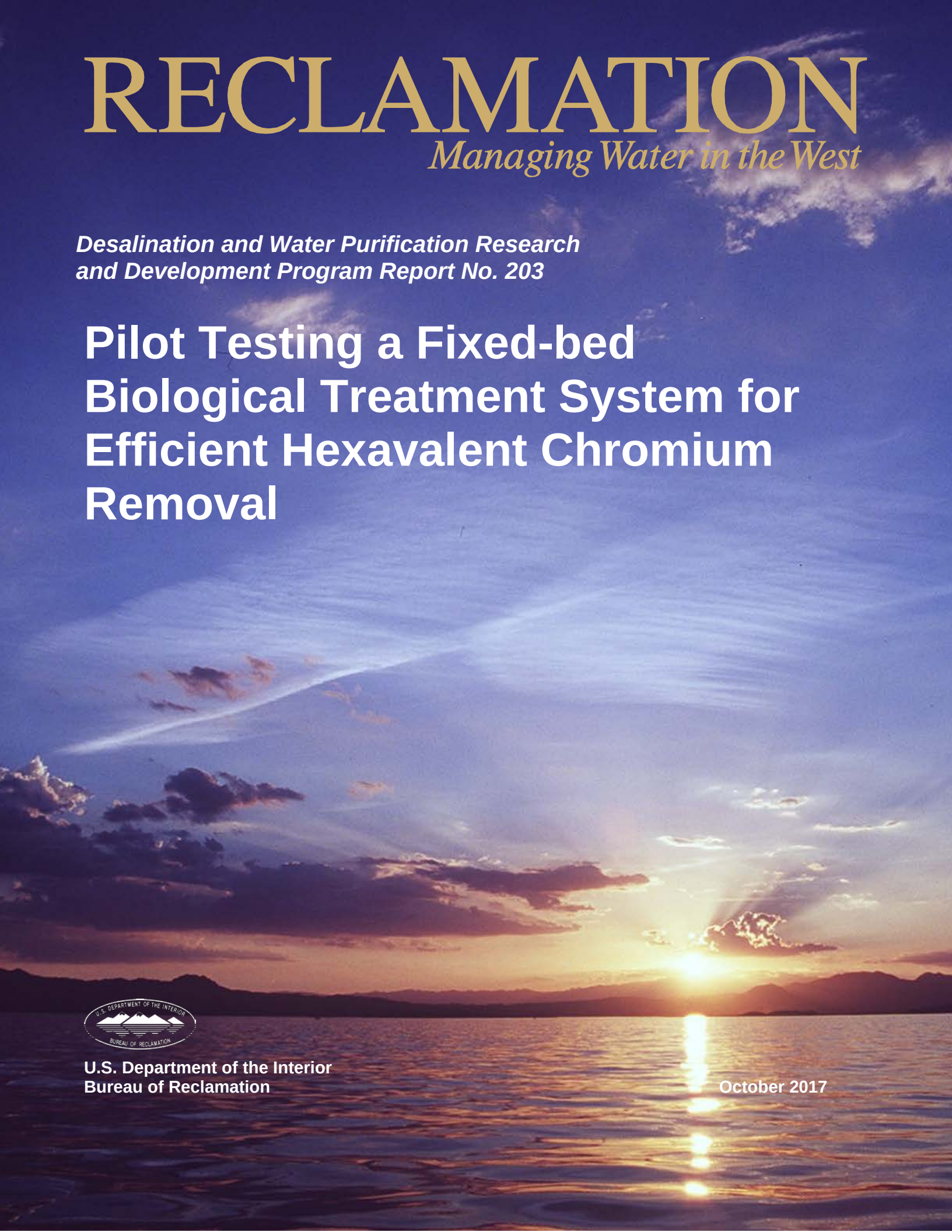

RECLAMATION

Managing Water in the West

Desalination and Water Purification Research and Development Program Report No. 203

Pilot Testing a Fixed-bed Biological Treatment System for Efficient Hexavalent Chromium Removal

U.S. Department of the Interior
Bureau of Reclamation

October 2017

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14. ABSTRACT

This study assessed hexavalent chromium Cr(VI) removal efficiency by using a two-stage, fixed-bed (FXB), biologically active carbon bioreactor system (BAC to remove Cr(VI) from groundwater containing approximately 75 micrograms per liter (µg/L) Cr(VI). Raw water was supplemented with acetic acid (ACA) and phosphoric acid (PHA), and fed into two columns (i.e., bioreactor and biofilter) operated in series. Hydrogen peroxide and a coagulant were injected into the bioreactor effluent to reoxygenate the water and to meet the turbidity treatment goal, respectively.

Overall, the study demonstrated that a two-stage, FXB BAC treatment system may provide an economical solution for Cr(VI) removal from contaminated water sources. While biological acclimation took approximately 4 months, when optimized for operating parameters, such as empty bed contact time (EBCT), backwash protocol, and chemical feed doses, the system effectively removed Cr(VI) to less than 7 µg/L with an EBCT of 10 minutes. Greater than 3 milligrams per liter (mg/L) concentrations of dissolved oxygen (DO) was effectively maintained in the final effluent while ensuring turbidity less than 0.3 NTU for more than 95 percent of the time. The system was resilient and not affected by operational disturbances, including a 3-day shutdown, intermittent operation, and a 24-hour disruption in the PHA feed. The absence of ACA for 24 hours resulted in increased Cr(VI) levels in the effluent. However, the system recovered within 6 hours, lowering the effluent concentration to less than 10 µg/L. Molecular biology revealed that previously described Cr(VI)-reducing bacteria were present in the system. Low strength backwash wastewater was generated during a bioreactor or biofilter backwash. The bioreactor backwash wastewater had significantly low levels of arsenic and chromium compared to the biofilter backwash wastewater. This would result in further diluted waste when the backwash water from the bioreactor and biofilter are blended together.

15. SUBJECT TERMS
hexavalent chromium, trivalent chromium, biological removal, biological acclimation, microbial community, backwash, backwash wastewater

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Prepared for Reclamation Under Agreement No. R16AP00028

by

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Water Treatment Engineering Research Team
Denver, Colorado

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The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Acronyms and Abbreviations

Chemical

ACA	acetic acid
ACH	aluminum chlorohydrate
As	arsenic
C	carbon
Cl^-	chlorine
$Cr(OH)_3$	chromium hydroxide
Cr	chromium
Cr(III)	trivalent chromium
Cr(VI)	hexavalent chromium
Fe(II)	ferrous iron
Fe(III)	ferric iron
$FeCl_2$	ferric chloride
H_2O_2	hydrogen peroxide
N	nitrogen
N_2	nitrogen gas
NH_4Cl	ammonium chloride
NO_3^--N	nitrate as nitrogen
O_2	oxygen
P	phosphorus. A molar ratio of 1 (C):100 (P) was used to determine the PHA dose.
PHA	phosphoric acid
U(IV)	tetravalent uranium
U(VI)	hexavalent uranium

Acronyms

AMS	Aqua Metrology Systems
AWWA	American Water Works Association
BAC	biologically active carbon
BWW	backwash wastewater
BDOC	biodegradable organic carbon
Carollo	Carollo Engineers, Inc.
CFU	colony forming unit
City	City of Norman
COD	chemical oxygen demand
CT	concentration * time
DBCP	dibromochloropropane
DBP	disinfection byproduct
DBPFP	disinfection byproduct formation potential
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DOC	dissolved organic carbon
EBCT	empty bed contact time
E. coli	*Escherichia coli*
EEA	Eurofins Eaton Analytical
EPA	United States Environmental Protection Agency
FXB	fixed bed
GAC	granular activated carbon
HAA_5	haloacetic acids
HMI	human-machine interface
HPC	heterotrophic plate count
IX	ion exchange

MCL	maximum contaminant level
MPN	most probable number
MRL	maximum reporting limit
NSF	National Sanitation Foundation
NTP	National Toxicology Program
O&M	operation and maintenance
ODEQ	Oklahoma Department of Environmental Quality
OTU	operational taxonomic units
PCR	polymerase chain reaction
RCF	reductive coagulation/filtration
RCRA	Resource Conservation and Recovery Act
RDP	Ribosomal Database Project
RO	reverse osmosis
SBA	strong base anion
SM	standard method
STLC	soluble threshold limit concentration
TCLP	toxicity characteristics leaching procedure
TDS	total dissolved solids
TTHM	total trihalomethanes
TTLC	total threshold limit concentration
TSS	total suspended solids
VOC	volatile organic compound
WBA	weak base anion

Measurements

gpm	gallon per minute
gpm/ft^2	gallons per minute per square foot
kg	kilogram
L	liter
L/s	liter per second
L/m^2-s	liter per square meter per second
M	meter
m^3/day	cubic meters per day
mg	milligram
mg/kg	milligram per kilogram
mg/L	milligram per liter
mL	milliliter
NTU	nephelometric turbidity unit
pCi/L	picocuries per liter
s	seconds
scfm	standard cubic foot per minute
µg	microgram
µg/L	microgram per liter

1. Executive Summary

The City of Norman (City) supplies water to its customers using water from Lake Thunderbird and groundwater from the Garber-Wellington aquifer. The City augments these sources with treated water from Oklahoma City via a system interconnect.

The Garber-Wellington aquifer has elevated levels of hexavalent chromium (Cr[VI]), and some wells in the aquifer have also exhibited elevated levels of arsenic (As) and other constituents. Currently practiced technologies for Cr(VI) removal include adsorption, ion exchange (IX), electrocoagulation, membrane-based processes, and chemical reduction to trivalent chromium (Cr[III]). However, these abiotic technologies may require additional treatment (e.g., pH adjustment) and may produce waste that requires disposal or management. Conversely, biological process obviates these additional challenges and may provide a more economical approach for Cr(VI) removal.

We conducted a 9-month pilot study with the water from the City's Well 5, which receives water from the Garber-Wellington aquifer, to evaluate the effectiveness of a two-stage, fixed-bed (FXB) biologically active carbon (BAC) system for the removal of Cr(VI) The pilot skid consisted of two 2 feet (0.61 meters [m]) diameter columns (i.e., bioreactor and biofilter) operated in series. The skid was equipped with automatic backwash capabilities and chemical feed systems for electron donor (i.e., acetic acid [ACA]), nutrient (i.e., phosphorus [PHA]), coagulant, and hydrogen peroxide (H_2O_2). The skid also included in-line monitoring and data logging for flow rate, headloss, dissolved oxygen (DO), nitrate, and turbidity.

The raw water contained approximately 75 µg/L Cr(VI), 0.7 mg/L nitrate as nitrogen (NO_3^-N), and 6 µg/L As. As expected, nitrate removal was observed within a week, whereas biological acclimation for Cr(VI) removal took approximately 4 months. Once complete biological acclimation was achieved, operating parameters such as empty bed contact time (EBCT), backwash protocol, and chemical doses were optimized.

The optimized system effectively removed Cr(VI), lowering it from approximately 75 µg/L to less than 7 µg/L with an EBCT of 10 minutes. While Cr(VI) reduction to Cr(III) occurred in the bioreactor (i.e., the first-stage column), the biofilter (i.e., the second-stage column) effectively retained Cr(III) solids. The optimized chemical feed systems allowed maintaining greater than 3 mg/L DO and less than 0.3 nephelometric turbidity units (NTU) in the final effluent. Previously described Cr(VI)-reducing bacteria were identified in the system through high throughput deoxyribonucleic acid (DNA) sequencing. The bioreactor and biofilter backwash wastewater contained 286 and 4,100 milligrams per kilogram (mg/kg) total chromium (Cr), respectively, and <36 and 59 mg/kg total As, respectively. Mass balance from the experimental run shows that the

blended bioreactor/biofilter wastewater would have significantly lower levels of arsenic and chromium. The system was resilient and was not affected by a 24-hour disruption in phosphorus feed, a 3-day system shutdown, Cr(VI) spike in the raw water or intermittent, on-demand operation. As expected, the effluent Cr(VI) increased when the ACA feed was disrupted for 24 hours, but the system rapidly recovered upon restoring the feed.

Collectively, the pilot performance suggests that a two-stage, FXB BAC system (Biotta®) can provide an effective and robust option for the removal of Cr(VI) from water sources.

2. Background

2.1. Need for the Project

Communities across the Reclamation's 17 western states are facing unprecedented challenges in providing safe, reliable water supplies to their customers. Recent year's drought across much of California, Texas, Oklahoma, and portions of other Western States has been daunting to countless water providers. This extended drought has reinforced the importance of a diverse supply portfolio and the critical role groundwater can play in reliably meeting demands. The City of Norman (City) recognized the importance of maintaining a significant proportion of its water supply from groundwater in its 2060 Strategic Water Supply Plan.

At the same time, more stringent drinking water quality regulations threaten the viability of the very same groundwater sources needed to mitigate the drought's impacts. Tightening the arsenic standard several years ago forced many communities to make difficult decisions between abandoning wells, finding alternate sources of supply, and expensive treatment. The City abandoned several wells rather than treating the groundwater.

Currently, Oklahoma regulates total chromium in drinking water with a maximum contaminant level (MCL) of 100 µg/L. Even though California's MCL for Cr(VI) of 10 µg/L was revoked in September 2017, both Federal and State level regulations may be implemented in near future.

