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September 2022

Environmental Laboratory

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Final report

Prepared for US ARMY ENVIRONMENTAL COMMAND
2450 CONNELL ROAD
FORT SAM HOUSTON, TX

Under Project CEERD-EL-17-69, "Longhorn Army Ammunition Plant Perchlorate System Assessment, Augmentation, and Analysis"

Abstract

Optimization of operation and performance of the groundwater treatment system regarding perchlorate removal at Longhorn Army Ammunition Plant (LHAAP) is dependent on specific conditions within the reactor and the larger groundwater treatment process. This study evaluated the microbial community compositions within the plant during periods of adequate perchlorate degradation, sub-adequate perchlorate degradation, and non-operating conditions. Factors affecting the performance of the LHAAP ground water treatment system (GWTS) perchlorate de-grading fluidized bed reactor (FBR) are identified and discussed. Isolation of the FBR from naturally occurring microbial populations in the groundwater was the most significant factor reducing system effectiveness. The microbial population within the FBR is highly susceptible to system upsets, which leads to declining diversity within the reactor. As designed, the system operates for extended periods without the desired perchlorate removal without intervention such as a seed inoculant. A range of modifications and the operation of the system are identified to increase the effectiveness of perchlorate removal at LHAAP.

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Preface

This study was conducted for the US ARMY ENVIRONMENTAL COMMAND, 2450 CONNELL ROAD, FORT SAM HOUSTON, TX under Project CEERD-EL-17-69, “Longhorn Army Ammunition Plant Perchlorate System Assessment, Augmentation, and Analysis” The technical monitor was Mr. Nick Smith.

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1 Introduction

1.1 Background

Longhorn Army Ammunition Plant (LHAAP) is 3.5 miles west of the Louisiana/Texas border in Karnack, approximately 40 miles northwest of Shreveport, Louisiana and 12 miles northeast of Marshall, TX. It includes 451 buildings on 8,493 acres of land. Built in 1941, this facility's mission included production and demilitarization of World War II (WWII) era missiles. Contamination at the site resulted from production of various defense items (such as explosives, pyrotechnics, illuminants, and rocket motors) beginning near the start of WWII and continuing through the early 1990s. LHAAP was placed on the National Priorities List on 09 August 1990 as a superfund site. In 1990, the United States Environmental Protection Agency (USEPA) listed methylene chloride and trichloroethene as the primary contaminants of concern (COC) at the site. It was not until 2002 that perchlorate was identified as a COC. The cleanup process includes remediation of site groundwater using a pump and treat system. Cleanup is governed by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). The US Army is the lead agency performing the remediation in coordination with the Texas Commission on Environmental Quality (TCEQ) and the USEPA. Full-scale cleanup activities have been ongoing since 25 October 1996 and continue to this day. This cleanup system has experienced several upsets since it's modification for treating perchlorate as well as the tri-chloroethylene the plant was originally designed and constructed to treat.

Longhorn AAP was established to support mobilization requirements for WWII and was placed in standby mode from 1945 to 1952. In 1952, it was reactivated to support Korean War activities, at that time the plant was operated by Universal Match Corporation. During this time, the industrial activities of the plant expanded to include loading, assembling, and packing (LAP) of rocket motors and pyrotechnic ammunition. In the 1970s, the Thiokol Corporation received the contract to repurpose longhorn from a WWII era liquid fuel facility into a solid fuel rocket motor plant. In 1955, Longhorn Plant 3, which was operated by Thiokol Corporation (later Morton Thiokol, Inc.), was designated to produce solid propellant rocket motors. In 1956 the Nike-Hercules program began which produced sustainer motors. Additionally, propellants and motors for

missiles (Falcon, Lacrosse, Honest John, and Sergeant) were produced at the plant. In 1959, a Main Rocket Motor Assembly Building (45E) was constructed along with a Static Test Building (25T).

During the Vietnam War, the plant produced illuminating, pyrotechnic ammunition and solid propellant rocket motors including first and second stages of the Pershing IA missile. LHAAP was one of two facilities that destroyed Pershing IA and II missiles following the Intermediate-Range Nuclear Forces Treaty of December 8, 1987, which prohibited such missiles. Demilitarization of the missiles was achieved by static burn and crushing of the missiles.

Figure 1. Map of LHAAP showing operable units LHAAP-18/24 and the associated interception trenches which feed the GWTP*.

As part of the ongoing cleanup effort, groundwater extraction wells were installed on three sides of the LHAAP-18.24 operable units. The system used to perform treatment of the extracted groundwater was originally designed to treat volatile solvents, primarily trichloroethylene and methylene chloride, and heavy metals including barium in groundwater

* US Army. 2019. "Final Proposed Plan LHAAP-18/24, Burning Ground No. 3 and Unlined Evaporation Pond." February.

from LHAAP operable units 18 & 24 (DOW Environmental 1995) shown in Figure 1. The design of the original water treatment system configuration was based on the results of groundwater concentration measurements using 48 individual monitoring wells. Perchlorate was not included in the analysis of these groundwater samples (DOW Environmental 1995). In 2002, a Human Health and Ecological Risk Assessment was performed which identified perchlorate as a COC for groundwater at operable unit 17. As a result, a site-wide baseline ecological risk assessment was conducted*.

The treatment plant construction was completed in December 1996 with subsequent installation of approximately 5,000 ft of collection trenches to intercept contaminants migrating toward Caddo Lake from Unit 18 (Burning Ground No. 3) in 1997. These trenches collect the extracted groundwater from the contaminant plume for transport to the treatment system. Extraction and treatment of the contaminated shallow groundwater began in April 1998† . It operated for a year before perchlorate treatment was included in 1999.

In early 2000, treatment options and treatability studies were initiated to determine the modifications needed for the existing treatment system to provide the perchlorate treatment. A fluidized bed reactor (FBR) was selected as the remedy addition to the treatment system. The FBR was designed to treat 50 gallons per minute (gpm) and was placed online in February 2001. (Polk et al. 2001).

Multiple industrial activities occurred at the LHAAP site over the five decades in operation. The groundwater being treated at the LHAAP GWTP contains several contaminants which require varied treatment processes. To produce a water acceptable for release, several unit operations occur as the water is moved through the plant (Table 1). Figure 2 shows a schematic of the process and the order of the unit operations. The groundwater is treated for metals and volatile organic compounds (VOCs) prior to the FBR bioremediation step. Both treatment steps contain multiple processes, all of which affect the microbial population present in the bioreactor and thereby the effectiveness of this unit for perchlorate degradation.

* US Army. 2010. “Final Proposed Plan for LHAAP-17, Burning Ground No. 2/Flashing Area Group 2.” May, 00098226.

† Longhorn Army Ammunition Plant Texas. EPA ID # TX6213820529. July 2004. caddolakedata.us/media/498/site%20map-longhorn%20ammunition%20plant.pdf

Table 1. Effluent limitation for discharge per record of decision (ug/L) (Shaw 2006).

Category-Analyte	Daily Average	Daily Maximum
Metals		
Aluminum (total)	777	1,644
Arsenic (total)	365	772
Barium (total)	1,000	2,000
Cadmium (total)	2	3
Chromium (total)	355	752
Chromium III	297	628
Chromium VI	58	124
Cobalt (dissolved)	5,433	11,495
Iron (dissolved)	1,132	2,395
Manganese(dissolved)	7,323	15,494
Nickel (total)	87	184
Selenium (total)	6	12
Silver (total)	1	3
Vanadium (dissolved)	1,698	3,592
Zinc (total)	146	310
Perchlorate		
Perchlorate	6	13
Volatiles		
1,1,1-Trichloroetane	3,417	7,230
1,1,2-Trichloroethane	103	217
1,1-Dichloroethane	6,633	4,032
1,1-Dichloroethene	119	253
1,2-Dicchloroethane	85	181
Acetone	1,132	2,395
Benzene	85	181
Carbon Tetrachloride	85	181
Chlorobenzene	22,300	47,180
Chloroform	1,708	3,615
Ethylbenzene	26,954	57,025
Methylene Chloride	803	1,699
Styrene	2,829	5,987
Tetrachloroethene	85	181
Toluene	1,980	4,189
Trichloroethene	85	181
Vinyl Chloride	34	72
Xylene	40	84

The groundwater requiring treatment is pumped from approximately 5,000 ft through a collection trench from operable units LHAAP-18 and LHAAP-24 (Figure 1). Each trench delivers a portion of the water treated at the LHAAP GWTF. In the LHAAP GWTP treatment system, the water treatment stream (pH 6.3) flows from the influent tank to a pH balance tank where caustic solution (NaOH) is added to bring the pH to 10 as it is fed into a flocculation tank where polymer is added to promote flocculation. The flocculated material is removed during a clarification unit operation which achieves metals removal. After the clarifiers, the stream is fed to an air stripper for VOCs removal. The water leaving the air stripper is stored in a holding tank and then fed into the fluidized bed reactor (FBR) for perchlorate removal. Nutrients and acetic acid are added to the water to encourage biological activity within the FBR. The FBR contains coconut husk biochar to which the perchlorate degrading bacteria can attach. The FBR effluent must meet discharge limits of a Daily Average concentration of under 279 µg/L and a under the daily maximum concentration of 591 µg/L. The plant runs in a steady state flow operational mode until it reaches the holding tank. It is run as a batch system with feed from the holding tank in a recycle loop until determined that the perchlorate concentration has met specifications.

1.2 Problem Statement

The operation and performance of the FBR at LHAAP was not continuous. Extended periods of non-treatment occurred regularly. The causes of these disruptions included plant shutdown, large temperature swings, acetate feed spills, power outages, freezing, and natural shifts in the biological consortia within the FBR over time.

1.3 Objectives

A thorough survey of the system with regards to the amount and type of biological activity within the system and the FBR was conducted to alleviate treatment interruptions at the LHAAP GWTS. The objective of the research presented in this report is to identify the plant conditions and operating procedures that cause interruptions and plant conditions and operating procedures that will reduce treatment interruptions.

1.4 Approach

We conducted sampling of the key plant processes under various operation conditions. These samples were analyzed by genetic sequencing for microbial populations.

