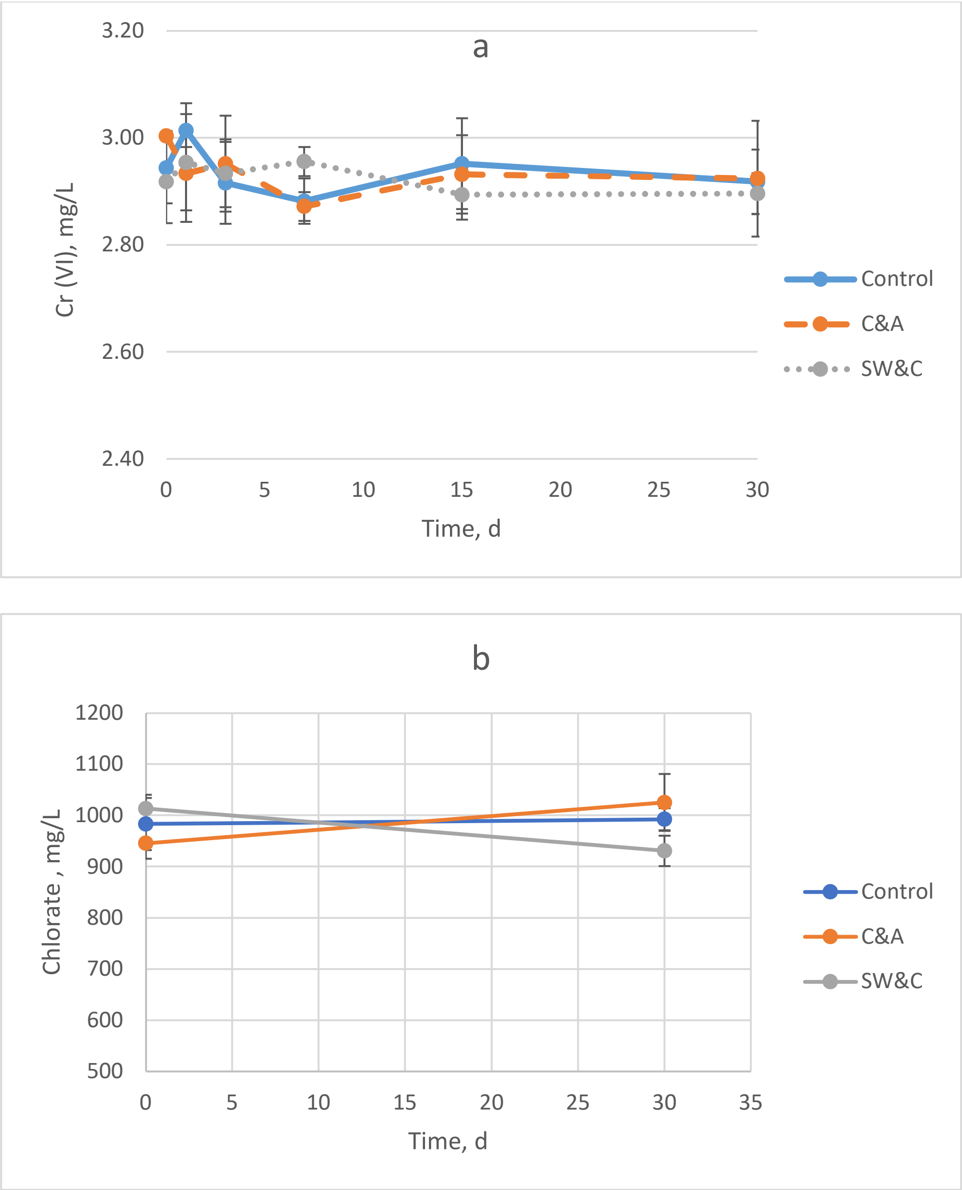

Figure 14 - Sterile condition result (a, chromium hexavalent concentration, b, chlorate concentration)

5.4.2 Batch microcosm

5.4.2.1 Chlorate

Based on the ion chromatography of chlorate, the control bottles without inoculum did not have any change after 10 and 20 days. Fresh manure and digested activated sludge reduced the chlorate ion after 10 days, while sediment and activated sludge gradually reduced in 20 days. Sediment and combined activated sludge with acetate revealed better results with 100% removal in 20 days and activated sludge without acetate just removed 72%. The concentration of chlorate for soil and digested manure for that period does not indicate any meaningful change.