Cr(III) and Cr(VI) are the predominant chromium species present in water (Bartlett 1991). Cr(III) is an essential micronutrient and considered benign at the concentrations found in natural water sources (Anderson 1995 and National Toxicology Program 2008). In contrast, uncertain conclusions have been reported regarding human health effects from Cr(VI) exposure through drinking water (Brandhuber et al. 2005)—even though it has been identified as a carcinogen when inhaled (IARC 1990). The fact that Cr(VI) is reduced to Cr(III), which does not easily diffuse across cell membranes (Ramírez-Díaz et al. 2008), in saliva and the digestive tract adds further uncertainty on Cr(VI) toxicity. Considering these facts, the United States Environmental Protection Agency (EPA) is further reviewing additional information on Cr(VI) toxicity. Concurrently, utilities and public health officials are proactively investigating the feasibility of limiting Cr(VI) concentrations in drinking water to very low levels.

Cr(VI) can be removed from water sources by using either technologies that remove Cr(VI) directly (i.e., adsorption, anion exchange, membrane filtration, or electrocoagulation) or those that chemically reduce Cr(VI) to Cr(III) and then subsequently remove Cr(III) solids through precipitation or by using membranes. Reverse osmosis (RO), weak base anion (WBA) exchange, strong-base anion (SBA) exchange, and reductive coagulation/filtration (RCF) may effectively remove Cr(VI) to low levels (i.e., less than 5 µg/L) with minimal water losses

(Blute et al. 2014). While effective, these physical-chemical treatment technologies have several disadvantages, including:

- Requirements for upstream and downstream treatment steps (e.g., pH adjustments)
- Difficulties disposing of the resulting solid and/or liquid wastes
- High costs of chemicals and/or energy needed

In contrast, biological reduction of Cr(VI) to Cr(III) may provide an effective and economical approach for removing Cr(VI) from drinking water sources.

2.2. Hexavalent Chromium Removal Process

In the presence of an electron donor (e.g., acetate) and nutrients (e.g., phosphorus [P]), microorganisms in an engineered bioreactor can degrade a contaminant to innocuous, less toxic, or easily separable end products. For example, nitrate and perchlorate can be biologically converted to nitrogen gas (N_2), and chlorine (Cl^-) and oxygen (O_2), respectively. Similarly, biological reduction of hexavalent uranium [U(VI)] and Cr(VI) results in the production of tetravalent uranium [U(IV)] and Cr(III) solids, which can be easily separated from the water through filtration. Furthermore, biological processes may allow simultaneous removal of a variety of contaminants, such as nitrate, perchlorate, arsenic, and volatile organic compounds (VOC) (Li et al. 2010; Upadhyaya et al. 2010, and Upadhyaya and Brown 2016).

Over the last 19 years, Carollo has developed and optimized a two-stage, FXB BAC treatment system (Biotta®) to remove oxidants from groundwater. It uses two FXB biological reactors in series (i.e., an anoxic bioreactor and an aerobic biofilter) and includes specialized monitoring and chemical dosing algorithms, tailored media selection and configuration, and multiple biomass control tools (Figure 1).

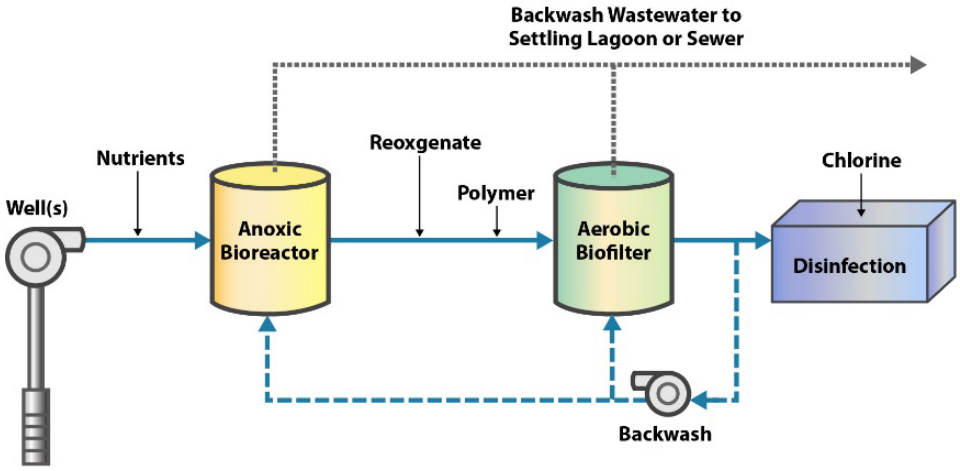

Figure 1. System process flow.

For treating Cr(VI), the Cr(VI) would enter the anoxic bioreactor. Bacteria would reduce Cr(VI) to insoluble Cr(III) within the bioreactor. The Cr(III) would precipitate, likely as chromium hydroxide, $Cr(OH)_3$, and get trapped within the media of the bioreactor. Any Cr(III) not filtered out in the bioreactor would get filtered in the aerobic biofilter (Figure 2). This two-stage process removes chromium from the treated water, leaving no chromium in the finished water. Cr(III) solids are removed from the bioreactor and biofilter during the regular backwash events. During backwash events (which typically happen every 24 - 48 hours), the system is taken off line, and water is pumped at a high rate in the reverse direction up through the FXBs. The backwash wastewater is collected and discharged to a settling basin or settling lagoon. After separating the sludge in the settling basin or lagoon, the water can be recycled to upstream of the bioreactor in the treatment system.

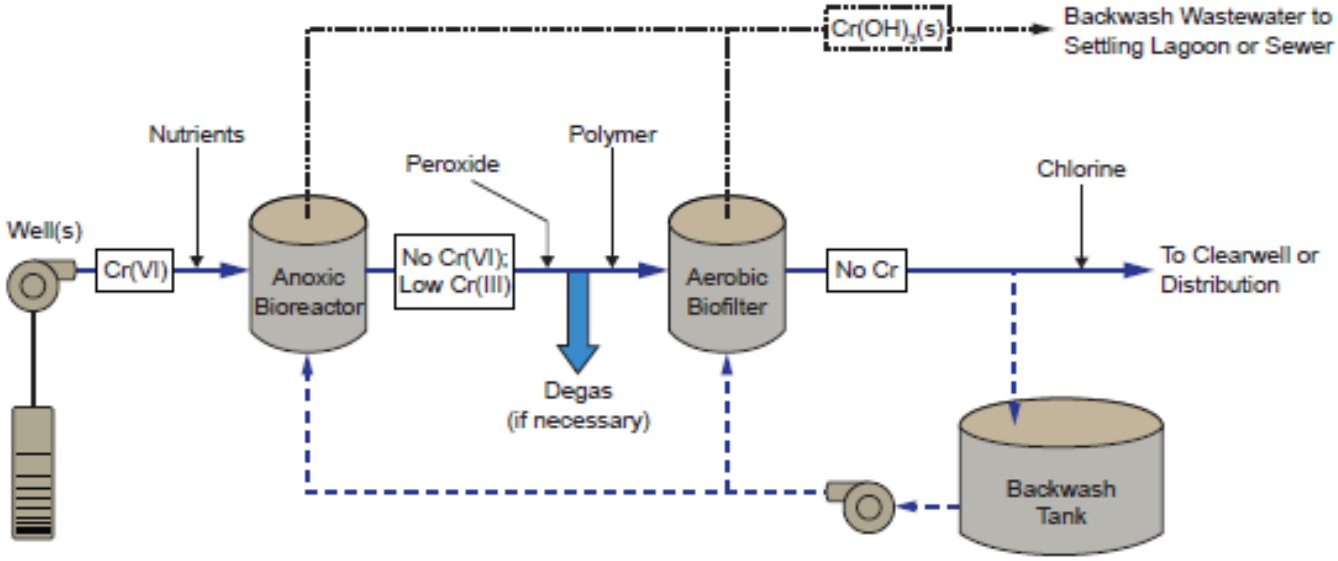

Figure 2. Process flow diagram for chromium removal in a two-stage, FXB BAC treatment system.

2.3. System Advantages

Observed strengths of the system include:

- **Flexibility in target contaminant removal:** In addition to the above features, the two-stage, FXB BAC treatment system offers considerable flexibility. Multiple contaminants can be removed across the system including Cr(VI), perchlorate, VOCs, uranium, selenium, and arsenic.
- **Robust performance:** Biomass control system helps maintain stable performance. The system is independent of raw water quality and treatment goals as well as insensitive to wide swings in operating conditions.

- **Green operation:** The system does not require much energy, produces minimal waste, and does not create a high-strength concentrate or brine waste. Instead, multiple contaminants are destroyed to innocuous and/or less toxic and stable end products. Compared to ion exchange systems, the system reduces salt loading to the potable water distribution system.

- **Low operating costs:** Operation and maintenance (O&M) and life-cycle costs can be low. Tailored chemical feed algorithms minimize the largest component of O&M costs: chemicals.

2.4. Study Objectives

We conducted a 9-month pilot study to evaluate the effectiveness of the two-stage, FXB treatment system for Cr(VI) removal from the groundwater extracted from the City's Well No. 5, which has elevated levels of chromium and arsenic.

The primary objective of this study was to evaluate the effectiveness of a two-stage, FXB BAC treatment system for Cr(VI) removal. The specific project objectives included:

- Confirm site-specific design and operating criteria for Cr(VI) removal, including empty bed contact time (EBCT), electron donor (i.e., ACA) dosage, and phosphoric acid (PHA) dosage. These criteria will serve as the basis of design for a potential future full-scale facility.

- Demonstrate sustained Cr(VI) removal under steady-state operation.

- Demonstrate the stability of the system under forced operating disturbances.

- Provide sufficient operational and performance data to support the potential future application for a water supply permit from the Oklahoma Department of Environmental Quality (ODEQ) to operate a full-scale system.

- Familiarize operations staff with the system.

3. Conclusions and Recommendations

The following key conclusions and recommendations were drawn based on the results of the pilot study:

- Biological acclimation for Cr(VI) removal may take longer than the acclimation time required to achieve nitrate removal.

- Biological reduction of Cr(VI) to Cr(III) takes place in the bioreactor, while the biofilter effectively retains Cr(III) solids.

- The system performance will not be affected by a short-term (up to 24 hours) PHA feed failure.

- In the absence of ACA, Cr(VI) removal will diminish. However, the system rapidly recovers once the chemical feed is reestablished.

- The system readily handled fluctuations in raw water concentrations of Cr(VI), from 0 - 100 µg/L.

- The system will perform equally well when operated in an intermittent, on-demand mode, suggesting that it can also be used in isolated rural areas.

- Low strength backwash wastewater will be the only waste generated during treatment. Bioreactor and biofilter backwash wastewater (BWW) should be blended for dilution prior to discharge.

- Overall, a two-stage, fixed-bed BAC treatment system can provide effective removal of Cr(VI) and may provide a robust alternative to existing Cr(VI) removing technologies.

4. Materials and Methods

4.1. Source Water

The City receives water from three sources

- Lake Thunderbird outside Norman
- Groundwater from the Garber-Wellington aquifer
- Treated water via an interconnect with Oklahoma City

The Garber-Wellington aquifer has elevated levels of Cr(VI) and arsenic. This pilot study evaluated groundwater from Well 5, which pumps water from the Garber-Wellington aquifer. Cr(VI) and arsenic levels in Well 5 are presented in Table 1. Currently, water produced from Well 5 is blended with finished water from Norman's water treatment plant (WTP) to achieve compliance with MCLs before distribution.

Table 1. Cr(VI) and Arsenic Levels in Well 5 (Sept 2016 - July 2017)

Contaminant		Concentration (µg/L)
Cr(VI) (n = 80)	Minimum	66.0
	Average	74.4
	Maximum	79.0
Total Cr (n = 96)	Minimum	65.2
	Average	72.7
	Maximum	84.0
Arsenic (n = 69)	Minimum	1.3
	Average	4.9
	Maximum	6.7
n = number of samples		

4.2. Pilot Skid and Process Flow

The pilot skid shown in Figure 3 was used for this pilot study. The pilot skid operates between 3 and 25 gallons per minute (gpm) (16.4 and 136.3 cubic meters per day [m^3/day]) and contains three 2 feet (0.61 m) diameter columns constructed from epoxy-coated steel that are 8 feet (2.44 m) tall and contained in a 40 foot (12.12 m) by 8 foot (2.44 m) by 8 foot (2.44 m) trailer. The first column (i.e., the anoxic bioreactor) is packed with granular activated carbon (GAC), whereas the third column (i.e., biofilter) contains GAC over sand.

In general, most of the contaminant is removed in the bioreactor. The second column serves as an open basin, which could be used for flocculation or degasification but was not used in this study. The biofilter polishes the bioreactor

effluent by removing turbidity, residual contaminants, and organic carbon. The skid is equipped with automatic backwash capabilities and chemical feed systems for electron donor, nutrient, coagulant, and H_2O_2. The skid also includes in-line monitoring and data logging for flow rate, headloss, DO, nitrate, and turbidity. A human-machine interface (HMI) can be used on site or remotely to monitor and control pilot operations.

Figure 3. Pilot testing skid.

As shown in the process-flow diagram (Figure 4), effluent from the bioreactor can travel directly to the biofilter or to an interstage degasification column ahead of the biofilter. However, the interstage column was not used during the pilot testing. Effluent from the biofilter was pumped into a backwash tank, which overflowed to the Norman Water Treatment Plant forebay.