2 Perchlorate Biodegradation

Over the last few decades, the specific microbial processes and microbes that are effective for perchlorate biodegradation have been studied. A high level of understanding of these details and how they can be incorporated into water treatment systems has been achieved. Several conditions are required for optimal biological degradation of perchlorate based on a review of research and operational details from a number of existing industrial perchlorate/groundwater remediation systems.

- Effective FBR perchlorate degradation requires anaerobic conditions.
- Nitrate and other electron acceptors present in groundwater can be used during biological respiration more readily than perchlorate and high concentrations of these electron acceptors can reduce the effectiveness of perchlorate degradation.
- The organic substrate used as the electron donor can affect rates.

Some bacteria can use perchlorate as an electron acceptor while oxidizing a large range of substrates. Perchlorate-respiring bacteria (PRB) are widely distributed in the environment and are enriched at perchlorate-contaminated sites. The microbial population in the LHAAP groundwater feed was determined.

Table 2 summarizes many of the critical biodegradation of perchlorate techniques and mechanisms.

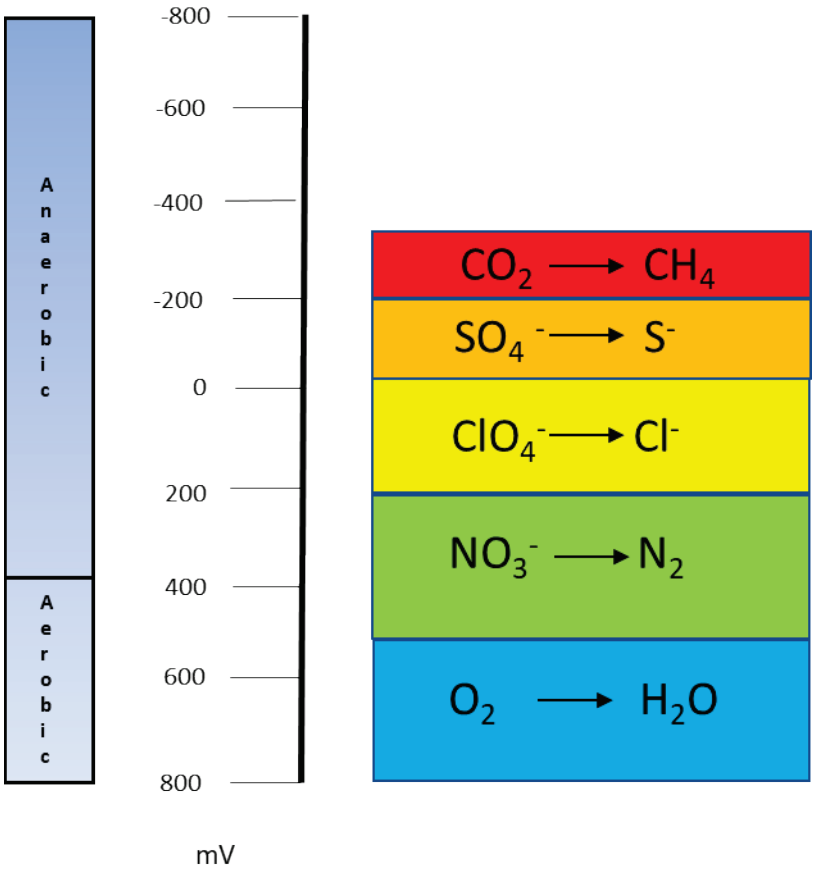
Perchlorate-reducing organisms are commonly found in soil and the environment performing this type of process. Anoxic respiration couples the oxidization of an organic substrate (substrate that can be referred to as an electron donor) to the reduction of an oxyanion (oxyanion can be referred to as an electron acceptor, Nijack [2012]). Microbes capable of degrading perchlorate can use the perchlorate oxyanion as an electron acceptor and are useful within FBRs for treating perchlorate contaminated groundwater (Kengen et al. 1999; Song and Logan 2004; Zhang et al. 2002). Many of the microbes capable of degrading perchlorate are also able to use nitrate as an electron acceptor. Figure 2 illustrates the redox potential at which a range of electron acceptors can be reduced. The redox potential at which perchlorate can be reduced is higher than the potential nitrate can be reduced. Microbes capable of using both nitrate and perchlorate as electron acceptors for the anoxic respiration process will preferentially use available nitrate over

perchlorate. When these multi-substrate reducing microbes are present, the nitrate concentrations are usually reduced to low levels before significant perchlorate reduction occurs.

Table 2. Biodegradation of perchlorate.

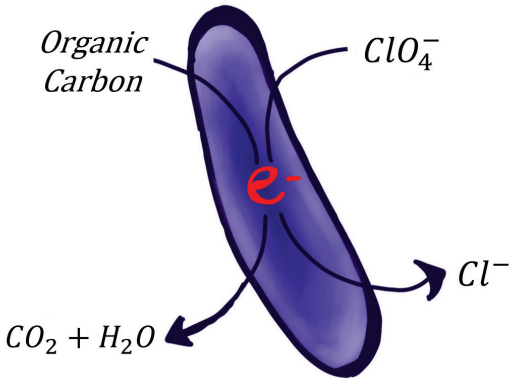
Topic	Description	Reference
Optimal Growth Conditions for Perchlorate Reducing Bacteria (PRB)	Most PRB prefer neutral pH and mesophilic temperature	Balk et al. 2008
	All are strict respires and require an e^- acceptor for growth. Most can utilize alternate e^- acceptors such as O_2, nitrate and chlorate in preference to perchlorate. All perchlorate reducers completely reduce perchlorate to O_2 and Cl^- without accumulation of chlorate, chlorite and O_2.	Wallace et al. 1996 Coates et al. 1999
	Growth coincides with oxidation of e^- donor and production of Cl^-.	Bardiya and Bae 2011
	Although, perchlorate reducing bacteria can use wide variety of organic and inorganic e^- donors, majority of them are unable to use carbohydrates, benzoate, catechol, glycerol, citrate, and benzene. By far, acetate is the most commonly used single e^- donor except *M. perchloratireducens* which cannot utilize acetate.	Balk et al. 2008 Coates et al. 1999 Shrout and Parkin 2006
Perchlorate Reduction by PRB	Perchlorate reduction rates are significantly influenced by presence of alternate e^- acceptors [O_2 and nitrate but not SO_4^-, Fe(III) and Mn(IV)], temperature, salinity, pH, ammonium ion and availability of molybdenum.	Kengen et al. 1999 Chaudhuri et al. 2002
	Perchlorate reducing bacteria utilize O_2 in preference to perchlorate. In *A. suillum* dissolved oxygen at 2 mg L^{-1} caused complete inhibition of perchlorate reduction. Severe inhibition has also been reported with microaerophilic *W. succinogenes* HAP^{-1}, *D. anomalous* strain WD and strains JDS5 and JDS6.	Chaudhuri et al. 2002 Wallace et al. 1996 Coates et al. 1999 Shrout et al. 2005

Figure 2. Redox potential diagram.



The degradation of perchlorate (or nitrate) requires the concurrent oxidation of a co-metabolite. Acetic acid and ethanol were evaluated as growth (i.e., electron donor) substrates for the FBR at the LHAAP. Acetic acid was chosen as the preferable substrate based on a treatability study by Polk et al. (2001). Figure 3 illustrates the co-metabolic process by which perchlorate is degraded through anoxic respiration.

Figure 3. Co-metabolic process for perchlorate degradation through anoxic respiration using acetate as a carbon source.

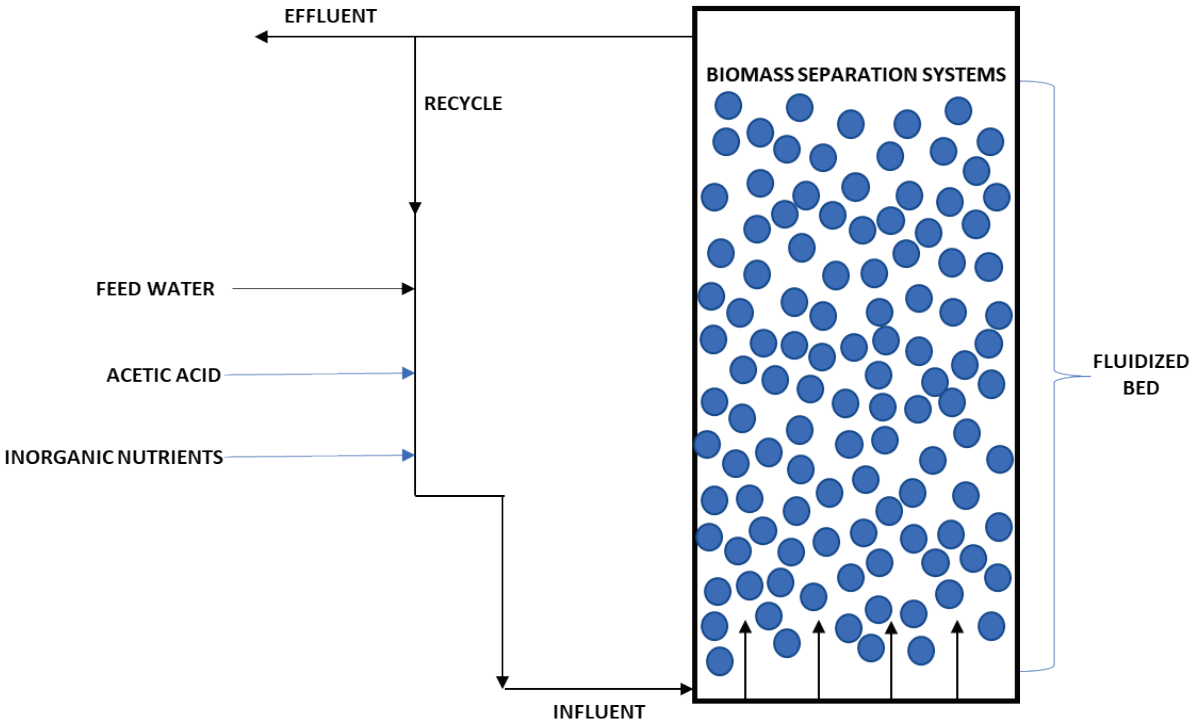


3 Fluidized Beds for Biological Treatment

FBRs have been employed for the treatment of waters containing a wide range of contaminants. These systems operate by taking advantage of specific biological processes that directly or indirectly degrade contaminants. FBRs have been used on industrial scales for the treatment of explosives (Fuller et al. 2007; Rodgers and Bunce 2001), chlorinated solvents (Suidan et al. 1996; Segar et al. 1997), polychlorinated biphenyls (Borja et al. 2006), polyaromatic hydrocarbons (Hickey et al. 1995), pesticides (Bilal et al. 2019), and herbicides (Sandoval-Carrasco et al. 2013).