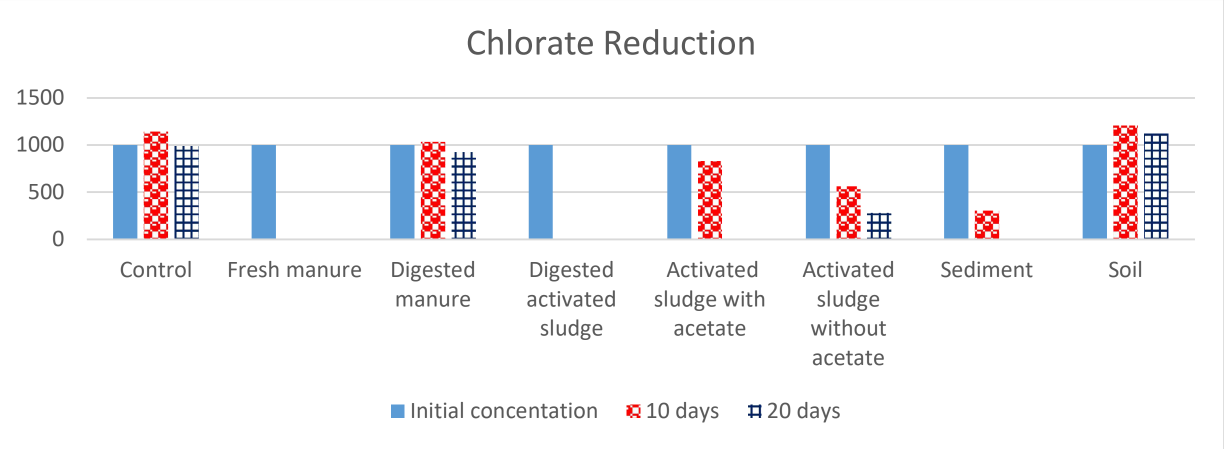

Figure 15 - Chlorate concentration after 10 and 20 days

5.4.2.2 Chromium

Chromium (VI) concentration in all samples dropped significantly after 10 days. The bottles inoculated with the soil sample entirely transformed the chromium ion, although they could not remove chlorate in that period. Figure 16 illustrates Cr(VI) concentration in the filtered samples after 10 and 20 days compared to the initial value (3 mg/L). Since chromate reduction is rather unspecific while chlorate reduction is quite specific, it is more likely that some media did not contain chlorate reducing bacteria.