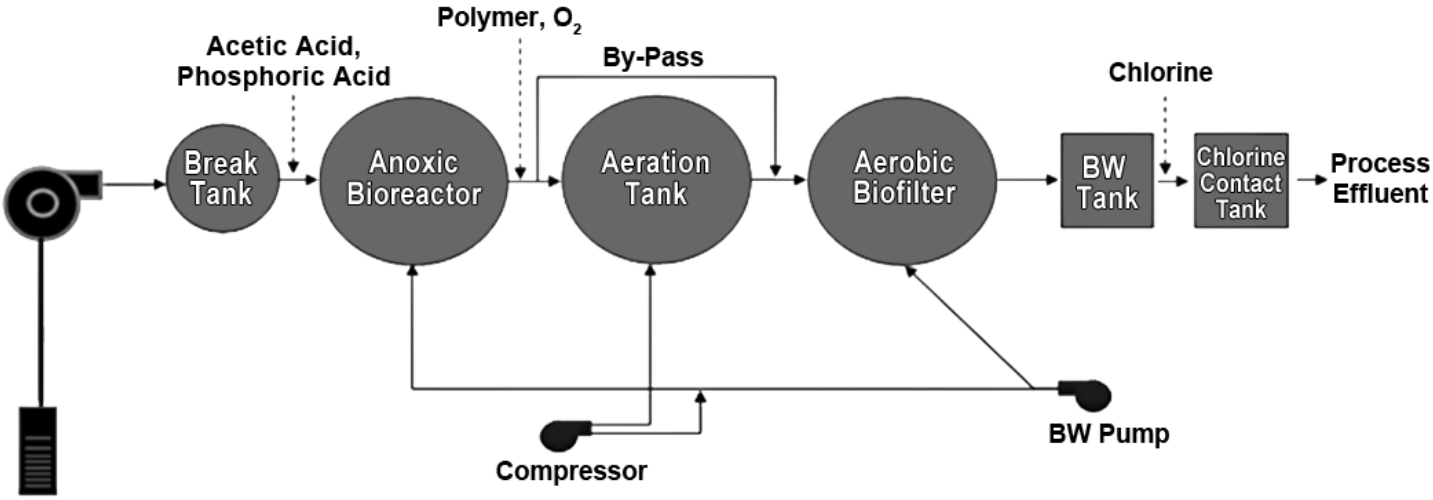

Figure 4. FXB biological pilot process flow diagram.

4.3. Experimental Design

To evaluate the effectiveness and robustness of the two-stage, FXB treatment system for Cr(VI) removal, the pilot testing was conducted in five phases:

- Biological acclimation
- Process optimization
- Sustained optimal removal
- Robustness evaluation
- Intermittent, on-demand testing

These phases are described below.

4.3.1. Phase I—Biological Acclimation

The purpose of this phase was to develop efficient Cr(VI)-reducing biological activity in the system using microorganisms indigenous to Well 5. A flow rate of approximately 11.76 gpm (0.44 liters per second [L/s]) was used, resulting in an EBCT of 10 minutes and hydraulic loading rate of 3.74 gallons per minute per square foot (gpm/ft^2) 2.54 liters per square meter per second [L/m^2-s]). The system was operated under bypass mode (i.e., the middle column was not used), and H_2O_2 was dosed upstream of the polishing biofilter to re-oxygenate the water. To meet effluent turbidity targets, doses of aluminum chlorohydrate (ACH) were provided upstream of the polishing biofilter. Samples were collected and analyzed throughout the study based on the schedule provided in Section 4.4.1.

4.3.2. Phase II—Process Optimization

The purpose of this phase was to determine the optimal system operating conditions for backwash parameters, EBCT, and chemical doses.

4.3.2.1. *Establishing Backwash Criteria*

Headloss and contaminant removal data were monitored closely and were used to establish backwash design criteria (i.e., frequency, air scour rate/duration, and fluidization rate/duration). Backwashes were performed at set time intervals (i.e., 12 to 22 hours for the bioreactor and 48 hours for the polishing biofilter). The treated effluent was used to backwash the columns. The optimized bioreactor backwash sequence is:

1. Drain the columns to leave an air gap

2. Air scour

3. Combine air (1.27 standard cubic foot per minute [scfm]/ft^2; 6.45 L/m^2-s) with a water wash (3.18 gpm/ft^2 [2.16 L/m^2-s]) for 132 seconds.

4. Perform hydraulic fluidization of the bed at 9.55 gpm/ft^2 (6.48 L/m^2-s) for 10 minutes.

5. Use a reactor settle time of 180 seconds.

4.3.2.2. *Testing Other Potential Performance Enhancement Strategies*

To evaluate the possibility of enhancing Cr(VI) removal performance, nitrogen supplementation was evaluated. Ammonium chloride (NH_4Cl) was added to the raw water with a target concentration of 1 to 2 milligrams per liter (mg/L) nitrogen (N) for approximately 3 weeks.

4.3.2.3. *EBCT Optimization*

Initially, the system was operated with an EBCT of 10 minutes. After 3 months, the EBCT was raised to 20 minutes to expedite the biological acclimation. Once complete Cr(VI) removal was observed, the EBCT was lowered to 16 minutes. Further evaluation of EBCTs suggested that less than 7 µg/L Cr(VI) could be achieved with EBCTs as low as 7 minutes. However, considering some additional safety factors, 10 minutes was considered as the optimal EBCT.

4.3.2.4. *ACA and PHA Dose Optimization*

Initially, ACA dose was determined based on the stoichiometric requirement considering a net biomass yield of 0.4 milligrams (mg) biomass chemical oxygen demand (COD)/mg COD of acetate. A molar ratio of 1 (C):100 phosporus (P) was used to determine the PHA dose. After the EBCT optimization, ACA and PHA doses were adjusted to determine the minimum concentration required without compromising Cr(VI) removal performance. The optimal PHA dose was 1 mg/L.

4.3.2.5. *H_2O_2 and ACH Dose Optimization*

While the first stage bioreactor was being optimized for Cr(VI) removal, H_2O_2 and ACH doses were also adjusted in attempt to meet final effluent DO and turbidity goals.

4.3.3. Phase II—Sustained Optimal Removal

The purpose of this phase was to demonstrate sustained Cr(VI) removal for approximately one month using the design criteria developed in phase II. The following additional tests were also performed during this phase.

4.3.3.1. *Backwash Wastewater Characterization*

We characterized backwash wastewater collected during a bioreactor or biofilter backwash through three different testing methods as discussed below.

4.3.3.1.1. General Characterization

On two different occasions, two separate backwash wastewater composite samples were collected during backwashing of the bioreactor and biofilter. The samples were analyzed for total suspended solids (TSS), COD, Cr(VI), metals, and gross alpha.

4.3.3.1.2. Jar Testing

Backwash wastewater (BWW) generated during a bioreactor or biofilter backwash was collected to determine settling characteristics. To collect a representative composite BWW sample from the entire period of a wash flow (i.e., 10 minutes at a flow rate of 30 gpm [1.89 L/s]), the 10-minute period was divided into 8 equal segments of 'collecting periods' (10 seconds) and 7 equal segments of 'wasting periods' (70 seconds) in a 40-gallon (151.4 liter [L]) trashcan. After collection, the wastewater was properly mixed before transferring the wastewater into 2-L jars in the jar testing equipment.

Three separate jar tests were performed using either :

- Wastewater collected during a bioreactor backwash
- Wastewater collected during a biofilter backwash
- Blended wastewater

The blended wastewater was prepared by combining the bioreactor and biofilter backwash wastewater at a volumetric ratio of 2:1.

For each set of jar tests, four to five 2-L jars were filled with backwash wastewater and the testing was performed using a Phipps & Bird six-paddle jar tester following this protocol:

1. Add flocculant (i.e., ACH) to all jars simultaneously.

2. Start pre-programmed mixing sequence (Table 4.2).

3. Collect approximately 50 mL sample from all jars simultaneously at 1, 2, 4, 8, and 16 minutes after conclusion of step 2.

4. Measure the turbidity in all samples, and the pH for the first sample from each jar.

Table 2. Mixing Sequence Used for Jar Tests

Stage	G Value (s^{-1})	Duration (s)
Flash Mix	300	30
First	60	600
Second	40	600
Third	20	600

G = mean velocity gradient: velocity (feet/second)/distance (in feet)

4.3.3.1.3. Hazard Assessment

Oklahoma follows Resource Conservation and Recovery Act (RCRA) regulations to manage hazardous wastes. For the purpose of RCRA, the EPA has developed and implemented hazardous waste identification regulations, outlining the toxicity characteristics leaching procedure (TCLP) to determine hazard characteristics of a waste. The California Code of Regulations (Title 22, Chapter 11, Article 3) provides additional procedures (i.e., soluble threshold limit concentration [STLC], and total threshold limit concentration [TTLC]) to determine hazard characteristics of a solid waste.

To assess the potential hazardous nature of the wastewater generated during the study, BWW grab samples were sent to Eurofins Eaton Analytical (EEA) through TCLP, STLC, and TTLC. Metals of interest for this evaluation were: arsenic, barium, cadmium, chromium, mercury, selenium, and silver. The protocol described in Section 4.3.3.1.2 was used to collect a representative BWW sample.

4.3.3.2. *Disinfection Tests*

Disinfection tests were conducted with the biofilter effluent after determining the instantaneous chlorine demand. Batch tests were conducted targeting a free chlorine dose corresponding to 4-log virus inactivation after satisfying instantaneous chlorine demand. Chlorinated and unchlorinated biofilter effluent samples were analyzed for heterotrophic plate counts (HPC), total coliform, and *Escherichia coli* (*E. coli)*. For comparison, tests were also conducted with the raw water.

4.3.3.3. *DBPFP Tests*

Two separate disinfection byproduct formation potential (DBPFP) tests were conducted with the raw water and biofilter effluents. Chlorine was added to the samples to achieve a target free chlorine dose of 2 mg/L after satisfying the instantaneous demand. Total trihalomethanes (TTHM) and haloacetic acids (HAA_5) samples were collected at sample time points of 15 minutes, 1 day, 5 days, and 7 days after the chlorine addition. Residual chlorine, pH, and temperature were also measured at these sampling time points. The residual chlorine was quenched immediately after collecting the samples.

4.3.3.4. *Microbial Community Characterization*

Media samples were collected from the bioreactor and biofilter for the characterization of microbial community present in the system. The samples were shipped overnight on ice to the University of Texas at Austin for deoxyribonucleic acid (DNA) extraction and DNA sequencing. The DNA was extracted and polymerase chain reaction (PCR) amplification was performed, and multiplexed 16S amplicon sequencing was performed on MiSeq (Illumina, San Diego, California) after constructing a dual-index library (Kozich et al. 2013). All Miseq data were analyzed in QIIME (Caporaso et al. 2010). After filtering the sequence reads to remove low-quality sequences and chimeras, the sequences were clustered into operational taxonomic units (OTU) at 97 percent sequence identity (Edgar 2010 and Edgar et al. 2011). Taxonomy was assigned to each

representative sequence to determine the phylogenetic affiliation of the bacteria present in the system by using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) against the Greengenes reference database (McDonald et al. 2012).

4.3.4. Phase IV—Robustness Testing

The purpose of this phase was to evaluate the process performance under forced system disturbances. Design criteria established during phase II were used throughout these tests. The following disturbances were tested.

4.3.4.1. *Effects of Backwashing on Cr(VI) Removal*

Because portions of the established microbial population are removed from the bioreactor and biofilter during backwashing, effluent Cr(VI) and turbidity were measured immediately following backwash events to evaluate the impacts of periodic biomass removal. This test also allowed determining whether a re-acclimation (ripening) or filter-to-waste period was required following backwashing.

4.3.4.2. *Raw Water Quality Fluctuation*

For approximately one week, Cr(VI) was spiked to the raw water to achieve an influent concentration of approximately 100 µg/L. Cr(VI) and turbidity were closely monitored during this period.

4.3.4.3. *ACA or PHA Feed Failure Simulation*

The ACA or PHA feed was turned off for a 24-hour period to simulate a full-scale chemical dosing system failure. Cr(VI) and turbidity were closely monitored during the feed shutdown and after resuming the ACA or PHA feed.

4.3.4.4. *System Shut-Down Simulation*

The pilot system was completely shut down for three days. Cr(VI) monitoring was performed upon system restart, and performance recovery period for Cr(VI) removal was quantified.

4.3.5. Phase V—Intermittent Operation

Three intermittent operating conditions (listed below) were evaluated. The optimized design criteria established during phase II were used during this testing. Performance was compared to the data collected in phase III.

- On one week, off one week. This condition was evaluated for approximately 25 days.

- 100 hours on (equivalent to 8 am Monday to noon Friday) then off 68 hours (equivalent to Friday afternoon through Monday morning). This condition was evaluated for 14 days.

- On 60 minutes, off 60 minutes, repeated for 12 hours, then off 12 hours (to simulate systems that run to meet intermittent demands). This evaluation was conducted for 3 days.

4.4. Water Quality Monitoring

4.4.1. Sampling Schedule

Table 3 provides the sampling plan developed for the pilot study. The pilot skid was equipped with an in-line data collection system that generated real-time data on nitrate + nitrite, DO, turbidity, headloss, and flow rate. To expedite the optimization process, an in-line Cr(VI) analyzer was installed 4 months into the 9-month pilot testing. In addition, grab samples were collected per Table 3 to determine various water quality characteristics at a local lab or on site.