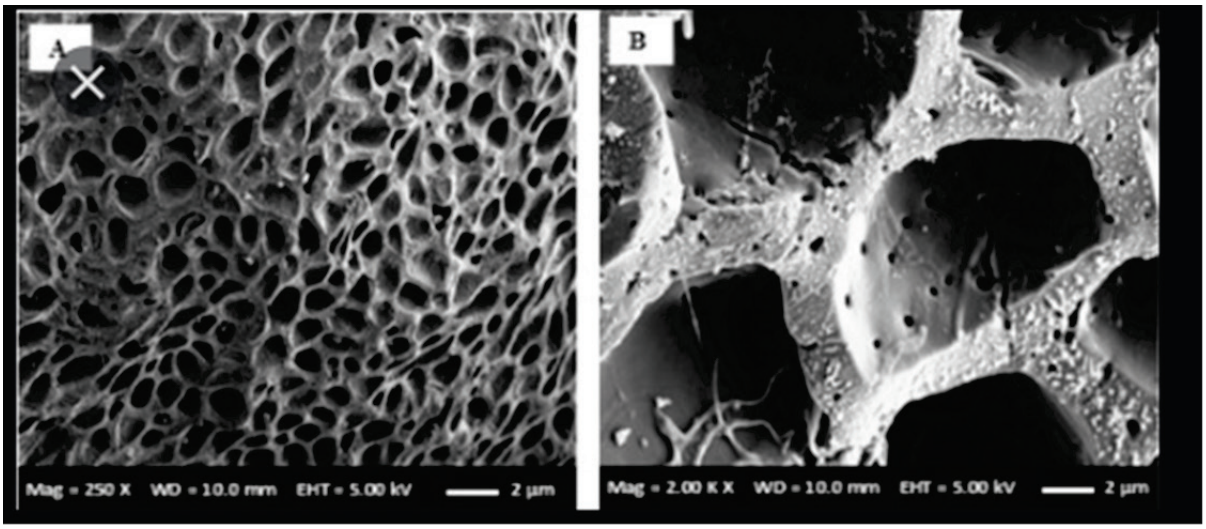
A typical FBR (Figure 4) is a fixed-film reactor that utilizes stationary microbes on a hydraulically fluidized bed of media particles. A solid media such as coconut shell biochar (Figure 5) is used as the fluidized bed media providing the means of integrating the removal capabilities of biotreatment and physical chemical adsorption within this type of FB reactor. The LHAAP FBR was initially inoculated with a proprietary perchlorate degrading microbial community in 2001 (Polk et al. 2001). Since then, the plant has experienced numerous operational upsets (CES 2004, 2005) and temporary shutdowns (CES 2004, 2005). The positioning of the FBR within the treatment plan does not provide natural re-inoculation of the reactor with indigenous groundwater microorganisms. This has resulted in a community of only a few microbes dominating the system rather than a broad spectrum of contributors that would strengthen the health of the reactor. During shutdowns the reactor is starved of perchlorate due to the isolation of the reactor at the tail end of the plant.

Figure 4. Fluidized bed reactor schematic.



A material with perchlorate sorptive capabilities such as bio char (21.97 mg perchlorate/gram of biochar) (Mahmudov and Haung 2010) provides a secondary removal function of the reactor by adsorbing the contaminant. This increases the ability of the system to deal with the issues of microbial inhibition due to toxic inputs and allows for the treatment of recalcitrant compounds (Envirogen 2001). In Figure 5, scanning electron microscopy reveals the high porosity and surface area of the pyrolyzed coconut biomass associated with perchlorate sorption capacity of the media used in the Longhorn FBR system.

Figure 5. SEM images of Longhorn Coconut Husk.



The media is suspended or fluidized within the reactor vessel by the upward flow of water through the system. Suspension of the media allows for mixing, which results in extensive contact between the contaminated water and biologically active surfaces* (Sutton and Mishra 1994). The groundwater containing the perchlorate contamination at LHAAP has low levels of organic matter and requires the addition of electron donor material for the perchlorate degradation to occur. When biological growth occurs on the fluid bed media, the diameter of the media increases and its effective density is reduced, resulting in an expansion of the media bed beyond the expansion of the bare carbon media (Envirogen 2001). An inbed carbon cleaning system works at deeper levels in the carbon bed by shearing the biomass from the carbon using an eductor powered by service water. The excess biomass separated from the media exits the system through the effluent collection system.

The electron donor, acetic acid, is metered into to the FBR. Microorganisms within the reactor perform oxidation/reduction reactions, consuming dissolved oxygen (DO), nitrate, and perchlorate. The acetate electron donor is added to maintain the redox level at which perchlorate degradation occurs. Acetic acid dosing control is required to avoid excess electron donor which could promote sulfate reduction or methanogenesis thereby halting perchlorate degradation. The by-products of the perchlorate degradation are nitrogen gas, chloride ion, carbon dioxide, heat generation,

* US Environmental Protection Agency. 1993. *Nitrogen Control Manual. EPA/625/R-93/010*, Office of Research and Development. Washington, D.C.

and additional biomass. As the microorganisms acclimate and grow, increasing biomass quantities, the number of attached microbes per media particle increases. The presence of abiotic organic materials that make up biofilms has a large effect on the number of attached microbes per media particle.

Attached microbes reside in biofilms that coat the biologically active surfaces of the FBR media. To generate these biofilms, bacteria produce a gel-like, organic, hydroscopic exudate classified as an extracellular polymeric substance (EPS) (Gil-Serrano et al. 1990) and is known as a biopolymer (BP). EPS production will establish and maintain biofilms on surfaces (Davies and Geesey 1995; Heilmann et al. 1996). The EPS often makes up a large fraction of the biofilm mass. Depending on bacteria type, microbial colonies can consist of 10–25% cells and 75–90% EPS as dry mass (Costerton 1999; Flemming et al. 2000). EPS also plays a role in several biofilm characteristics that microbes rely on to develop and maintain communities. Some of the functions of EPS within biofilms are: water retention, including moisture retention in a solid matrix (Larson et al. 2012), surface adhesion, self-adhesion of cells into biofilms (Sutherland 2001), formation of protective barriers, enhanced scavenging of anions and cations from bulk liquid and surfaces, nutrient accumulation (Laspidou and Rittmann 2002), enhanced growth rates, resistance to antibiotics and disinfectants (Goldberg 2002; Peng et al. 2002), greater local diversity, physical protection and stabilization (Chen et al. 1998), and providing a framework for coordinated behavior (Bais et al. 2006). The presence of these biofilms within FBRs relates directly to operational factors such as media density and the size and diversity of microbial population.

Biotreatment of water using FBRs have been shown to be effective and low-cost techniques for perchlorate degradation in pumped groundwater. The operation and performance of the FBR at LHAAP, however, is dependent on specific conditions within the reactor and the larger groundwater treatment process. By evaluating the microbial community compositions at periods of adequate perchlorate degradation, sub-adequate perchlorate degradation, non-operating conditions, and increased understanding of the factors affecting the performance of the LHAAP perchlorate degrading FBR can be achieved.

4 Results and Discussion

The concentration of perchlorate at five locations within the water treatment facility at Longhorn was measured during a period of effective water treatment. Table 3 presents the perchlorate levels measured at these locations within the treatment system during a period of effective plant operation. As would be expected, measurable levels of perchlorate were measured in the sample of the input water and before and after the air stripping process. The samples taken downstream from the FBR contained non-detect levels of perchlorate (<5 ppb). These values are typical, but the concentrations of perchlorate and other contaminants vary as factors such as pumping rates, groundwater recharge rates, and seasonal variations in groundwater levels vary.

Table 3. Perchlorate concentration measured in LHAAP samples collected during normal operation at various locations within the treatment system.

Sample Location	Perchlorate (mg/L)	Sample Location	Perchlorate (mg/L)
Influent	*12.8*	After FBR	*ND*
Before Stripper	*12.4*	After IX	*ND*
After Stripper	*13.3*		

Table 4 describes the water chemistry at six locations within the system during effective operations. Specific conductance, sulfate, and sulfide levels increase steadily throughout the system under these operating conditions. The pH, ammonia, and phosphorous vary at these points. Manganese and zinc levels decrease as water passes through these points in the treatment system.

Table 5 provides a list of genera of interest to the genus level present in these consortia. The percentage is an indication of the fraction of DNA obtained from all microbial species identified as being unique to the particular genus. Many of these genera have been shown to contain species demonstrated to degrade perchlorate in anaerobic conditions.

Table 4. Water chemistry during steady state operations.

Analyte	Units	RESULTS					
		Groundwater	After Clarifier	After Stripper	After FBR	IX Column	Plant Effluent
Specific Conductance (EC)	uS/cm	1540	1850	1960	2260	2220	2410
pH	pH Units	6.34	10.1	9.44	7.57	7.45	8.35
Ammonia as N	mg/L	0.201	0.219	0.154	17.3	17	16.8
Sulfate	mg/L	70.2	73.5	74.1	90.5	90.9	122
Sulfide	mg/L	ND	ND	ND	5.18	8.15	19.6
Phosphorus	mg/L	0.178	0.175	0.0817	3.91	3.77	3.48
Manganese	mg/L	0.54	0.268	0.107	0.0724	0.0759	0.0755
Zinc	mg/L	0.112	0.0708	0.0411	0.0396	0.0354	0.0252

Table 5. Genera representing at least 2% of the consortia using 16S MiSeq under different plant conditions*.