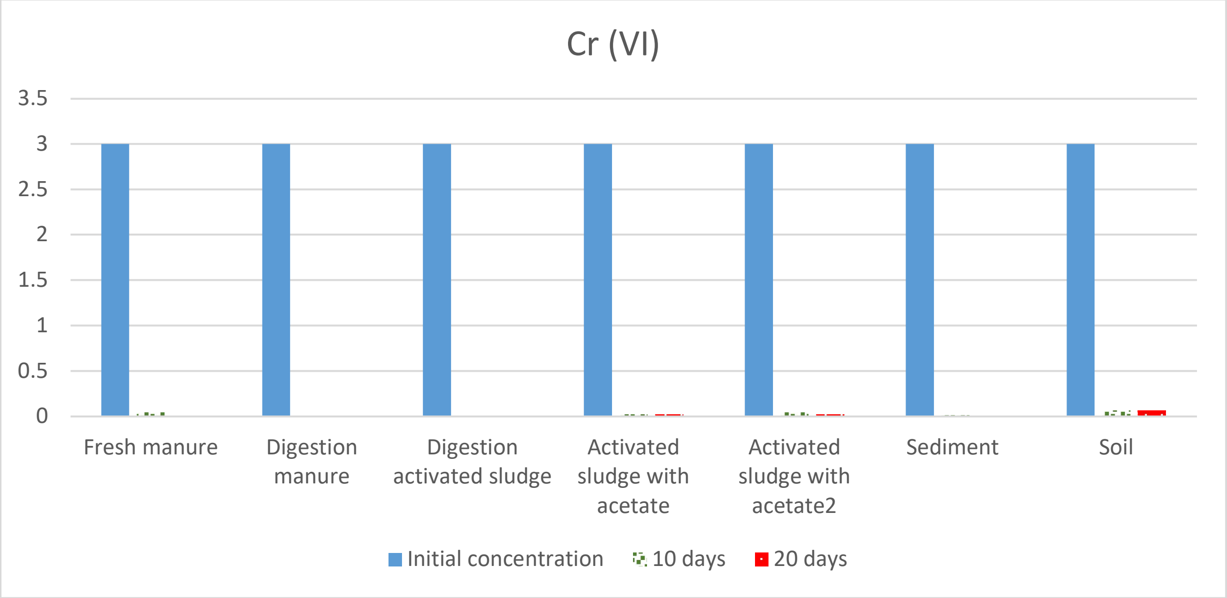

Figure 16 - Chromate concentration after 10 and 20 days

Total chromium concentration in the filtered solution (0.45 micron) indicates the soluble chromium in the aqueous phase. Figure 17 compares the soluble chromium in the samples after 10 and 20 days. The amount of soluble chromium ions decreased as Cr(VI) was removed in all bottles. In neutral pH, the most stable form of remediation product, Cr(III), is solid and stays in the filter during the filtration process.

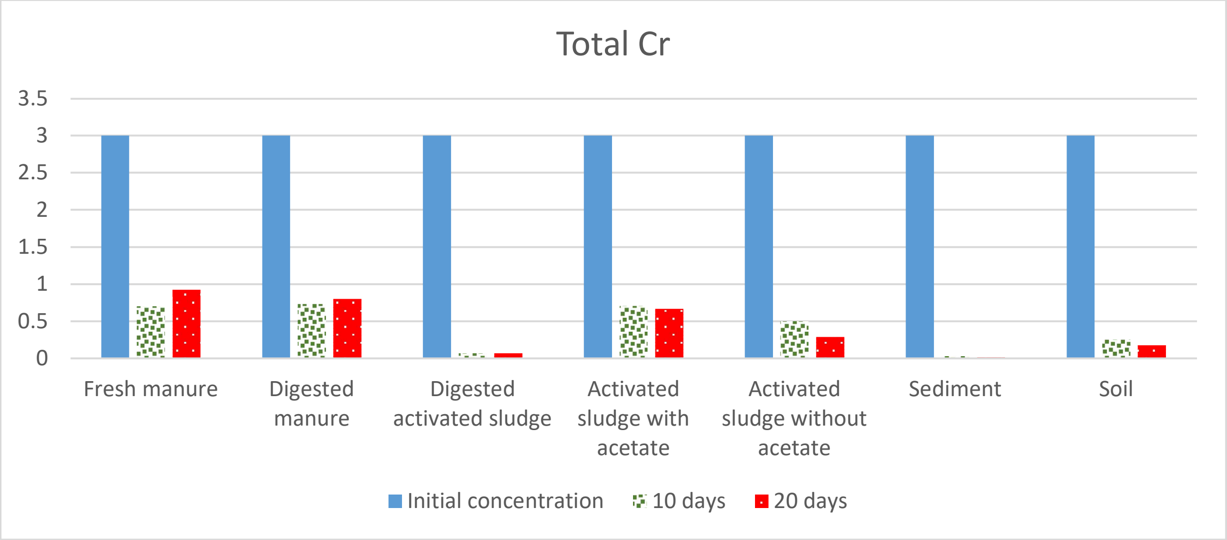

Figure 17 - Total dissolved chromium concentration

5.4.2.3 Carbon Dioxide Formation

GC data from the gas phase on top of the bottles, presented in Figure 18, indicate that the higher carbon dioxide production occurs when the chlorate remediation happens. Fresh manure produced the most significant CO_2 amongst the bottles with 1.1 millimoles and activated sludge plus acetate samples and sediment followed. Soil and digested manure exhibited the lowest gas production with just 0.2 and 0.4 millimoles without removing chlorate.