Table 3. Water Quality Monitoring Schedule

Parameter	Sampling Location	Sampling Frequency	Lab/Analytical Source
Regular Samples			
Nitrate/Nitrite	1F[(1)], 1E[(2)], 2E[(3)]	Continuous	In-line Probe
Dissolved Oxygen	1F, 1E, 2E	Continuous	In-line Probe
Turbidity	1E, 2E	Continuous	In-line Probe
Headloss	1F to 1E, 1E to 2E	Continuous	In-line Pressure Gauge
Flow	1F to 1E, 1E to 2E	Continuous	In-line Flow Meter
Total Cr	1F, 1E, 2E	2/week	EEA[(4)]
Cr(VI)	1F, 1E, 2E	3/week, continuous[(6)]	EEA
Arsenic	1F, 2E	2/week	EEA
Dibromochloropropane (DBCP)	1F, 1E, 2E	3/week	EEA
Sulfate	1F, 2E	1/week	EEA
Orthophosphate	1F, 2E	1/week	EEA
Dissolved Organic Carbon (DOC)	1F, 1E, 2E	2/week	EEA
Additional Samples			
Turbidity	1F, 1E, 2E	1/week	Field Probe
pH	1F, 1E, 2E	3/week	Field Probe
Temperature	1F, 1E, 2E	3/week	Field Probe
Gross alpha	1F, 2E	1X during phase III	
Biodegradable organic carbon (BDOC)	1F, 1E, 2E (duplicate samples)	1/week during phase III	EEA

Parameter	Sampling Location	Sampling Frequency	Lab/Analytical Source
HPC[7]	1F, 2E (duplicate samples)	1/week during phase III	EEA
Total Coliform[7]	1F, 2E (duplicate samples)	1/week during phase III	EEA
Escherichia coli (*E. coli*)[7]	1F, 2E (duplicate samples)	1/week during phase III	EEA
TTHMs[8]	1F, 2E (15 min, 1 day, 5 days, 7 days, duplicate samples)	2X during phase III	EEA
HAA_5[8]	1F, 2E (15 min, 1 day, 5 days, 7 days, duplicate samples)	2X during phase III	EEA
Total Cr	Bioreactor and biofilter BWW[6] (duplicate samples)	2X during phase III	EEA
Arsenic	Bioreactor and biofilter BWW (duplicate samples)	2X during phase III	EEA
Cr(VI)	Bioreactor and biofilter BWW (duplicate samples)	2X during phase III	EEA
Gross alpha	Bioreactor and biofilter BWW	1X during phase III	EEA
TSS	Bioreactor and biofilter BWW (duplicate samples)	2X during phase III	EEA
COD	Bioreactor and biofilter BWW (duplicate samples)	2X during phase III	EEA

Notes:
(1) 1F = Bioreactor feeds
(2) 1E = Bioreactor effluents
(3) 2E = Biofilter effluent.
(4) EEA = Eurofins Eaton Analytical
(5) BWW = Backwash wastewater
(6) An in-line Cr(VI) analyzer from Aqua Metrology Systems (AMS), California was installed 4 months into the 9-month pilot testing to help expedite process optimization.
(7) Microbiological parameters were measured during batch concentration * time (CT) tests.
(8) Disinfection byproduct (DBP) samples were collected after chlorinating the samples to achieve 4-log virus inactivation and then quenching remaining chlorine at 15 minutes, 1 day, 5 days, and 7 days.

4.4.2. Analytical Methods

Analyses of water quality parameters were conducted using EPA or American Water Works Association (AWWA) approved standard methods (SM). Table 4 lists the analytical methods used during this study.

Table 4. Analytical Methods

Parameter	Analytical Method	Parameter	Analytical Method
Nitrate	EPA 300.0A	pH	4500-H+ B
Nitrite	EPA 300.0A	Temperature	SM 2550 B
DO	SM 4500-O G	BDOC	Servais Method
Cr(VI)	EPA 218.6	HPC	SM 9215
Total Cr	EPA 200.8	Total Coliform	SM 9221B
Arsenic	EPA 200.8	*E. coli*	EPA 1603
Sulfate	EPA 300.0A	TTHMs	EPA 502.2
Orthophosphate	EPA 300.0A	HAA_5	EPA 552.3
DOC	SM 5310 C	TSS	SM 2530 D
Turbidity	SM 2130 B	Metals	EPA 200.8

4.4.3. Treatment Chemicals

Chemicals used in this pilot study are presented in Table 5. All chemicals were National Sanitation Foundation (NSF) -60 certified.

Table 5. Treatment Chemicals

Name	Specific Gravity	pH
ACA, Glacial (100 percent)	1.04	2.4
PHA (75 percent)	1.69	1.0
H_2O_2 (30 percent)	1.1	2.0
ACH (50 percent)	1.3	5.0 - 7.0

5. Results

This section presents and discusses the results collected using in-line analyzers for nitrate, DO, and turbidity (Appendix A), the Cr(VI) results obtained in house using Hach DR900 (Appendix B), the Cr(VI) in-line analyzer provided by AMS (Appendix C), and grab samples analyzed at EEA (Appendix D).

5.1. Biological Acclimation

5.1.1. Biological Acclimation for Nitrate Removal

The pilot testing officially started on September 17, 2016. The system was supplied with ACA and PHA to enhance the growth and activity of nitrate and/or Cr(VI)-removing microorganisms indigenous to the Well 5 groundwater. Figure 5 presents nitrate + nitrite concentrations (collected through in-line nitrate + nitrite analyzer) in the raw water and the final effluent during biological acclimation for nitrate removal.

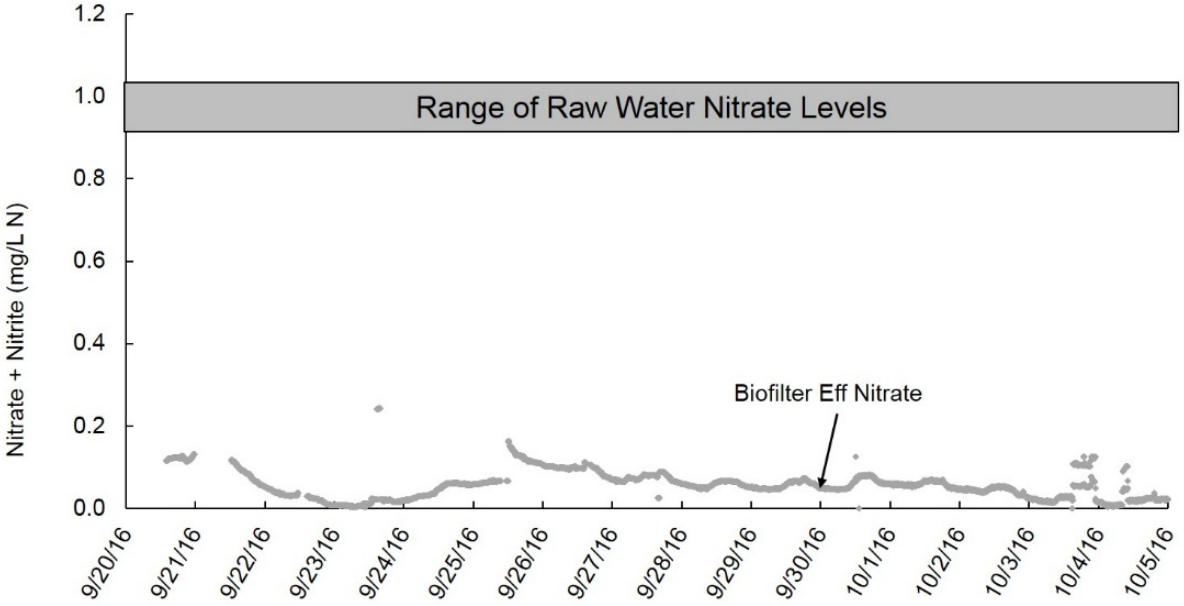

Figure 5. Nitrate concentrations in the raw water and biofilter effluent during biological acclimation.

Figure 6 shows nitrate removal after complete biological acclimation for nitrate removal. To evaluate the possibility of expediting biological acclimation for Cr(VI) removal, nitrogen supplementation (1 to 2 mg/L N in the form of NH_4Cl) was evaluated from November 29 through December 19, 2016.

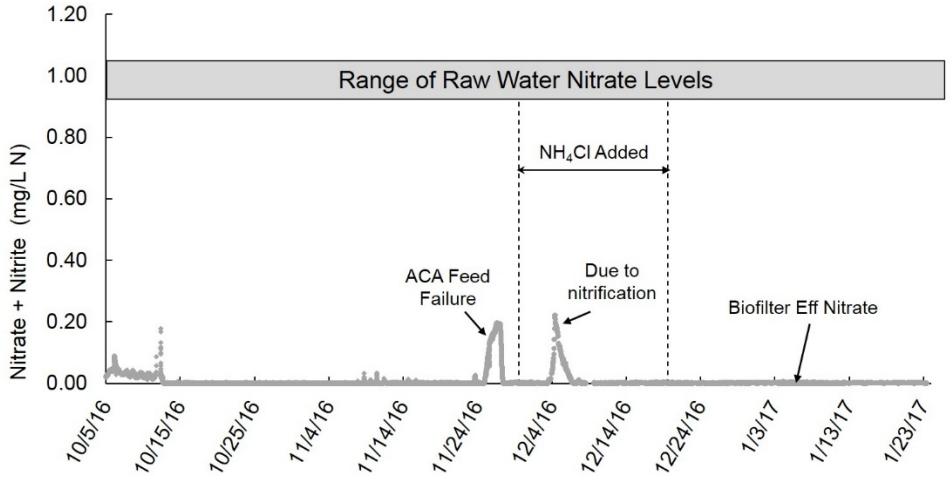

Figure 6. Sustained nitrate removal.

Based on Figure 5 and Figure 6, the following observations can be made:

- Biological acclimation for nitrate removal occurred within 2 weeks, lowering the influent nitrate (0.7 to 1 mg/L N) to less than 0.02 mg/L N (detection limit).

- Sustained nitrate removal to below detection was observed after biological acclimation,.

- As expected, disruptions in ACA feed resulted in nitrate breakthrough, but once the feed was re-established, the system recovered within hours.

- Ammonium chloride injection resulted in nitrification in the biofilter, but denitrification was rapidly established, resulting in nitrate below detection in the final effluent.

5.1.2. Biological Acclimation for Cr(VI) Removal

Figure 7 and Figure 8 present the in-house Cr(VI) analysis results (measured onsite using Hach DR900) and EEA lab Cr(VI) analysis results, respectively.

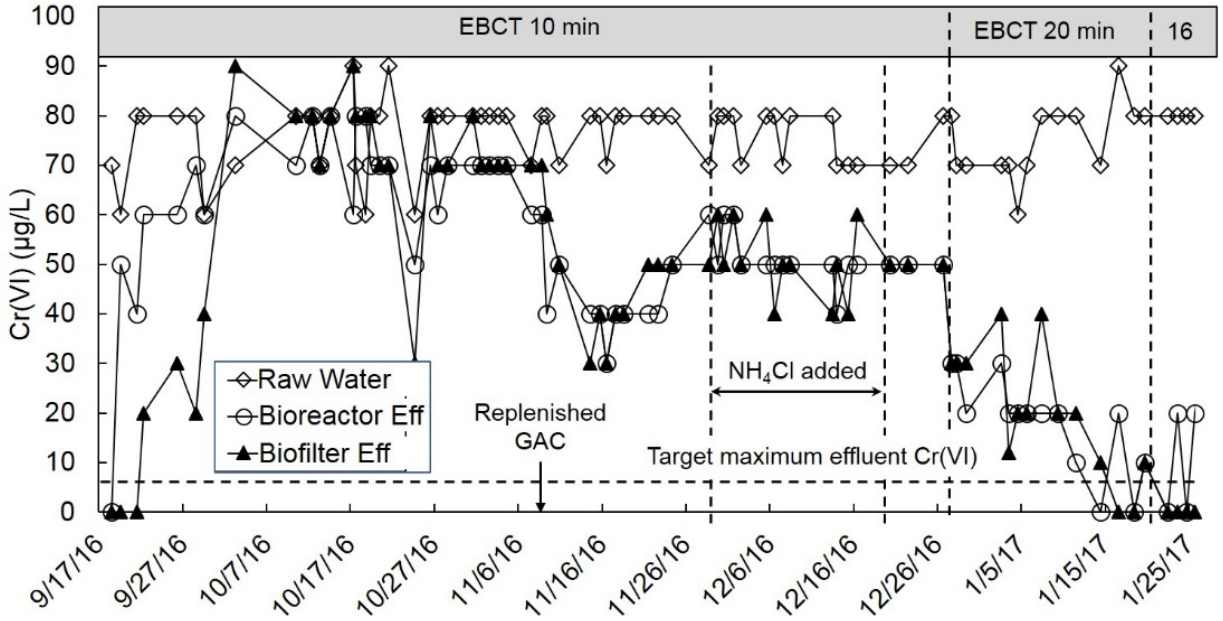

Figure 7. In-house Cr(VI) results (measured using DR900)