Sample Point	Groundwater	FBR influent	FBR effluent	IX effluent	FBR effluent	FBR effluent
Plant condition	Normal Operation				On Hold	Total Shutdown
Campylobacteraceae Arcobacter	0.5%	1.1%	2.2%▲	1.0%	6.2%	31.5%▲
Comamonadaceae Hydrogenophaga	26.1%▲	1.7%▲	15.3%▲	2.0%▲	0.0%	0.1%
Comamonadaceae Limnohabitans	0.1%	8.9%▲	1.7%	6.0%▲	0.0%	0.0%
Geobacteraceae Geobacter	0.1%	0.2%	1.4%	0.5%	1.8%	9.9%▲
Helicobacteraceae Sulfurimonas	0.0%	0.0%	0.0%	0.0%	9.1%▲	0.0%
Pseudomonadaceae Pseudomonas	0.6%▲	5.8%▲	21.7%▲	7.5%▲	10.0%▲	0.0%
Rhodocyclaceae Dechloromonas	0.2%	0.2%	0.7%	0.3%	55.1%▲	0.2%
Rhodocyclaceae Thauera	4.2%▲	0.0%	0.0%	0.0%	0.3%	0.3%▲
Sum	31.80%	17.90%	43.00%	17.30%	82.50%	42.00%

Dark text indicates >2%, ▲ – Top three

The values listed in Table 5 represent the percentage of the total amplified ribosomal RNA sequences following nested RNA polymerase chain reaction (PCR) application. The interaction of these sequences with barcoded binding units is used for the attribution of these sequences to specific microbial genera (Illumina 2018). The percentages listed in Table 5 are not necessarily representative of the actual microbial community as the nested 16s RNA assay introduces acknowledged biases that have been shown to over or under proportion specific microbial groups. Inherent biases are introduced because of multiple aspects of the assay (Schirmer et al. 2015). Given that PCR biases are widely recognized, and further unknown biases may arise from the sequencing process itself, the use of the values presented must be qualified by the knowledge that these values are loosely representative of the microbial community at the LHAAP (Lee et al. 2012).

DNA extractions from sparse bacterial populations, such as groundwater are evaluated using nested PCR as a technique for increasing the concentration of microbial DNA. Without this step concentrations in the groundwater are typically several-fold lower than standard PCR. Nested PCR involves two rounds of PCR reactions with the first round targeting a wide DNA region and the second targeting a narrower sub-region of the products of the first round. In nested PCRs, barcoded primers are used in the second round of PCR. Nested PCR results in an unavoidable bias due to preferential amplification which will be greater when two successive PCR reactions are applied (Yu et al. 2015). The specific set of binders used during the second round of amplification affect the DNA amplified as the microbial DNA that does not match the binders well will not be fully represented. The representation of rare genera and species of microbes requires high depth of coverage/depth of sequencing. (Pinto and Raskin 2012). In addition to primer bias, the identification of microbes with specificity at the genera and species levels is dependent on the accuracy and completeness of the library the RNA sequences are queried against. Microbial genera less fully represented within the library may be underrepresented in the results compared to those more fully sequenced. The results summarized in Table 5 are specific to the taxonomic assignment from the QIIME II library. (Caporaso et al. 2010). The depiction of the LHAAP microbial communities from the nested PCR-based survey used is inherently biased against low abundant taxa which are required to fully perform taxonomic assignment of these communities (Gonzalez et al. 2012). During the presentation of results and discussion

that follows, the taxonomic assignments and relative number of amplified sequences attributed to each genera will be used acknowledging these unquantified biases.

The taxonomy of all the family/genera listed in Table 5 is: Kingdom-Bacteria, Phylum- Proteobacteria. The classes and orders represented within the eight genera listed in Tables 4-6 are: Under Class Betaproteobactera are the *Burkholderiales (Comamonadaceae Hydrogenophaga* & *Comamonadaceae Limnohabitans*) and *Rhodocyclales (Rhodocyclaceae Dechloromonas*

Rhodocyclaceae Thauera) Orders. Class Deltaproteobacteris Order Desulfuromonadales (Geobacteraceae Geobacter). Class Epsilonproteobacteria Order Campylobacterales (Campylobacteraceae Arcobacter & Helicobacteraceae Sulfurimonas) and Class Gammaproteobacteria Order Pseudomonadales (Pseudomonadaceae Pseudomonas). For each genus-level identifications, electron acceptor utilization (including perchlorate) is documented in Table 6.

Three of the genera have been shown to contain species capable of degrading perchlorate as either a metabolite or co-metabolite during microbial respiration. They all use oxygen, seven use nitrate, and three use sulfur. The presence of species capable of reducing the redox potential of the water within the treatment plant to levels at which perchlorate can be used as a metabolite or co-metabolite (Figure 3) is indicated by the effective perchlorate degradation observed during normal plant operational periods (Table 5).

The conditions associated with the microbial population metabolism with references contained within are listed in Table 7. At the genera level, all organisms discussed are classified as chemotrophs, which indicates the ability of an organism to oxidize inorganic or organic compounds as its principal energy source (McIlroy et al 2017), only Limnohabitans is classified as phototrophs and able to use light as an energy source. Two organisms can obtain their energy from inorganic compounds (lithotrophs) and five are able to obtain energy from electron transfer to organic compounds (organotroph) via electron transfer from that molecule (oxidation). One organism, Pseudomonas, had species within its genus able to use either inorganic or organic compounds as electron donors. Four organisms were classified as autotrophs, which obtain carbon from

inorganic sources like carbon dioxide (CO2) and five were classified as heterotrophs. Heterotrophic bacteria get their reduced carbon from other organisms (McIlroy et al 2017).

Table 6. Electron acceptor utilization by family/genera listed in Table 5.

Family Genus	O_2	NO_3^-	NO_2^-	N_2O	SeO_4^{2-}	Iron (III)	ClO_3^-	ClO_4^-	S	Oxic/ Anoxic	References
Campylobacteraceae Arcobacter	Y	Y						Y**			Carlström et al. 2013, Rice et al. 2002
Comamonadaceae Hydrogenophaga	Y	Y								aerobe	Zhao et al. 2011
Comamonadaceae Limnohabitans	Y									aerobe	Kasalický et al. 2018
Geobacteraceae Geobacter	Y	Y				Y			Y	anaerobe	Röling 2014
Helicobacteraceae Sulfurimonas	Y	Y	Y						Y		Han and Perner 2015 Cheng et al. 2018
Pseudomonadaceae Pseudomonas	Y	Y						Y	Y	Obligate aerobe or facultative anaerobe	Steinberg et al. 2005 Coates et al. 1999
Rhodocyclaceae Dechloromonas	Y	Y	Y				Y	Y		Facultative anaerobe	Coates 2015
Rhodocyclaceae Thauera	Y	Y	Y	Y	Y						Xing et al. 2018, Anupama et al. 2015

In which: Oxygen (O_2), Nitrate (NO_3^-), Nitrite (NO_2^-), Nitrous Oxide (N_2O), Selenate (SeO_4^{2-}), Iron (III), Chlorate (ClO_3^-), Perchlorate (ClO_4^-), and Sulfur (S)

* Obligate aerobe

** Only one species capable of perchlorate degradation in saline conditions

Table 7. Metabolism classifications for each genera listed in Table 5.

Family Genus	Energy source		Electron Donor		Carbon Source		Reference
	Photo-troph	Chemo-troph	Organo-troph	Litho-troph	Hetero-troph	Auto-troph	
Campylobacteraceae Arcobacter		x				x	Carlström et al. 2013
Comamonadaceae Hydrogenophaga		x		x		x	Xing et al. 2018 Willems and Gillis 2015
Comamonadaceae Limnohabitans	x	x	x		x		Kasalický et al. 2018 Hahn[1] et al. 2010
Geobacteraceae Geobacter		x	x		x		Röling 2014
Helicobacteraceae Sulfurimonas		x				x	Cheng et al. 2018 Han and Perner 2015
Pseudomonadaceae Pseudomonas		x	x	x	x		Coates et al. 1999 Madigan and Martinko 2005 Nozawa-Inoue, et al. 2005 Rowe et al. 2015
Rhodocyclaceae Dechloromonas		x	x		x		Coates 2015 Nozawa-Inoue, et al. 2005 McIlroy et al 2017 Sun et al. 2009
Rhodocyclaceae Thauera		x	x		x	x	Xing et al. 2018 Oren 2014 McIlroy et al 2017

5 Summary of Known Properties for the Genera Selected

The specific aspects of each of the genera listed in Table 5 with regards to water treatment plants in general and bio-reactive processes for perchlorate removal are described.

5.1 Arcobacter

Arcobacter, from the Latin arcus meaning "bow" and the Greek bacter meaning "rod," so "bow-shaped rod" or "curved rod." Most *Arcobacter* cells possess a characteristic curved shape. This genus of spiral-shaped bacteria is found in a wide range of habitats, and some species can be human and animal pathogens. Three of the five known species within this genus are pathogenic and as an aero tolerant species these microbes have the capability to survive in air.

The *Acrobacter* species, *A. sulfidicus* is an obligate micro-aerophile that oxidizes sulfides. High salt tolerance is characteristic of these microbes. *Arcobacter* species can be isolated from water collected from hypersaline lagoons. Another *Arcobacter* species, designated strain CAB, was isolated from marine sediment and found to have the capacity to grow via perchlorate reduction. This is the only member of the *Arcobacter* genus that has been shown to degrade perchlorate (Carlström et al. 2013). Dissimilatory perchlorate-reducing bacteria (DPRB) like Arcobacter CAB exist largely in freshwater, mesophilic, neutral-pH environments.

The species *Arcobacter butzleri* was isolated from a contaminated groundwater source. These microorganisms, while found in soils and sediments, can survive in the groundwater environment. *A. Cryaerophilus* is commonly found in wastewater treatment systems. A study of wastewaters identified two distinct subgroups of this species, one was predominant in samples with temperatures above 20°C and another in less than 20°C systems. Fisher et al. (2014) concluded that this finding is relevant because understanding the ecological factors affecting the fate of *Arcobacter spp*. in wastewater is dependent on the wastewater temperature. The species *A. skirrowii* is a potentially pathogenic microbe predominantly observed in mammals and food products. *A. nitrofigilis* is a halotolerant microbe tolerant of salinity levels above that of seawater. It can be a symbiotic organism in a marine environment, infecting plant

roots and fixing nitrogen. *A. sulfidicus* is an autotrophic obligate that oxidizes sulfides and that can produce filamentous sulfur (Sievert et al. 2007; Rice et al. 2002; Madigan et al. 2009).