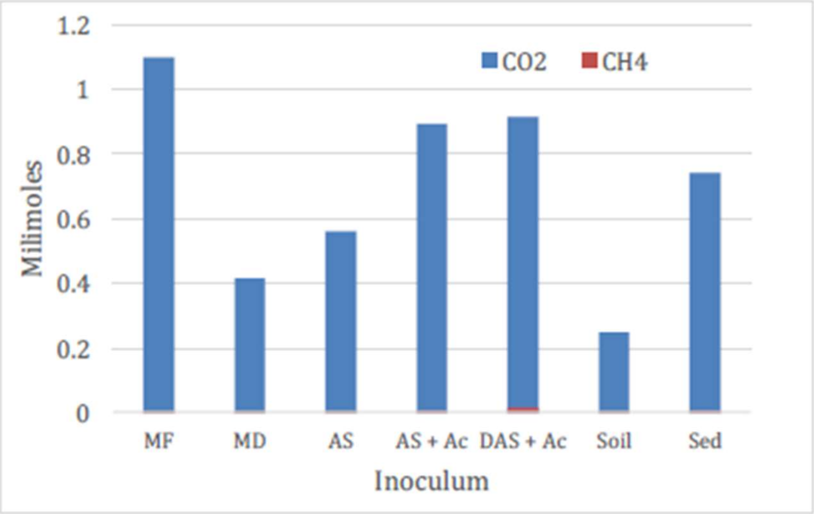

Figure 18 - GC measurements taken after ten days on primary enrichment bottles (one replicate bottle per sample). From left to right, samples were fresh manure (MF), manure digestate (MD), activated sludge (AS), activated sludge + acetate (AS+Ac), digestate activated sludge + acetate (DAS+Ac),,soil (Soil), and sediment (Sed). [1]

5.4.3 Continuous column test

5.4.3.1 Chlorate

The chlorate concentration in the columns feeding synthetic water with acetate decreased gradually

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[1] *The graph is reported by Kieran A. Milner, in undergraduate course report "Enrichment and isolation of oxyanion degrading bacteria: bioremediation of chlorate and chromate contamination using microbial reduction" for MBIO 4030, Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada*

from 1000 mg/L to 708 mg/L after 88 hrs. After that, it fell faster and reached 137 mg/L at 136 hrs which shows the reduction reactions occur four times more quickly. Increasing the population of reducing microorganisms in the log phase is most likely the main reason for changing the reaction rate. Figure 19 illustrates these changes over time.

Although the chlorate concentration shows a slight drop initially, it remains constant after that and does not fall below ninety percent of the initial. The packing soil possibly contains tiny amounts of carbon sources that the reducing bacteria could utilize as an electron donors. This resource is not enough to complete the reaction and help the reducing microorganisms multiplied to the extent they can significantly reduce chlorate.