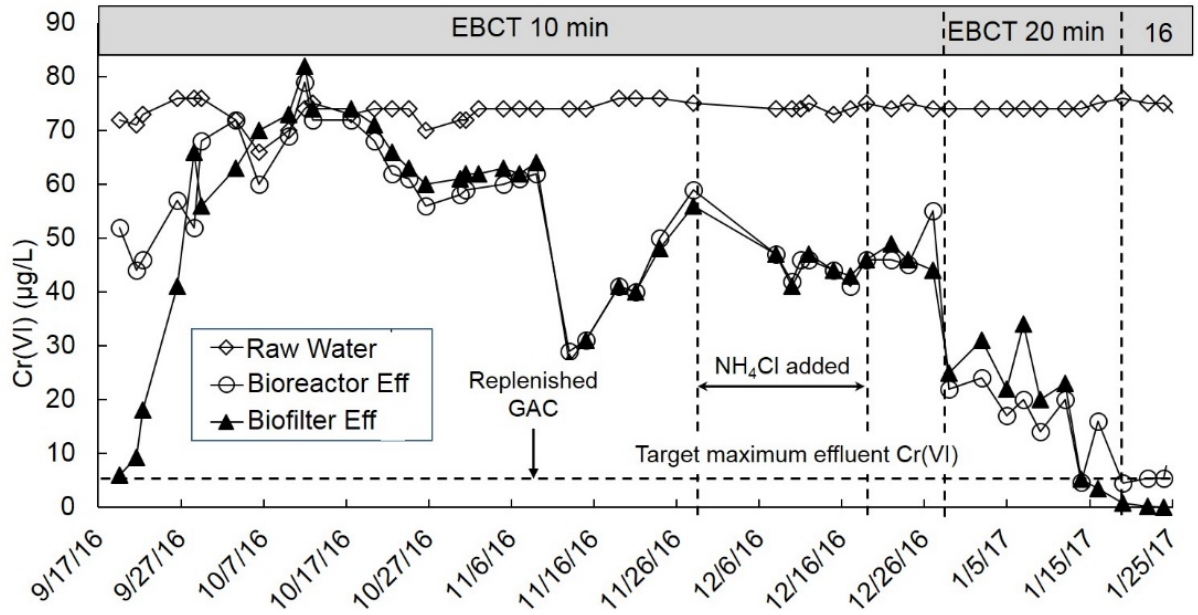

Figure 8. Cr(VI) results obtained from eurofins eaton analytical

The following observations can be made based on Figure 7 and Figure 8:

- In general, the in-house Cr(VI) results closely matched the lab Cr(VI) results. Raw water Cr(VI) concentrations averaged at 72 ± 10 micrograms per liter (µg/L) (average ± standard deviation) and 75 ± 11 µg/L for laboratory and DR900 measurements, respectively.

- Initially, Cr(VI) removal occurred through adsorption onto the GAC in the reactor.

- The raw water contained 0.7 to 1.0 mg/L N nitrate and adding NH_4Cl to the influent did not enhance Cr(VI) removal, suggesting that the system was not nitrogen-limited. In addition, the system was also not P-limited as the biofilter effluent contained 0.32±0.19 mg/L phosphate as P.

- Additional Cr(VI) removal was observed in the biofilter, which could be mediated by bacteria that can reduce Cr(VI) under aerobic conditions. Bacteria from the genera *Acinetobacter*, *Azohydromonas*, and *Zoogloea* were abundant in the biofilter (discussed below).

- After complete biological acclimation, lowering the EBCT to 16 minutes did not affect Cr(VI) removal.

Figure 9 through Figure 11 compare lab results on Cr(VI) with total Cr results.

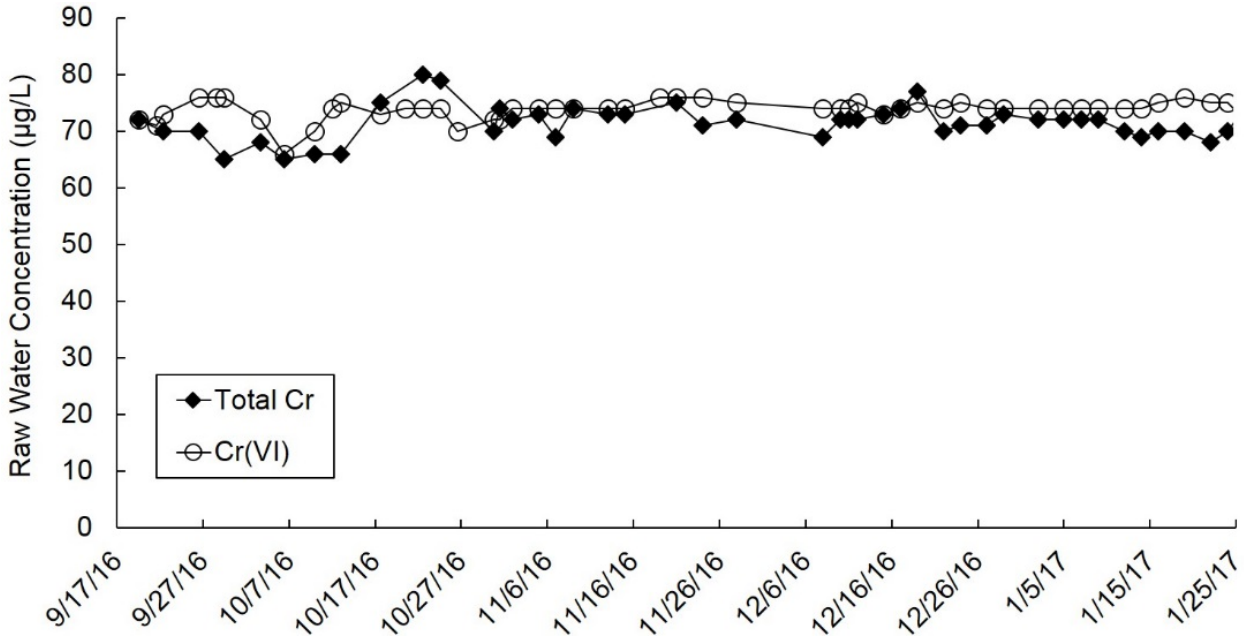

Figure 9. Total Cr versus Cr(VI) in the raw water.

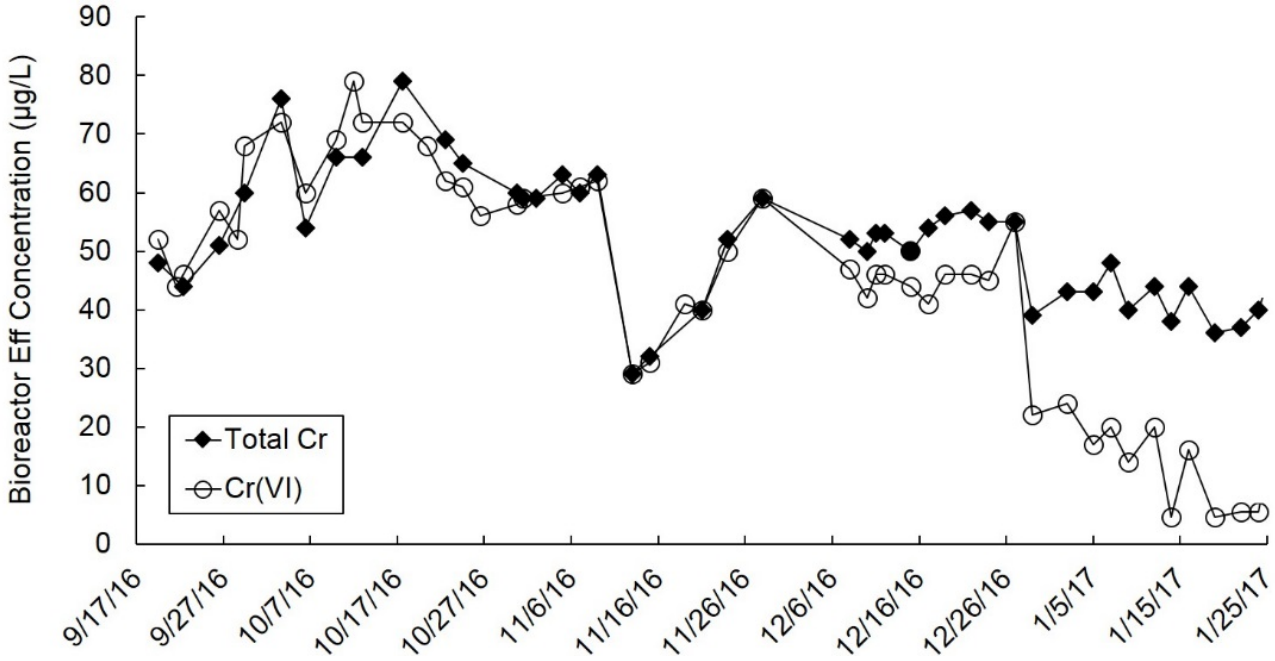

Figure 10. Total Cr versus Cr(VI) in the bioreactor effluent.

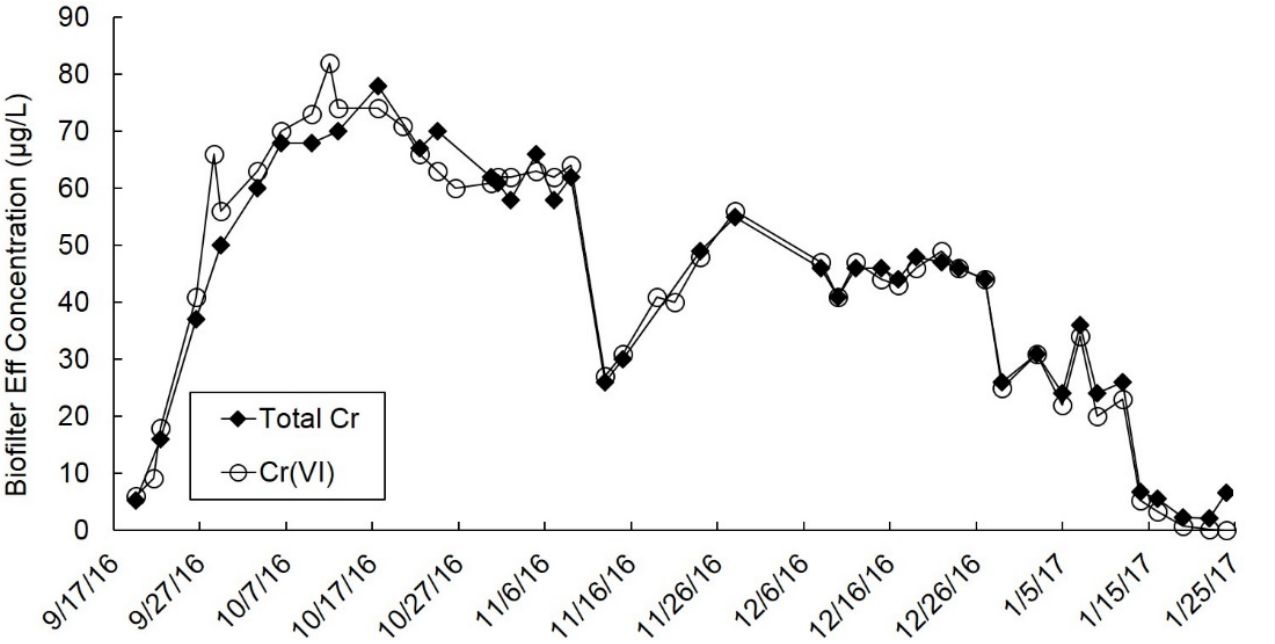

Figure 11. Total Cr versus Cr(VI) in the biofilter effluent.

The following observations can be made based on Figure 9 through Figure 11:

- Cr(VI) was the major chromium species in the raw water.
- In the bioreactor effluent, total chromium levels were significantly higher than Cr(VI) levels, suggesting the presence of Cr(III) in the bioreactor effluent. This is expected since the bioreactor is not designed for filtration.
- The biofilter effectively retains Cr(III) present in the bioreactor effluent.

Overall, biological acclimation for Cr(VI) removal took significantly longer compared to nitrate removal. Longer EBCT likely expedited biological acclimation for Cr(VI) removal. After complete acclimation, Cr(VI) reduced in the bioreactor to Cr(III), which was removed in the biofilter.

5.2. Optimal Process Parameters

Process optimization was undertaken once effective Cr(VI) removal to less than 7 µg/L was observed. Process optimization included chemical dosing and backwash settings. Chemical doses were optimized by determining the lowest doses required to meet all water quality objectives. The optimum process parameters are summarized in Table 6.

Table 6. Optimized Process Parameters for Biottta® Pilot System Treating the Groundwater from Well 5

Type	Criteria	
	Bioreactor	Biofilter
Chemical Doses		
ACA	15–17 (mg/L)	
PHA	1.0 (mg/L)	
H_2O_2	12–14 (mg/L)	
ACH	1.5 (mg/L)	
EBCT	10 min	10 min
Runtime	20 hours	48 hours
Backwash Settings		
Drain		
Air Scour	7 minutes, 7 scfm	7 minutes, 7 scfm
Combined flow (air + water)		
Air scour	4 scfm/ft^2	3 scfm/ft^2
Water flow	10 gpm	10 gpm
Hydraulic fluidization of the bed	30 gpm, 12 minutes	30 gpm, 10 minutes
Media settling	180 seconds	180 seconds

5.3. Sustained Contaminant Removal

5.3.1. Cr(VI) Removal during Sustained Contaminant Removal Phase

The pilot system was operated for approximately 30 days under steady state operating conditions using the optimal design criteria determined during phase II (Table 6). Figure 12 through Figure 14 present in-line and lab Cr(VI) results collected from April 14 through May 18, 2017. The in-line results were collected using an Aqua Metrology Systems (AMS) in-line analyzer. The raw water Cr(VI) concentrations ranged from 74 to 77 µg/L (data not shown).