5.2 Dechloromonas

This is an important group of microbes with regards to biodegradation of perchlorate in engineered systems (Sun et al. 2009; Nozawa-Inoue et al. 2005). Within this genus exists microbes capable of effectively degrading a range of contaminants including perchlorate. One widely studied species is a facultative anaerobe bacterium that occurs in soil environments, *Dechloromonas aromatica*. This species within the *Dechloromonas* genus can degrade benzene anaerobically, degrade perchlorate and oxidize chlorobenzoate, toluene, and xylene. This species is unique as it is one of the few anaerobes capable of degrading contaminants containing aromatic rings. Mutants of this specie that do not contain genes associated with the productions of perchlorate reductase enzyme did not show the ability to degrade perchlorate, which indicates this gene is critical for perchlorate biodegradation by these microbes. These mutants retained the ability to use nitrate as an electron acceptor. Microbes of this genus are chemo-organo-heterotrophs with a strictly respiratory metabolism. They have been shown to be capable of oxidizing acetate with O2, chlorate, perchlorate, or nitrate as electron acceptors. In systems shown to degrade perchlorate, *Dechloromonas, Sulfuricurvum,* and *Hydrogenophaga* existed in biofilms.

5.3 Geobacter

Species within the *Geobacter* genus perform anaerobic respiration. This capability makes them useful in perchlorate bioremediation despite their apparent inability to directly degrade perchlorate. The production of extracellular polymeric substances (EPS) critical to biofilm construction and reducing oxygen and nitrate species favors perchlorate degradation by other species of bacteria. *Geobacter* species oxidize organic compounds and metals, including iron, radioactive metals, and petroleum compounds into environmentally benign carbon dioxide while using iron oxide or other available metals as electron acceptors. They have been found in anaerobic conditions in soils and aquatic sediment. *Geobacter* has been identified in previous studies on the microbial reduction of nitrate or perchlorate, however, there is little evidence that microbes within this genus are active in degradation of perchlorate. These microbes have shown

the ability to produce EPS for the formation of thick, stable biofilms. This propensity for biofilm production has resulted in the use of *Geobacter* as part of supported bioelectrical current generation studies (Wan et al. 2018; Wen et al. 2017; Diaz 2008). Electric currents observed at anodes of electrochemical cells result from the oxidation of organic substrates. Researchers have proposed that *Geobacter* biofilms can be used to power microbial fuel cells and generate electricity from organic waste products (Malvankar et al. 2012). The potential of species within these genera within the bio-generation of electricity has resulted in extensive study of this genus in wastewater treatment plan environments.

5.4 Hydrogenophaga

Hydrogenophaga (eater of hydrogen) is a genus that has been studied due to the autotrophic growth of these aerobic hydrogen oxidizers. Research has been directed to the utilization of these bacteria to produce biomass or particular materials by CO2 fixation. Other potential applications of the enzyme hydrogenase found in these species have been explored in a wide range of practical technologies such as hydrogen production as a fuel supply of reducing power for bioremediation. In water treatment systems shown to degrade perchlorate, biofilms have been identified in which *Sulfuricurvum, Hydrogenophaga*, and *Dechloromonas* dominate the biofilm consortia. *Sulfuricurvum* and *Hydrogenophaga* were associated with decreases in the nitrate concentration and *Dechloromonas* with the direct degradation of perchlorate. Hydrogenophaga appears to act as a promoter of perchlorate degradation by other microbial species (Zhao et al. 2011).

5.5 Limnohabitans

Limnohabitans is a genus of Betaproteobacteria established by (Hahn et al. 2010b). The genus contains four species that represent planktonic bacteria dwelling in the water column of freshwater lakes, reservoirs, and streams (Hahn et al. 2010a). As part of freshwater, bacterioplankton community's species within these genera show high rates of substrate uptake and growth on algal-derived substrates. The bacteria from the R-BT lineage are known to inhabit a broad range of freshwater habitats within at least three continents and can constitute up to 30% of free-living bacteria in freshwater systems. In lakes, they inhabit both oxic and anoxic environments. In aquatic systems, the microbes have shown a

vulnerability to protozoan grazing, flagellates, and virus infection (Buck et al. 2009; Hahn et al. 2010a; Hahn et al. 2010b).

5.6 Pseudomonas

Pseudomonas is a genus of Gram-negative, Gammaproteobacteria, belonging to the family *Pseudomonadaceae* and containing nearly 200 species. These species have a wide range of metabolic activities and are present in a wide range of environments. While some species within this common genus of bacteria can cause infections in mammals, there are many different types of *Pseudomonas* bacteria; only a few types can cause an infection. *Pseudomonas* bacteria tend to live and breed in water, soil, and damp areas. Some members of the genus can metabolize chemical pollutants in the environment, and as a result, be used for bioremediation. Notable species demonstrated as being suitable for use as bioremediation agents include:

- *Pseudomonas sp. PDA* is an unusual bacterium due to its ability to respire using chlorate under aerobic conditions. Oxygen is known to prevent chlorate reduction by all chlorate and perchlorate reducing strains except for Pseudomonas sp. PDA.
- *P. chloritidismutans* differed from other (per)chlorate-reducing bacteria in that it was only able to use chlorate as a terminal electron acceptor. Other chlorate-reducing bacteria can also couple the reduction of perchlorate or nitrate to growth.
- *P. alcaligenes*, which can degrade polycyclic aromatic hydrocarbons (O'Mahony et al. 2006).
- *P. mendocina*, which can degrade toluene (Yen et al. 1991).
- *P. pseudoalcaligenes*, which can use cyanide as a nitrogen source (Huertas et al. 2006).
- *P. resinovorans*, which can degrade carbazole (Nojiri et al. 2002).
- *P. veronii*, which has been shown to degrade a variety of simple aromatic organic compounds (Nam et al. 2003; Onaca et al. 2007).
- *P. putida*, which can degrade organic solvents such as toluene (Marqués and Ramos 1993).
- *P. stutzeri* can degrade carbon tetrachloride (Sepulveda-Torres et al. 1999).

The breadth of metabolic processes associated with species within this genus makes the presence of these microorganisms with water treatment systems of interest. The ability to use chlorate and perchlorate as electron

acceptors exhibited by members of this genus may indicate these microbes playing a role in perchlorate degradation at LHAAP.

5.7 Sulfurimonas

Members of the genus *Sulfurimonas* have been shown to be effective denitrification bacteria capable of reducing perchlorate and are species of interest within systems for bioremediation of perchlorate contaminated groundwater. Within the genus *Sulfurimonas*, several non-pathogenic species are associated with hydrothermal vent environments. Other species within this genus have only been detected in terrestrial environments. The widespread occurrence of these species suggests an ability to successfully colonize a range of habitats during evolution.

Reduced sulfur species, when present in oil wells, result in an undesirable condition referred to as souring. Sour wells produce oil less valuable than non-sour wells, and a number of techniques have been attempted to sweeten these types of wells. One such approach is introducing perchlorate into these systems. The ability of sulfur reducing species such as some of the *Sulfurimonas* genus to use perchlorate as an electron acceptor during sulfur oxidation processes was shown as a means of lowering levels of reduced sulfur in some wells. The "sweetened" wells could produce higher value oils due to the lower levels of reduced sulfur in the product. (Takai et al. 2006; Cheng et al. 2018; Wan et al. 2017b; Haaijer et al. 2008; Han et al. 2012).

5.8 Thauera

Species in this genus occur in wet soil and polluted freshwater. The genus *Thauera*, like some dechloromonas species, has members that have shown the ability to use aromatic hydrocarbons as a substrate under anoxic conditions (McIlroy et al. 2017). These microbes are chemoorganoheterotrophs with a strictly respiratory metabolism. Molecular oxygen, nitrate, nitrite, and nitrous oxide are used as terminal electron acceptors. Selenate is used as the electron acceptor by some strains. Various organic acids, amino acids, and aromatic and aliphatic compounds are used as sole substrates. Only a few carbohydrates are utilized. Also, *Thauera* can grow chemolithoautotrophically, using hydrogen, carbon dioxide, and oxygen, or using simple organic compounds for growth.

This genus is a producer of extracellular polymeric substances known to be floc-forming materials. These biopolymers have the chemical form of polyhydroxyalkanoates. These polymeric materials have been studied as biodegradable alternatives to fossil-fuel based plastics. Geo-engineering processes employing biological carbon sequestration approaches have been pursued wherein these microbes are used to produce stable biopolymers and reduce the mass of biologically transformed carbon dioxide entering the atmosphere. These biopolymers have also been explored for potential as value-added products and remedial control substances for mobile metal and radionuclide contaminants (Wan et al. 2019a; Oren 2014; Garrity et al. 2005).

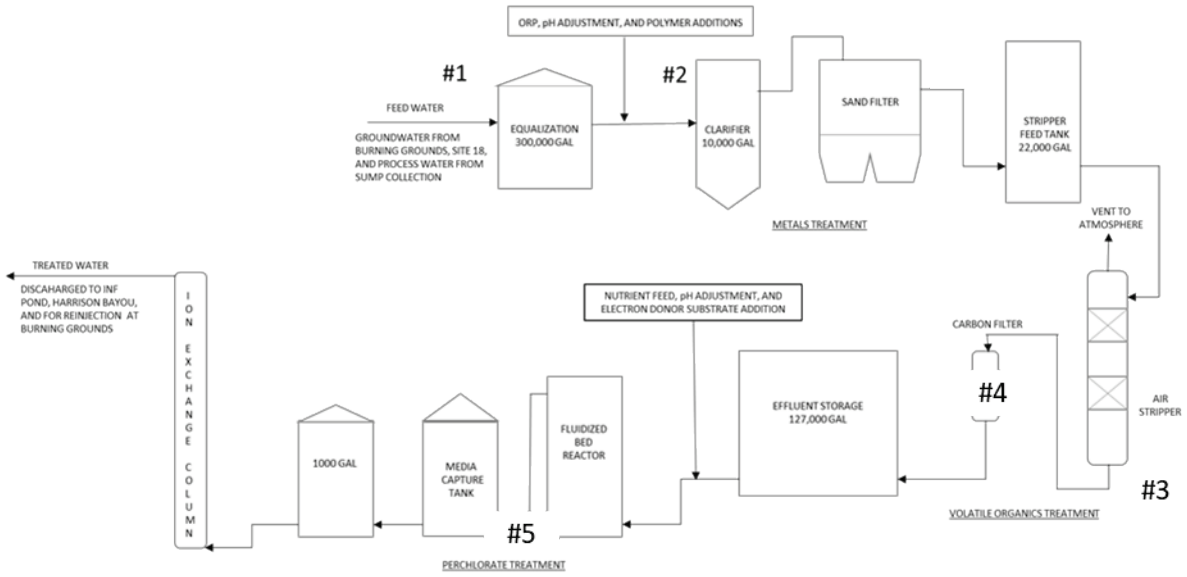
6 Water Treatment Operations Occurring Prior to the FBR Operation

The water pumped from the contaminated aquifer contains a broad range of genera. Among these are heterotrophic species that are useful for biological degradation of perchlorate within an FBR. Other species are useful for the removal of nitrate and other electron acceptor substrates that can be used preferentially over perchlorate for microbial respiration. The presence of a diverse bio consortia containing microbes capable of using nitrate as a substrate as well as perchlorate degrading microbes is critical if extensive reduction of perchlorate is to be achieved using a FBR for bioremediation.