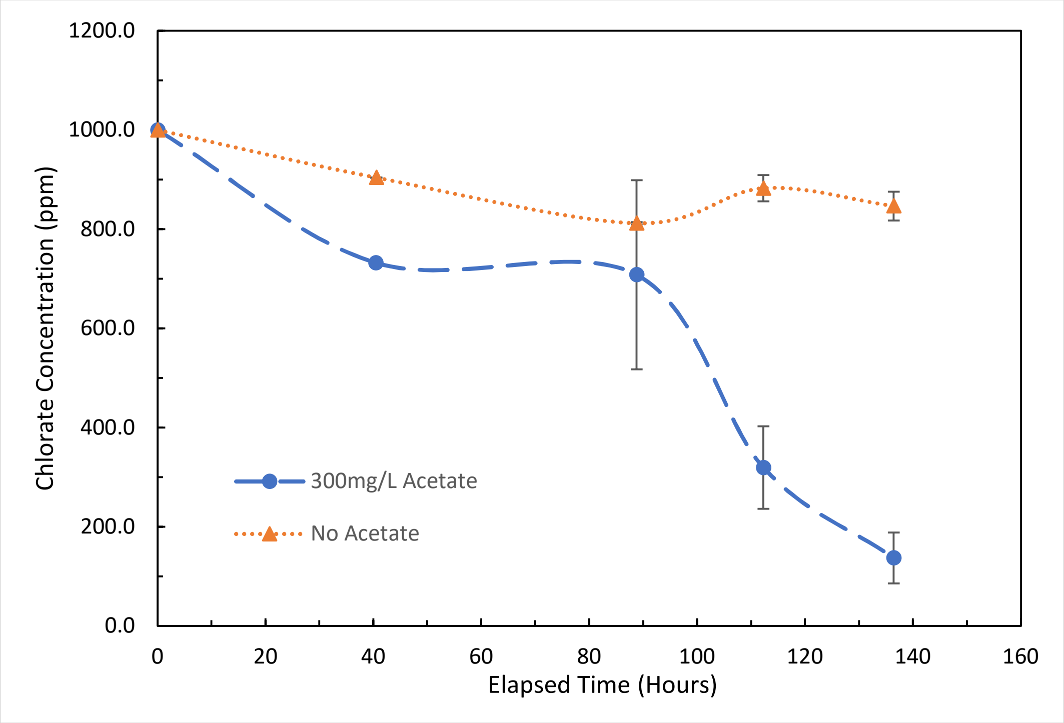

Figure 19- Chlorate concentration in continuous column study[2]

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[2] *The graph is reported by Emily Kiely-Smith, in undergraduate course report "The bioremediation of chlorate and hexavalent chromium co-contaminated water" for BIOE 4240: Graduation Project, Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba, Canada*

5.4.3.2 Chromium(VI)

Hexavalent chromium concentration decreased in both samples quicker in the columns, with acetate reaching 0.1 mg/L. In contrast, it drops to 1.2 mg /l after 136 hrs in the other columns, as shown in Figure 20. The amount of carbon source carried by the soil was enough to reduce some part of the chromate, considering the difference between initial values for both ions.

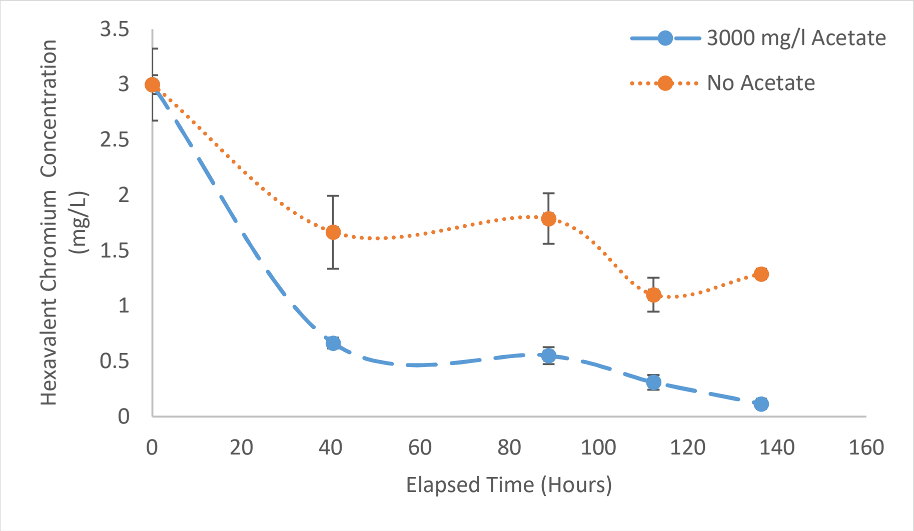

Figure 20 - Hexavalent chromium concentration in column samples

5.4.3.3 Flowrate

The flow rate of the synthetic water was measured every 12 hours by dividing the weight of fluid was collected by the elapsed time. The value showed a fluctuation initially for both series and then showed different trends for the columns. While the flow rate of synthetic water without acetate was around 0.5 l/week, it constantly dropped for the columns feeding with the whole composition of synthetic water containing acetate. Increasing microbial activity in the environment releases more particles that cannot pass 0.45-micron filters, affecting the flow rate. Insoluble Cr(III) salts cling to the cells, and that EPS (extracellular polymeric substances) would form as the biomass increases, thus changing the structure of the column packing material itself is most likely a reason for reducing flowrate. This insoluble solid phase was clearly visible in waste residue of the solution that contaminated with the soil microorganisms (*Figure 22*).