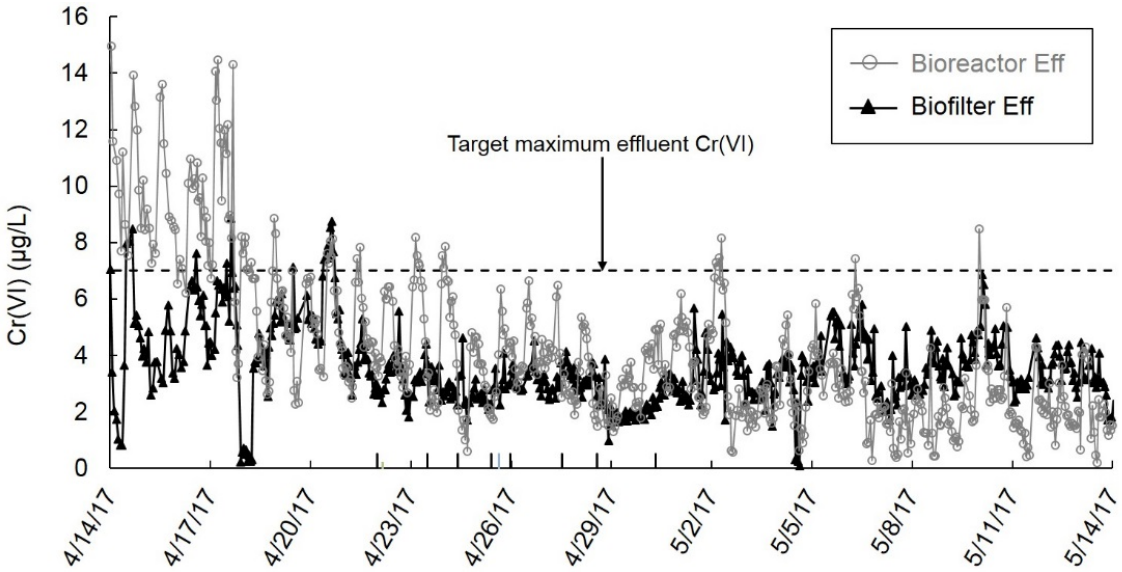

Figure 12. Cr(VI) concentrations in the bioreactor and biofilter effluents.

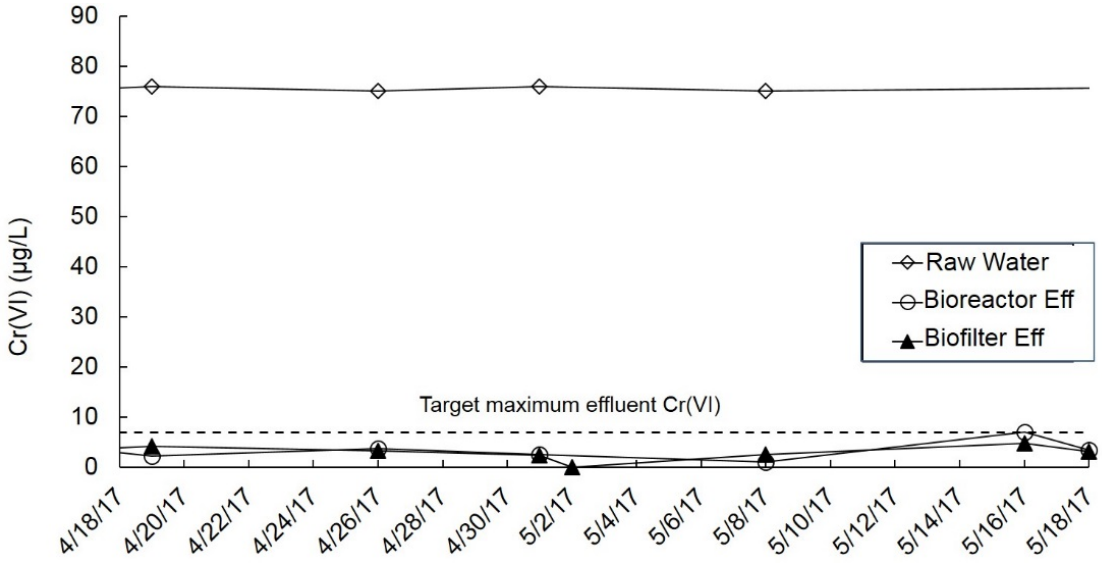

Figure 13. Laboratory results for Cr(VI) in the raw water, bioreactor effluent, and biofilter effluent.

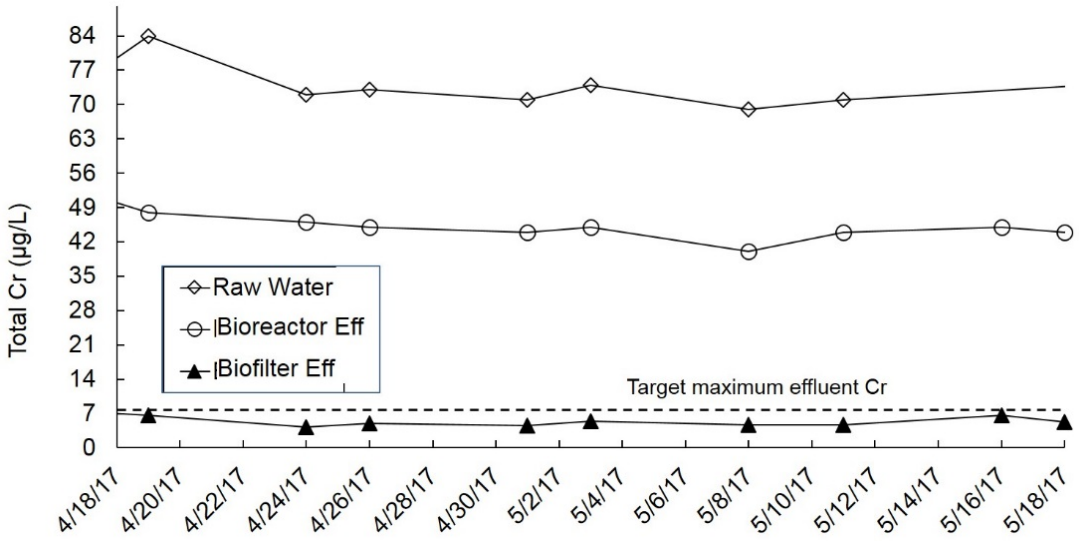

Figure 14. Total chromium in the raw water, bioreactor effluent, and biofilter effluent

The following observations can be made:

- Effective and sustained Cr(VI) removal was successfully demonstrated.
- The majority of Cr(VI) was converted to Cr(III) in the bioreactor, which was effectively filtered out in the biofilter.

5.3.2. Biofilter Effluent DO

Figure 15 shows DO concentrations in the biofilter effluent during the sustained contaminant removal phase (i.e., April 14 through May 18, 2017). In general, the biofilter effluent DO remained equal to or greater than the target DO concentration of 3 mg/L. The low DO observed between April 20 and May 1 could be attributed to inadequate H_2O_2 feed. GAC trapped in the H_2O_2 feed line restricted the chemical flow, limiting the H_2O_2 dose. Physical challenges associated with the hydrogen peroxide feed dosing location (i.e., at the top of the biofilter) were identified and addressed towards the end of the sustained contaminant removal phase, which allowed maintaining a dose of more than 3 mg/L DO. It is important to note that at a full-scale FXB BAC facility, these physical challenges would be less of a concern.

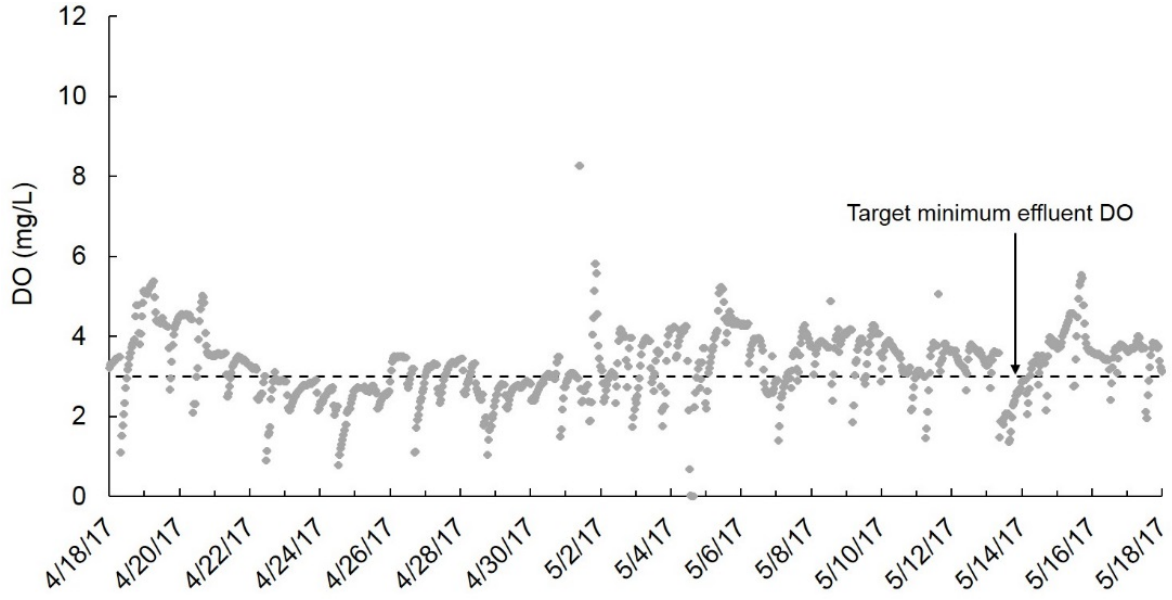

Figure 15. DO concentrations in the biofilter effluent.

5.3.3. Biofilter Effluent Turbidity

Figure 16 shows final effluent turbidity during the sustained contaminant removal phase (i.e., April 14 through May 18, 2017). In general, turbidity remained well below 0.3 NTU. Between April 14 and May 14, greater than 95 percent of the samples showed turbidity less than 0.3 NTU. During April 25 through May 3 testing, the turbidity remained equal to or greater than 0.3 NTU, likely due to higher ACA doses that resulted from an error in feed stock preparation. With an increased ACA dose, more ACA carried over to the biofilter—causing increased biogrowth in the biofilter and resulting in higher effluent turbidity.

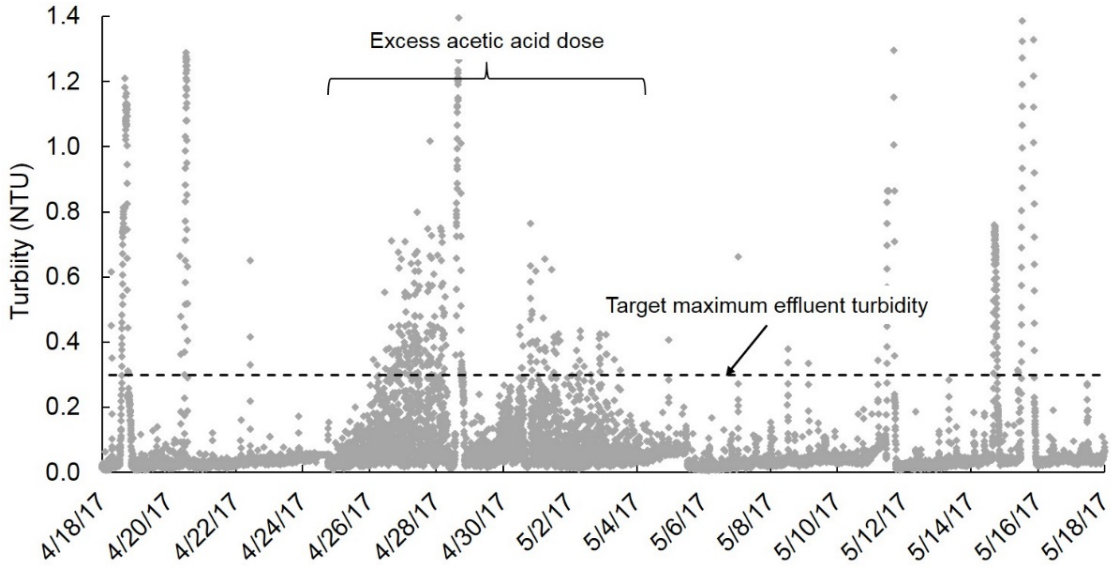

Figure 16. Biofilter effluent turbidity.

5.3.4. Backwash Wastewater Characterization

Three separate tests were conducted to characterize BWW collected during a bioreactor or biofilter backwash as discussed below.