Eight discreet operations occur prior to water entering the FBR. These locations are labelled 1-5 on Figure 6 and discussed with regards to possible effects on microbial population compositions.

The contaminated groundwater is collected by interceptor trenches as shown in Figure 1 and is pumped using a network of pumps. This water is delivered to the equalization tank (Figure 6, #1). The naturally occurring perchlorate degraders and support organisms present in the groundwater are immediately shifted as they enter the plant. Microbes present in the groundwater environment are present in this tank but not in a manner that represents the subsurface environment. Unattached microbes found in the aquifer may be overrepresented because they will move with the flow gradient established by the well configuration and pumping rates.

Figure 6. Locations in the treatment plant that can affect the microbial consortium within the treatment system.



The groundwater is first treated for metals content beginning at Site #2 in Figure 6. The streams Oxidation-Reduction Potential (ORP) and pH is monitored and adjusted with magnesium hydroxide and sodium hydroxide respectably as an anionic polymer (Magnafloc 110-L, BASF Corporation, Sufflolk, VA) is added as a floccing agent. The stream is then fed into a clarifier with the overflow feeding into a sand filter. The clarifier and sand filter physically remove the natural microbes.

The effluent from sand filters is collected in an air stripper feed tank and passed through an air stripping column (#3, Figure 6) for volatile organic removal. By forcing large volumes of air through the process stream, the air stripper operation will rapidly result in oxygen saturated aqueous conditions. Strictly anaerobic microbes such as *Dechloromonas* and many other known perchlorate degrading species will be in an unfavorable environment within oxygen saturated waters. Bacterial removal by air stripping documented as packed-tower designs have been shown to decrease heterotrophic bacteria by an average of 28% (Long 1997). Although no conclusive evidence has been presented regarding the mechanisms of bacterial removal by air stripping, the observed occurrences were generally accompanied by an increase in dissolved oxygen by 14% (Lingireddy 2002).

After the air stripper, the process stream is fed through a carbon filter (Site #4 in Figure 6) and into a storage tank. The carbon filter is used to

perform a polishing step to remove any remaining VOCs prior to microbial treatment of the perchlorate. Carbon filters can be a source of microbial pathogen forming communities. Several studies have been performed to understand the effect of carbon filters on microbial populations (Nriagu et al. 2018). In a study that used a water with low levels of total organic carbon, bacteria growth and biofilm formation were limited in the municipal main distribution and service line waters that occurred downstream of the carbon filters. Besides the low levels of organic carbon, it has been shown that microbial growth in systems following carbon filtration was suggested to be limited by lack of minerals, nutrients, the presence of residual disinfectants, etc. (Otterholt and Charnock 2011). Controlled experiments using carbon filters indicate that bacteria attachment to carbon within these filters is an important mechanism by which microorganisms penetrate treatment barriers and enter potable waters. Attachment to and formation of microbial communities on surfaces within such GAC filters has been shown to vary by microorganism based on the conditions within the filters. Because the carbon filters used for drinking water purification are effective accumulators of soluble and insoluble water constituents, accumulation of minerals, organic matter, and essential trace elements on filters have been shown to be sufficient to promote bacteria growth and biofilm formation on the carbon surfaces (Otterholt and Charnock 2011).

In addition to affecting the population of microbes in the effluent from carbon filters, the microbial community composition has been shown to be altered by these types of units (Wu et al. 2017). Wu et al. (2017) showed that influent, effluent, and media samples had distinct bacterial community structures, and the structure of effluent communities was more similar to that of the upstream filter fabric communities than that of influent communities. The bacterial community structure changed due to the filter environment itself. In this study, the relative abundance of individual microbes tolerant of filter environment, and the absolute abundance of these microbes was shown to increase in the effluent relative to the influent (Table 5). It is expected the carbon filter (#4, Figure 6) within the LWAAP GWTP alters the bacterial abundance and composition of the water. This alteration may not be one that promotes the presence of perchlorate degrading microbes or the microbes that serve a role in generating an environment within the FBR required for efficient perchlorate degradation.

The FBR (#5 in Figure 6), the systems designed and used to reduce perchlorate concentration are the last of the process. The effectiveness of the FBR operation is dependent on the microbial population and the specific microbes within the FBR. The effect of the earlier processes within the plant on the microbial community is a factor in the effectiveness of the FBR. The operation associated with the biological treatment of perchlorate at the LHAAP GWTS is primarily the FBR reactor itself. Anoxic respiration couples the oxidization of an organic substrate (substrate that can be referred to as an electron donor) to the reduction of an oxyanion (oxyanion can be referred to as an electron acceptor) (Nijack 2012). Microbes capable of degrading perchlorate can use the perchlorate oxyanion as an electron acceptor and are useful within FBRs for treating perchlorate contaminated groundwater (Kengen et al. 1999; Song and Logan 2004; Zhang et al. 2002). Many of the microbes capable of degrading perchlorate are also able to use nitrate as an electron acceptor. The FBR is an anoxic system; shown as a basic schematic in Figure 5. The reactor vessel consists of one 5-ft diameter by 21-ft high, stainless-steel vessel. Inside of the reactor is an inlet distribution header with laterals and nozzles, near the top is an effluent collector pipe and recycle collector pipe, and the reactor has an open top with a grating. Floatables are removed from the surface via the effluent collector pipe (Envirogen 2001).

7 Microbial Populations Within the LHAAP Groundwater Treatment System

To learn what factors affect the performance of the FBR operation within the larger treatment system, the composition of the microbial communities at different points within the plant and at varied operational states of the plant were determined. Understanding of the specific microbes and the ability of these communities with regards to supporting the species capable of degrading the perchlorate as well of those capable of directly degrading the perchlorate is required to optimize plant operation for effective perchlorate treatment within the FBR operation. Microbial identification using 16S rRNA for analysis provided indications of the presence of specific genera present at three distinct operating conditions of the LHAAP. These three conditions occurred over a period of ten months. The microbial community present within the equilibration tank during the condition of effective operation and normal flow is discussed below.

7.1 Microbial populations of the pumped groundwater as it enters the LHAAP groundwater treatment systems

In circumstances where aquifer contamination has persisted over many years, as is the case at the LHAAP, the aquifer microbial community often shifts in a way perchlorate utilizers are represented within the communities. The ability to directly use a contaminant such as perchlorate as an energy source is a means of limiting toxicity of the contaminant's present . In many cases, the requisite perchlorate degraders are naturally present and providing some level of natural attenuation within the groundwater. These processes are often inadequate for aquifer management as the degradation rates can be considerably slower than plume migration rates. These processes can be accelerated using a system like an FBR in which the conditions (pH, Temp, etc.) are controlled and the requisite nutrients and electron donor species are present.

Except for the initial inoculation with a proprietary perchlorate degrading microbial community in 2001 (Polk et al. 2001), the source of bacterial species entering the water treatment system is a result of the microbial community in the input water as it enters the system. A wide range of species were observed in this water with none representing greater than 1% of the consortium except for *Hydrophangia* which represents 26.1% and Thauera which represents 4.2% of the amplified DNA observed in the

MiSeq® (Illumina, San Diego, CA) amplification procedure. The remainder of species present include several known to be perchlorate degraders. The possible 75 years during which perchlorate is present in the aquifer may have played a role in the presence of these species. DNA sequences indicative of other species use nitrate, oxygen, and nitrite, and are represented within the amplified DNA sample. Sulfur reducing species are observed as well. Many species present assigned low percentages of amplified DNA used for sequencing are known producers of exopolymeric substances. The low level (percentages) in the material undergoing sequencing may not be representative of the aquifer microbial consortia makeup as attached species are less likely to be present in pumped groundwater relative to unattached species. The attached species within established biofilms have less mobility in pumped systems than unattached species that move freely along gradient flow lines.

Each genera listed in Table 5 except sulfurimonas is present in the pumped groundwater being used to feed the LHAAP groundwater treatment system. The percent of each genera present are generally low except for Hydrogenophaga and Thaura which are indicated at 26 and 4%. The point at which these pumped groundwater samples were taken is indicated on Figure 7. At this sampling point the pumped groundwater is combined and the pressure equalized in a 300,000-gal tank. The sample of this water was taken at the point immediately prior to it is entering the treatment plant from this equalization tank.

7.2 Microbial populations within the LHAAP groundwater treatment system

Each organism listed in Table 5 is present in samples taken from one or more of the sampling locations identified in the plant schematic below. Samples were taken during three operational conditions at the LHAAP water treatment plant and at one to four separate locations.