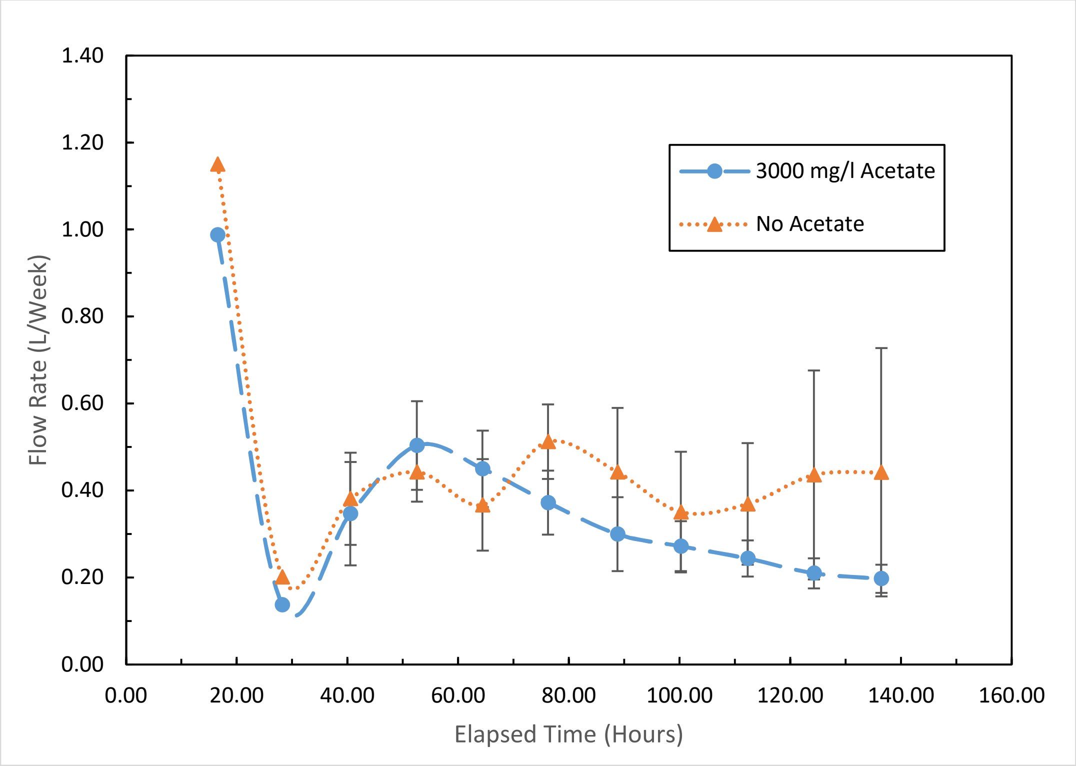

Figure 21- Flow rate over time

Figure 22- Solid precipitation formation in the waste solution

5.5 Conclusion

This study concluded that chlorate and chromium could not accept electrons from acetate at neutral pH under sterile conditions. Introducing and stimulating microorganism growth was necessary to reduce these ions to more benign species. Chromate reducing bacteria have been identified as ubiquitous in the environment and found in various sources like sediment, activated sludge, manure, digested manure, and some soils. The addition of acetate as an electron donor provided an appropriate electron donor for the reducing bacteria to complete the reduction of chlorate and chromium to below the applicable criteria.

The experiment with columns packed with the soil collected from a Manitoban site showed that reduction of red in continuous flow systems (similar to the batch tests). The effluent chlorate concentrations in the column effluent streams declined to 14 percent of the initial 1000 mg/L after 136 hours. In the columns that did not receive the electron donor (i.e., acetate), only 15 percent of chlorate was removed. The only available electron donor was carbon source from the soil for the reduction. This carbon content was enough to reduce chromate concentration to 1.2 mg/L at the sampling period because the initial concentration was way lower than chlorate (1000 mg/L). Chromium concentration for the columns that received acetate as electron donor was 0.1 mg/L, significantly lower than the other columns.