5.3.4.1. *General Characterization*

Two sets of backwash wastewater samples were collected for both the bioreactor and biofilter. Each sample was taken in duplicate (A and B) with the exception of gross alpha. All of the biofilter backwash wastewater samples had below detectable concentrations of Cr(VI) (detection limit 0.1 µg/L). However, the total chromium exceeded 8,000 µg/L in each biofilter BWW sample (Table 7). This was expected as Cr(III) solids generated in the bioreactor were filtered out in the biofilter, which were washed out during a biofilter backwash. The COD, TSS, and total dissolved solids (TDS) results suggest that the BWW had low concentrations and were similar to a regular municipal wastewater.

Table 7. Backwash Wastewater Characteristics

Analyte	Unit	Bioreactor BWW				Biofilter BWW			
		4/26/2017		5/2/2017		4/26/2017		5/2/2017	
		A	B	A	B	A	B	A	B
Cr(VI)	µg/L	17	3.3	7.6	8.4	1.7	ND	N/A	N/A
COD	mg/L	250	240	210	210	230	360	290	290
Total As	µg/L	4.6	5.4	5	5.6	96	100	90	92
Total Cr	µg/L	140	150	160	170	9,500	10,000	8,000	8,200
TSS	mg/L	110	140	110	110	560	580	550	530
Gross Alpha	pCi/L	7.5	N/A	N/A	N/A	870	N/A	N/A	N/A
TDS	mg/L	N/A	N/A	320	330	N/A	N/A	320	330

Notes:
BWW = Backwash wastewater
ND = Not detected
N/A = Not applicable
pCi/L = picocuries per liter

5.3.4.2. *Jar Testing*

Bioreactor Backwash Wastewater

ACH doses ranging from 0 mg/L (no ACH) to 100 mg/L were tested to generate a dose response curve for turbidity removal from the bioreactor BWW. Figure 17 shows turbidity removal (normalized to turbidity at 1 minute) as a function of ACH dose and time. The negative removal observed with a dose of 100 mg/L after 2 min of settling was likely related to analytical error. Based on the results, 15 mg/L ACH appears to be the optimal dose for the bioreactor BWW.

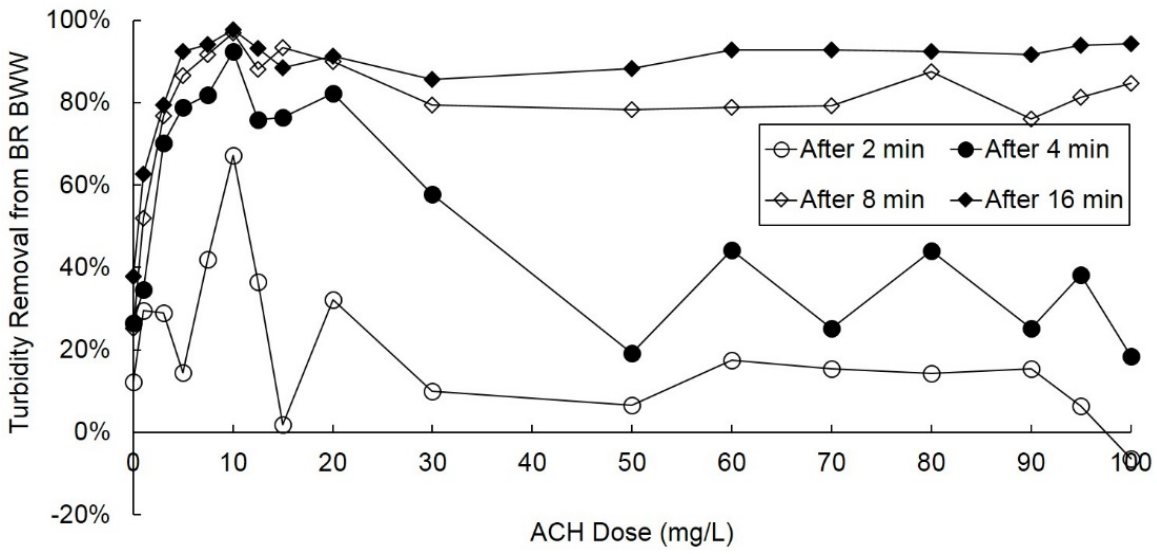

Figure 17. Turbidity removal from bioreactor BWW.

Biofilter Backwash Wastewater

Turbidity removal from biofilter BWW was evaluated with ACH dose ranging from 0 to 100 mg/L. Figure 18 presents the results of the jar testing. The negative values in the series were likely related to analytical error. While turbidity removals after 8 minutes were similar with ACH doses greater than 20 mg/L, the 20 mg/L dose provided maximum turbidity removal with a settling time of 4 minutes.

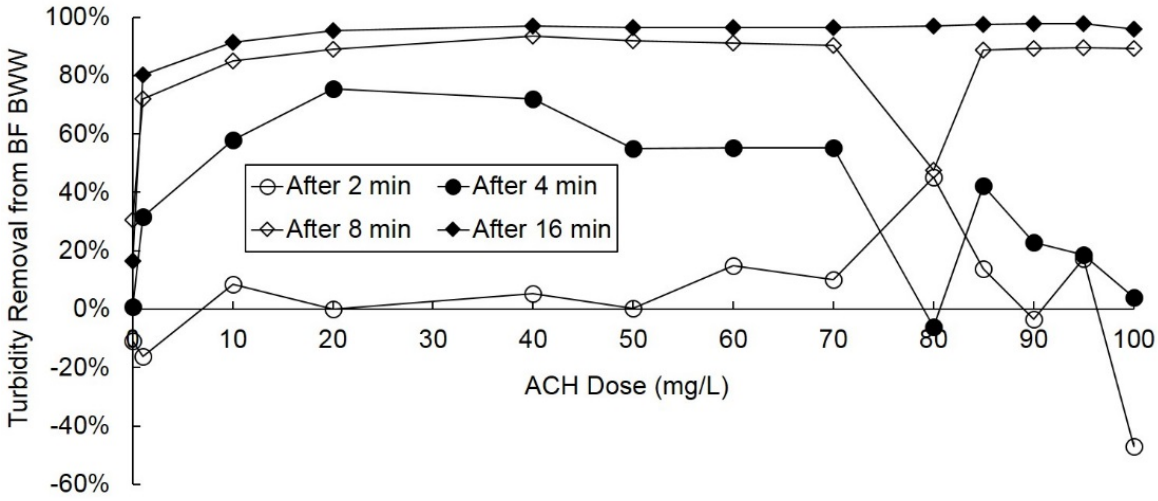

Figure 18. Turbidity removal from the biofilter BBW.

Blended Backwash Wastewater

For a full-scale system, the bioreactor and biofilter BWW can be sent to a settling basin to recover water. Therefore, jar testing was performed with the blended BWW to determine the optimal ACH dose for turbidity removal. The bioreactor and biofilter were backwashed every 20 to 24 and 48 hours, respectively, with the same amount of backwash waste produced during each backwash event. With a conservative approach, the bioreactor and biofilter BWW were blended at a 2:1 volumetric ratio. Figure 19 shows turbidity removal from the blended wastewater with the ACH doses evaluated. Even though ACH doses of 15 and 25 mg/L resulted similar turbidity removal after 4 minutes, 15 mg/L ACH may be an economical choice for turbidity removal from the blended BWW.

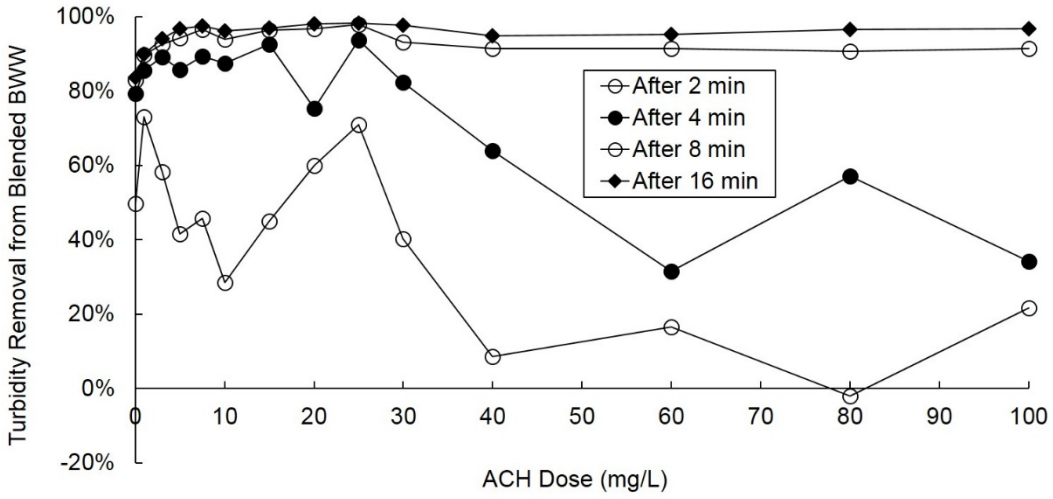

Figure 19. Turbidity Removal from Blended Backwash Wastewater

5.3.4.3. *Backwash Wastewater Hazard Assessment*

Table 8 presents the results from the TCLP, STLC, and TTLC analyses.

Table 8. Results of STLC, TTLC, and TCLP Analysis of the Bioreactor or Biofilter BWW

Parameter	STLC Results (mg/L)			TTLC Results (mg/kg)			TCLP Results (mg/L)		
	Regulatory Limits	BR - BWW	BF - BWW	Regulatory Limits	BR - BWW	BF - BWW	Regulatory Limits	BR - BWW	BF - BWW
Arsenic	5	<0.044	<0.044	50	<36	59	5	<0.044	<0.044
Chromium	5	0.032	1.1	2500	286	4100	5		0.43
Mercury	0.2	0.0005	0.00039	20	<0.0057	<0.0059			
Barium	100	0.079	0.15	10000	1.3	23			
Cadmium	1	<0.027	<0.027	100	<0.13	<0.13			
Lead	5	<0.041	<0.041	1000	<0.13	<0.13			
Selenium	1	<0.07	<0.07	100	<0.3	0.46			
Silver	5	<0.014	<0.014	500	<0.13	<0.085			

Notes:

BR BWW = Bioreactor backwash wastewater

BF BWW = Biofilter backwash wastewater

mg/kg = milligram per kilogram

The TTLC results for biofilter BWW exceeded the regulatory limits for arsenic and chromium, suggesting that it could be characterized as a hazardous waste. However, at a full-scale treatment system, a settling tank can be used to recover water from the BWW, and both bioreactor and biofilter BWW can be blended. If this approach is adopted, the biofilter BWW will be diluted when blended with the bioreactor BWW. Since the bioreactor and biofilter were backwashed every 20 to 24 hours and 48 hours, respectively, chromium and arsenic in the blended wastewater can be estimated by applying a dilution factor of 2.0 (i.e., 48 hours/24 hours = 2.0) to 2.4. Adopting a conservative approach, the ratio of 2 was used to estimate arsenic and chromium using Equation 1:

$$\text{Concentration in combined wastewater} = \frac{(BR\ BWW\ conc * 2.0 + BF\ BWW\ conc)}{(2.0+1)} \quad (1)$$

Using Equation 1, arsenic and chromium in the combined wastewater are estimated to be approximately 44 and 1,600 mg/kg, respectively, suggesting that the blended wastewater will be non-hazardous per California regulations.

5.3.5. Disinfection Tests

Disinfection tests were performed with the biofilter effluent and raw water samples. A concentration * time (CT) of 2 mg*min/L was used. Each sample was taken in duplicate (A and B). Table 9 presents the results of the disinfection tests. Unchlorinated raw water and biofilter effluent samples were used as the controls. The unchlorinated samples did not have *E. coli* or total coliform (maximum reporting limit [MRL] = 1 most probable number [MPN]/100 mL), but significant levels of HPC were observed. None of the chlorinated samples showed total coliform or *E. coli*, whereas very low levels of HPCs were observed in both the raw water and the biofilter effluent. The CT of 2 mg*min/L effectively disinfected the treated water.

Table 9.. Results of Disinfection Testing

Sample Location	Sample	Chlorinated Samples						Unchlorinated Samples		
		HPC (CFU/mL)		*E. coli* (MPN/ 100 mL)		Total Coliform (MPN/ 100 mL)		HPC (CFU/ mL)	*E. coli* (MPN/ 100 mL)	Total Coliform (MPN/ 100 mL)
Raw Water	A	3	6	<1	<1	<1	<1	22	<1	1
	B	<1	8	<1	<1	<1	<1	29	<1	<1
Biofilter Effluent	A	1	3	<1	<1	<1	<1	1500	<1	<1
	B	2	2	<1	<1	<1	<1	1100	<1	<1

CFU = colony forming unit

5.3.6. DBPFP Tests

DBPFP tests were performed with the biofilter effluent collected when the biofilter turbidity was expected to be the highest (i.e., shortly after and right before a bioreactor backwash). The samples were incubated with a target 7-day chlorine residual of 2 to 5 mg/L. The chlorine dose was adjusted based on the initial chlorine demand of the samples. Two separate DBPFP tests were conducted for both the raw water and biofilter effluent. Figure 20 and Figure 21 show TTHMs and HAA_5 formation, respectively, over the incubation period. The average of duplicate samples are shown in the Figures. Figure 22 and Figure 23 show the residual chlorine concentrations and pH of the samples, respectively.