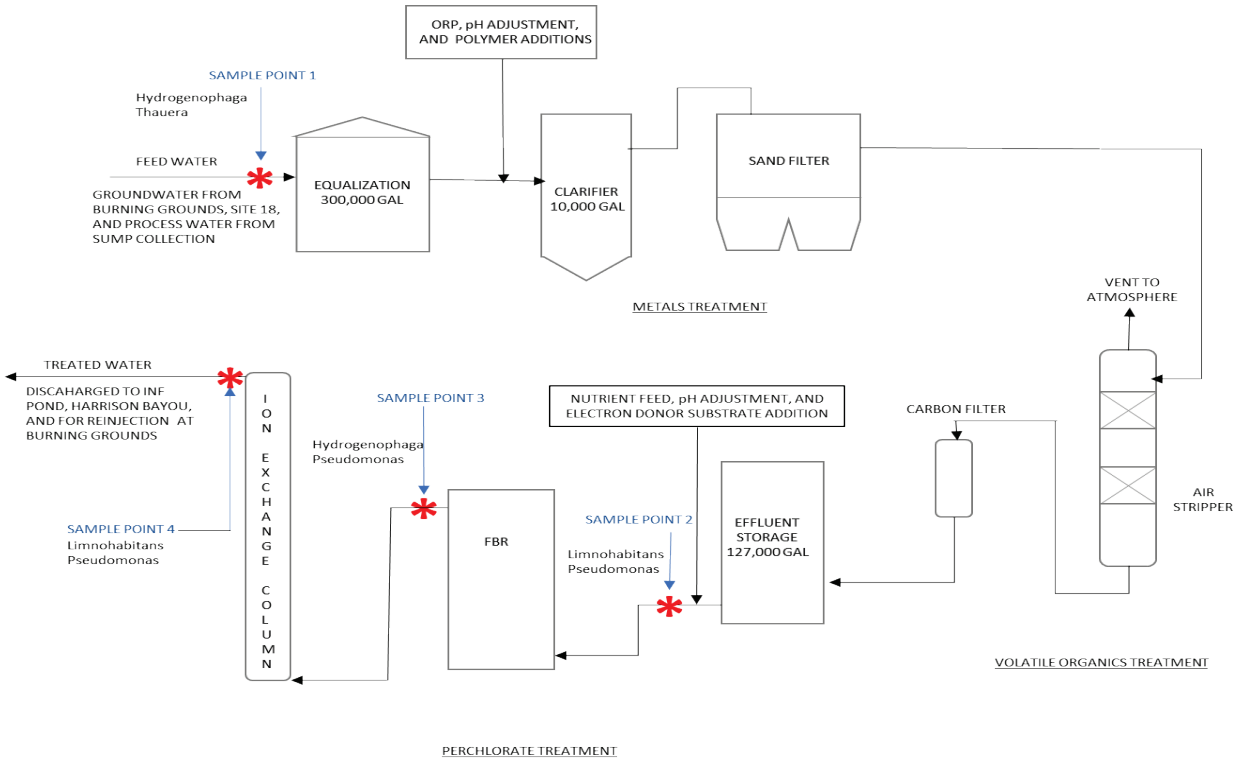
The three operating conditions were:

- Sample taken during plant hold condition. Reactor in recycle. 4/1/2017
- Sample taken during plant shutdown. No flow condition/ power outage/ no generator 9/12/2017
- Normal operational conditions on 1/9/2018

The four sample locations labelled as sampling points 1-4 in Figure 7 are:

- Sampling point 1 is groundwater feed into the GWTP.
- Sample point 2 is the input to the FBR
- Sample point 3 is at the Effluent of the FBR
- Sampling point 4 is after the ion exchange sorptive removal operation.

Figure 7. Genera identified at sampling points during steady state (normal) operations for perchlorate degradation.



Two genera were indicated as being present in the FBR at greater than 2% during periods of **successful perchlorate degradation**, *Limnohabitans* and *Pseudomonas* at the locations shown in Figure 7.

7.3 Limnohabitans and pseudomonas

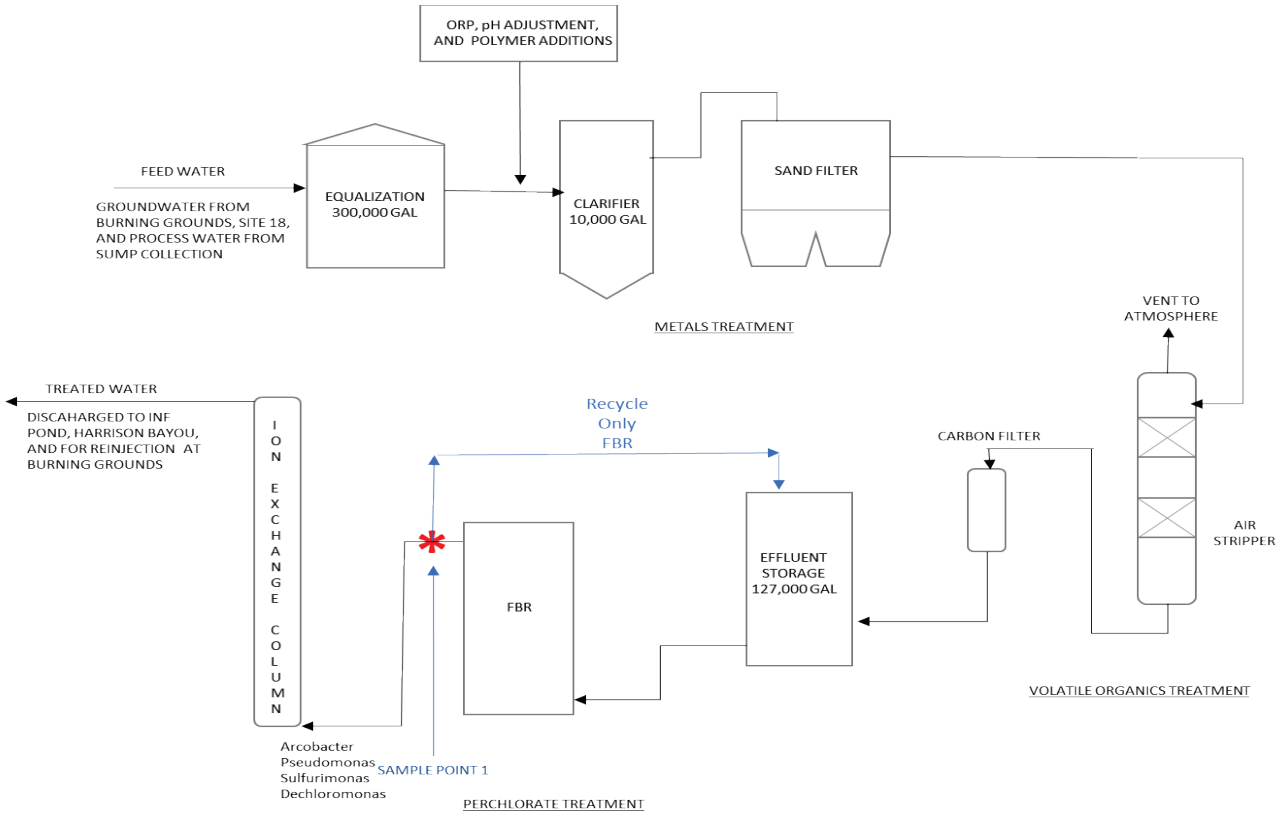
Pseudomonas contains many species and many of which are capable of biodegrading contaminants in FBR systems. The genus is observed in three of the sampling locations during a period of effective perchlorate degradation in the LHAAP GWTS. During the sampling period, the plant was operating at an average of 185 gallons per minute (Longhorn Admin Record 2018). The presence of *Pseudomonas* prior to, within, and following the FBR operation suggests the specific species present are there

because of those species' being found in the LHAAP groundwater itself. It is probable that the specific species of pseudomonas present in the groundwater can use of perchlorate as an electron acceptor because of the long-term contamination of these groundwaters with perchlorate.

Limnohabitans is also observed prior to, within, and after the FBR operation. *Limnohabitans* is not commonly associated with perchlorate biodegradation.

Limnohabitans is a freshwater bacterium effective in use of a wide range of substrates. These species show rapid growth and substrate use. They also have the capability of developing dense communities in both oxic and anoxic conditions. Unlike pseudomonas, *Limnohabitans* predominantly exist as unattached microbes. A benefit of *Limnohabitans* within the Longhorn FBR with regards to perchlorate degradation might be associated with utilization of non-perchlorate electron acceptors prior to and within the FBR as well as generation of organic substances as detrital materials.

Figure 8. Plant configuration during FBR recycle (hold) operation.



The **second** plant operating condition during which sample collection for determination of microbial consortia occurred was during a period of non-discharge under FBR recycling conditions (Figure 8). During this period, groundwater flow through the LHAAP GWTD was zero and the water present within the FBR was continuously recycled with acetate addition as required to maintain a reducing environment within the FBR[*]. This configuration is enacted during plant upsets that require effluent discharge analysis before water release[†], hold time is less than one week under normal operation.

The samples analyzed in this study under **plant hold condition** were unusual in that the fluidized bed reactor had been in recycle mode for four months (4/1/2017). During this period, the GWTP did not have any pH adjustment or nutrient feed. Sample points included the FRB influent and effluent water. The FBR effluent, which represents the active microbial populations in the reactor, had only four microbes present in the reactor above 2%. These were *Dechloromonas, Sulfurimonas, Pseudomonas,* and *Arcobacter* found at 55.1, 9.1, 10.0 and 6.2% in the FBR respectively.

The genus *Dechloromonas* is an important group of microbes with regards to the biodegradation of perchlorate in engineered systems. Species within this genus have been shown to be capable of oxidizing acetate with oxygen, chlorate, perchlorate, or nitrate as electron acceptors. The effectiveness of this system for perchlorate biodegradation is unclear as perchlorate concentrations in the recycled water will be substantially reduced even if the transformation occurs at an extremely slow rate under these conditions.

Microbes of the genus *Sulfurimonas* have been shown to be effective denitrification bacteria that are also capable of reducing perchlorate and are species of interest within systems for bioremediation of perchlorate contaminated groundwater. The use of the biological process of perchlorate degradation as a means of engineering systems for converting reduced sulfur species into oxidized forms is documented in Takai et al.