The reduction process increases solid particles in the media and drops the flowrate gradually.

Insoluble Cr(III) salts cling to the cells, and that EPS (extracellular polymeric substances) would form as the biomass increases, thus changing the structure of the column packing material itself is most likely a reason for reducing flowrate.

5.6 Supplemental document

Table 11- Vitamins solution

Material	conc (mg/l) in "1000x	Final conc (mg/l) in media
pyridoxine-HCl	100	0.1
riboflavin	50	0.05
nicotinic acid	50	0.05
lipoic acid	50	0.05
thiamine	50	0.05
p-aminobenzoic acid	50	0.05
folic acid	20	0.02
biotin	20	0.02
cyanocobalamin	10	0.01

Chapter 6: Summary and Conclusion

A set of experiments were designed to observe the ability of Manitoba's native microorganisms to reduce chlorate and chromium (VI), both of which are common by-products of sodium chlorate production. Acetate was selected as a cost-effective, readily obtainable electron donor since it is not fermentable, and two supplementary materials, macro-nutrients (phosphorus and nitrogen source) and micro-nutrients (ATCC 1191 minerals solution), were tested. Due to long winters, to investigate the effect of temperature drop for most Canadian sites, the test was done at 10 °C and 20 °C.

Chlorate and hexavalent chromium were entirely removed from the synthetic water containing acetate, nitrogen, and phosphorous sources at room temperature after 35 and 50 days. Although adding macronutrients is vital to complete the process, the solutions with the added minerals did not show any significant change and remained similar to the control system. The complete removal of the ions occurred after 80 days in the samples stored at 10 °C. Based on the ATP analysis, reducing temperature does not affect the total microbial activity but extends the time needed by the microorganisms to reach the proper growth rate.

Increasing solid particles is an undesirable consequence that may occur in bioremediation processes. Digestion of solid particles stuck in the filters in acidic solution proved that reducing the chromium (VI) to chromium (III) precipitates the ion in the neutral pH.

Chapter 7: Engineering Significance of Research

The results from this project can be applied to remove chlorate and hexavalent chromium from soil and groundwater in contaminated sites, including those in cold regions. The methodology described provides information about the substrate and nutrients needed for degrading the ions and the required amounts. To maximize bioremediation performance, the addition of an electron source is insufficient in groundwater without further addition of a nitrogen and phosphate source. Biodegradation can reduce high chlorate concentrations from 1000 mg/L to criteria protective of human and environmental health, and the final by-product from the process is chloride, a neutral ion in the environment.

Foreseeing possible future challenges of a remediation project is a key component of any engineering design. Reducing hexavalent chromium to innocuous trivalent form precipitates the chromium content of water and increases solid formation in the water path. The observed results suggest that any engineering design should consider clogging during the in-situ application and changes possible groundwater flow direction.

The results described herein showed that the native microorganisms could remove the oxyanions by receiving the electron donor material and nutrients. The difference between cold and warm conditions is only the required time to reach maximum microbial growth and entirely clean up the media.

Chapter 8: Recommendations and Future Research

Based on the laboratory and plant investigation, the following items are recommended:

- Acetate is an acceptable electron donor to remove chlorate and chromate if it is added in excess quantities to cover its consumption as a carbon source;
- Adding nitrogen and phosphorous sources are necessary if there is not adequate supply present on a given site; and,
- Any bioreactor or in-situ treatment design (using injection wells) may need to consider increasing solid particles and chromium residue, which may block the water path. The initial settling may have been a greater issue that chromium(III) precipitation or even cell biomass or EPS formation

Future research should investigate what happens to the mobility of these compounds once the acetate is used up? Do DO (dissolved oxygen) and pH return back to normal and does the precipitate remain a precipitate?

Other design questions would be – how long would this solution take, given the groundwater flow rate, consumption of acetate (modeled).

It may also include using different types of electron donors to see the effect of adding them.

Slurry mixing bioreactors could be used with screened microorganisms to find the exact reaction rate coefficient, which helps design ex-situ processes.

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