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[*] Longhorn Army Ammunition Plant Admin Record. 2018, Volume 6. *GWTP Quarterly Evaluation Report-1st Quarter 2017*. http://www.longhornaap.com/system/assets/AdminRecord/2018/2018_volume_6.pdf

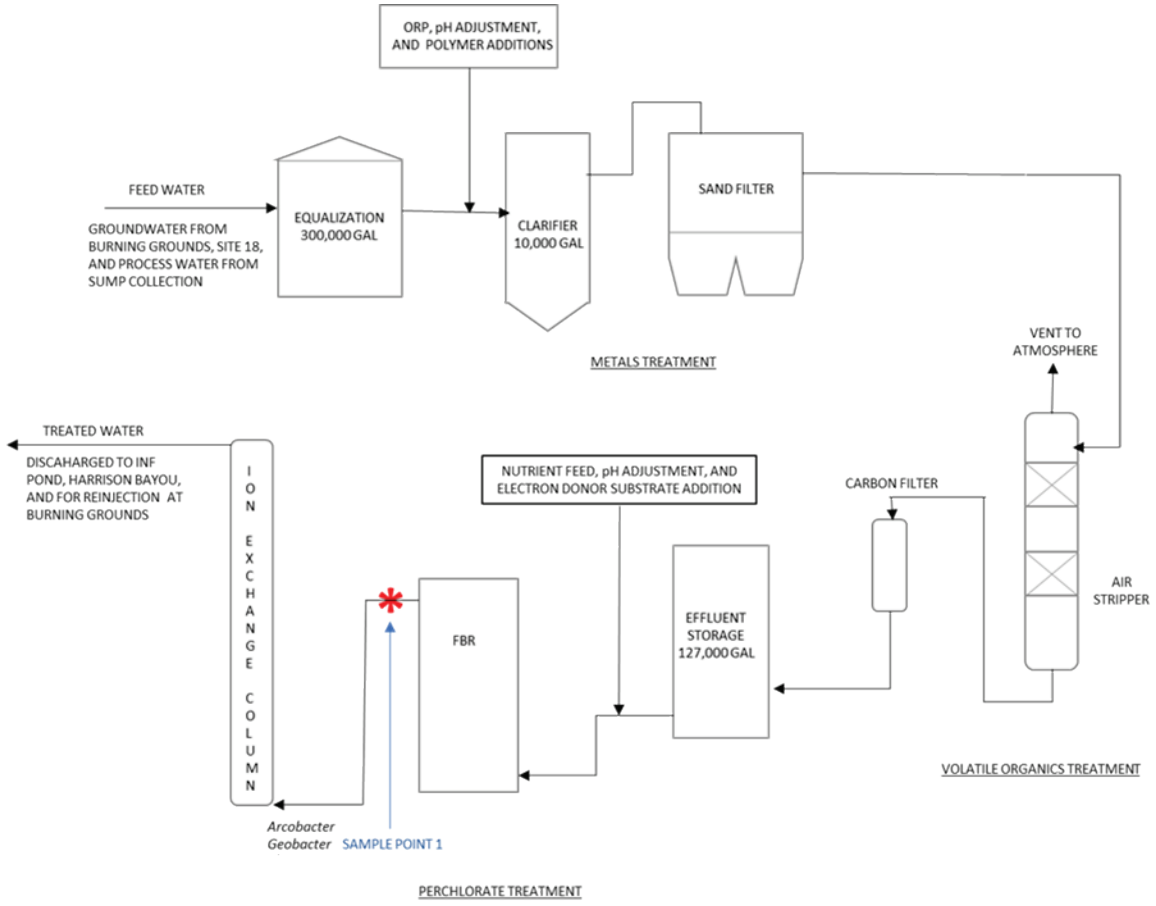
[†] Longhorn Army Ammunition Plant Admin Record. 2018, Volume 30. *GWTP Quarterly Evaluation Report-1st Quarter 2018*. http://www.longhornaap.com/system/assets/AdminRecord/2018/2018_volume_30.pdf

(2006), Cheng et al. (2018), Wan et al. 2017b), Haaijer et al. (2008), and Han et al. (2012).

Pseudomonas contains many species capable of biodegrading contaminants in FBR systems. The genus is observed in three of the sampling locations during a period of effective perchlorate degradation in the LHAAP GWTS. It is probable that the specific species of pseudomonas present in the groundwater have a capability for utilization of perchlorate as an electron acceptor because of the long-term contamination of these groundwaters with perchlorate.

The genus *Arcobacter* is found in the LHAAP GWTS during the FBR recycling conditions. Many species within this genus have been shown to be tolerant of high ion content as would be present during a long recycle period. One arcobacter species isolated, CAB, has been identified as being capable of degrading perchlorate (Sievert et al. 2007; Rice et al. 2002; Madigan et al. 2009).

Figure 9. Sample point during plant shutdown.



A **third** operational condition at the LHAAP GWTS plant was used to evaluate the microbial consortium within the plant under zero flow conditions with no FBR recycling as shown in Figure 9. A sample was taken from within the FBR after 30 days of zero flow. Under these conditions *Arcobacter* and *Geobacter* dominated the microbial populations at 31.5 and 9.9% respectively within the FBR.

8 Designing Redundancy into the Plant for FBR Disruptions

Biological systems used for water treatment have several advantages, which include low-cost operation, operation without introducing expensive or toxic chemical reagents, low capital costs, and minimal operator–required operations. One disadvantage of using biological systems for water treatment is the sensitivity of microbial populations to variability of the operating conditions of the reactor. Changes in conditions that can reduce the effectiveness or cause complete failure of treatment to occur can include fluctuations in temperature, salinity, pH, input water composition, reactor flow rate, and microbial community shifts. The inherent potential for system failure is often greater for biological treatment systems than non-biological treatment. One way to minimize the risk for system failure in a biological treatment system is to incorporate a backup process, such as a non-biological polishing process into the treatment system.

For a non-biological back-up system to be effective, system selection based on low capital costs and the ability to operate effectively on a discontinuous operating basis is necessary. These requirements for perchlorate removal can be satisfied by the use of sorptive technologies based on ion exchange materials. Ion exchange materials can be conditioned using an ion that has less affinity for the sorptive material than an ion for which removal is required. Perchlorate ion is effectively removed from water using this technique. The anionic nature of perchlorate over a wide pH range makes quaternary amine-based strong anion exchange resins useful as a sorptive media. The ion exchange process is more expensive than FBR biological treatment on a per gallon basis, but requires low capital resources for installation and rapid, effective perchlorate removal during periods of upsets. An Ion exchange scavenger system was installed downstream of the FBR in May 2017[*] . The LHAAP treatment system is designed such that an ion exchange operation can be placed online when the FBR experiences upsets that reduce the effectiveness of the biodegradation step. As shown in Figures 7, 8, and 9, an IX unit is in position immediately following the FBR reactor. When a

[*] Longhorn Army Ammunition Plant Restoration Advisory Board Meeting. July 27, 2017. longhornaap.com/system/assets/181/original/final_july_2017_rab_handouts.pdf?1501866669

biological upset occurs, the IX system provides perchlorate removal during the time between disruption and restoration of effective treatment at the FBR. During normal operation, the ion exchange process can be maintained using minimal costs and be available for temporary water treatment during a subsequent FBR upset.

9 Conclusions and Recommendations-

The FBR bioreactor requires a diverse microbial community in addition to perchlorate degraders for optimal perchlorate removal. Bacteria present in the extracted groundwater contained a wide range of organisms; some of which are known perchlorate degraders. As shown in Table 5, the total sum of the percentages of the most prevalent groundwater microbes is less than 32%. This is due to many other organisms being present at quantities below 2%. Within an FBR bioreactor, a complex mixture of support organisms as well as perchlorate degraders are preferred to reduce redox levels to those at which perchlorate can be used as an electron donor and enhance biofilm formation on biochar media which compensate for plant upsets. The presence of sulfate reducers as well as facultative anaerobes can reduce concentrations of sulfate or oxygen from levels that would otherwise cause disruptions. During plant upset, the diversity of the microbial populations in the reactor dropped with *Dechloromonas, Pseudomonas, and Sulfurimonas* having a cumulative 74.2% in the MiSeq analysis as compared steady state operation in which the same three organisms compose only 22.4% of the total population (see Table 5). The stress selected populations did recover during flow conditions indicating survival in niches of the bioreactor until process stream flow is reestablished and delivery of acetate, nutrients, and perchlorate is provided.

The LHAAP GWTP FBR for perchlorate degradation is at a disadvantage to other reactors placed at water treatment plants designed to treat waters for which perchlorate removal is the primary or only goal of the system. The post-design installation of the LH FBR in 2010 into a treatment system originally designed for solvent and metal removal complicates the perchlorate removal operations. Other versions of FBR bioreactors for perchlorate removal such as Area M located at the Naval Weapons Industrial Reserve Plant in McGregor, Texas (NAVFAC 2007), which is operating as a standalone unit, are perpetually fed groundwater containing both indigenous microbes as well as perchlorate. This maintains the microbial consortium required for effective perchlorate removal.

Because of the numerous processes that occur prior to the FBR at Longhorn, the microbial populations within the reactor vary over time and under different operational conditions. Three options for overcoming the issue are:

1. Regular use of a seed inoculant. This could be done by purchasing a seed inoculum or maintaining a seed at the process plant grown from the local groundwater.
2. Providing a slip stream around the metals and VOC treatments. This side-stream of GW could flow directly into FRB as a source of microbial consortia associated with the groundwater at LH which has been shown to contain a broad distribution of microbes that support and effect degradation of perchlorate.
3. Relocation of the FBR at a more effective position within the groundwater treatment train.

As the plant is currently in the tail end of its life cycle and is operated with minimal staff, a periodic reseed using an established inoculum and/or providing a side-stream of groundwater directly into the FBR is the most feasible remedy.

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14. ABSTRACT

Optimization of operation and performance of the groundwater treatment system regarding perchlorate removal at Longhorn Army Ammunition Plant (LHAAP) is dependent on specific conditions within the reactor and the larger groundwater treatment process. This study evaluated the microbial community compositions within the plant during periods of adequate perchlorate degradation, sub-adequate perchlorate degradation, and non-operating conditions. Factors affecting the performance of the LHAAP ground water treatment system (GWTS) perchlorate de-grading fluidized bed reactor (FBR) are identified and discussed. Isolation of the FBR from naturally occurring microbial populations in the groundwater was the most significant factor reducing system effectiveness. The microbial population within the FBR is highly susceptible to system upsets, which leads to declining diversity within the reactor. As designed, the system operates for extended periods without the desired perchlorate removal without intervention such as a seed inoculant. A range of modifications and the operation of the system are identified to increase the effectiveness of perchlorate removal at LHAAP.

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