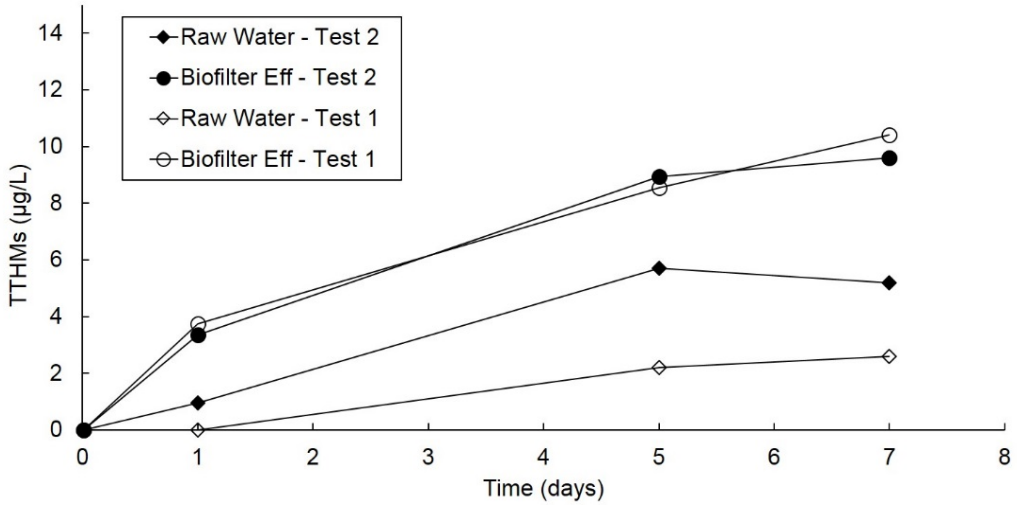

Figure 20. TTHM concentrations in the chlorinated water samples.

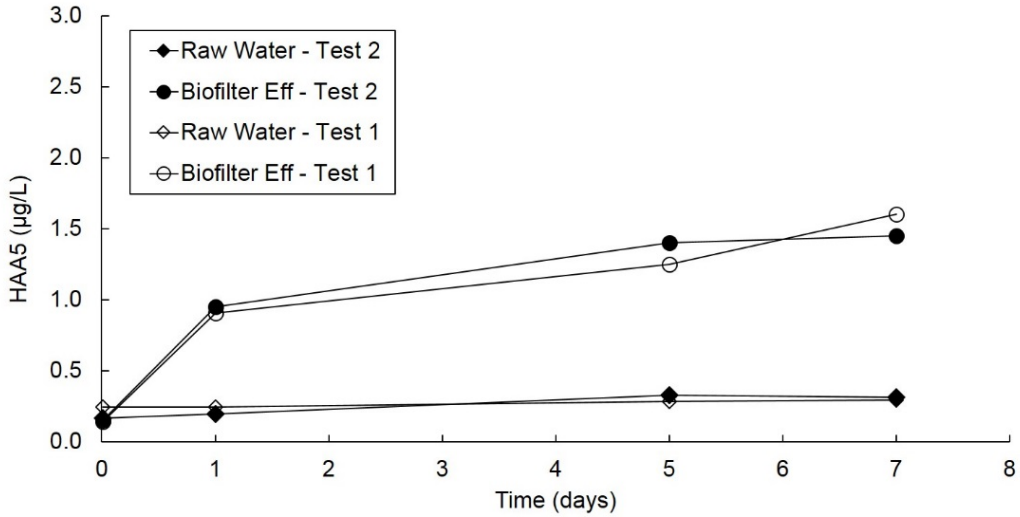

Figure 21. HAA5 concentrations in the chlorinated water samples.

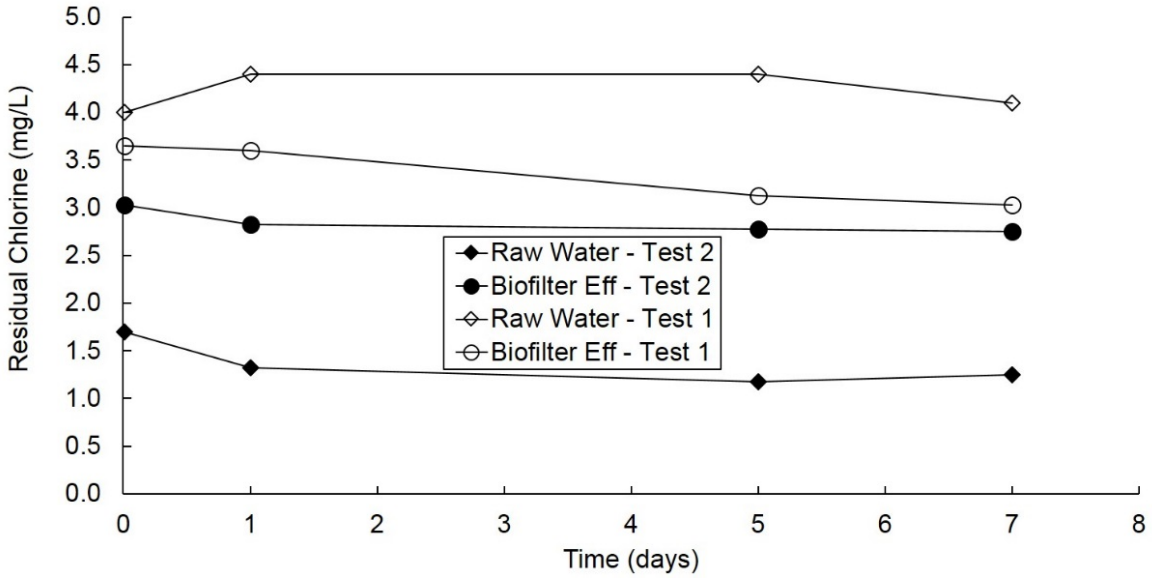

Figure 22. Residual chlorine concentrations in the chlorinated water samples.

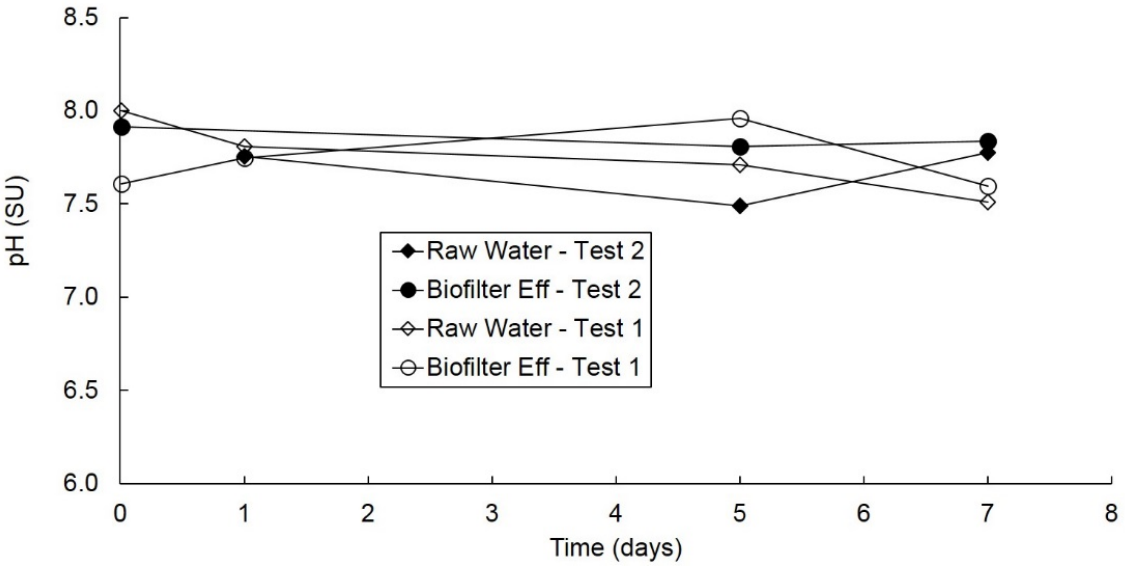

Figure 23. pH in the chlorinated water samples.

Based on these figures, the following observations can be made:

- pH remained relatively stable in all the samples throughout the incubation period.

- The final residual chlorine concentrations in the samples were 2.75 mg/L, except for one raw water sample.

- The samples had significantly lower DBPFP compared to the levels required in regulations (i.e., 80 µg/L TTHMs and 60 µg/L HAA_5).

5.3.7. Other Water Quality Parameters

Table 10 shows other water quality parameters monitored during the sustained contaminant removal demonstration phase.

Table 10. Other Water Quality Parameters

Parameter		Sample Location		
		Raw Water	Bioreactor Effluent	Biofilter Effluent
BDOC (mg/L) (n=3)	Average	0.13	0.27	0.29
	Maximum	0.21	0.6	0.34
	Minimum	0.1	0.1	0.22
Total Cr (µg/L) (n=9)	Average	74	46	5.4
	Maximum	84	53	7.4
	Minimum	69	40	4.2
Cr(VI) (µg/L) (n=4)	Average	75.5	2.4	3.1
	Maximum	76	3.7	4.1
	Minimum	75	1	2.4
DOC (mg/L) (n=5)	Average	0.27	0.92	0.27
	Maximum	0.47	2.7	0.36
	Minimum	0.17	0.36	0.22
Gross Alpha (pCi/L) (n=4)	Average	9.6	NA	6.1
	Maximum	10	NA	8.4
	Minimum	8.4	NA	4
Orthophosphate as P (mg/L) (n=4)	Average	0.011	NA	0.21
	Maximum	0.013	NA	0.29
	Minimum	0.009	NA	0.15
Sulfate (mg/L) (n=4)	Average	13.5	NA	13.5
	Maximum	14	NA	14
	Minimum	13	NA	13

The following observations can be made based on Table 10:

- The biofilter effluent contained very low levels of DOC.
- Gross alpha slightly decreased across the system, suggesting that the FXB system may also aid in removal of radioactive elements. This is in agreement with a previous study (Upadhyaya et al., 2012).
- Sulfate reduction was not observed, suggesting that sulfide production was negligible.

5.4. Robustness Testing

5.4.1. Effects of Backwashing

The bioreactor and the biofilter were backwashed every 20 to 24 hours and 48 hours of run time, respectively. To evaluate the effect of backwashing on Cr(VI) removal, Cr(VI) concentrations in the bioreactor and biofilter effluents were closely monitored immediately after a backwash from April 18 to April 22, 2017. Figure 24 shows Cr(VI) concentrations before and after the bioreactor and biofilter backwash.

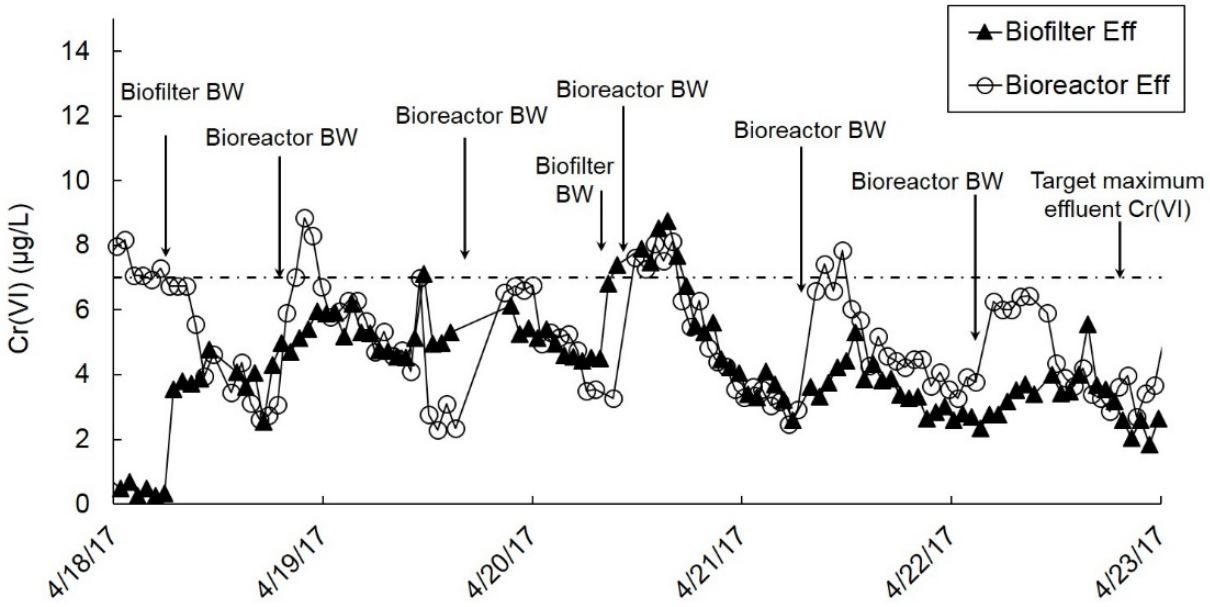

Figure 24. Effects of bioreactor and biofilter backwashing on Cr(VI) removal.

Biofilter effluent Cr(VI) levels remained less than 7 µg/L when the bioreactor or biofilter was backwashed. However, when the bioreactor was backwashed immediately after backwashing the biofilter, the biofilter effluent reached approximately 9 µg/L and the system required approximately 3 hours to reestablish the Cr(VI) removal performance. This suggests that the bioreactor and biofilter backwashes should be staggered. The results also suggested that a ripening period is not required as long as the bioreactor and biofilter backwashes are staggered.

5.4.2. Chemical Feed Failure

Both ACA and PHA feeds were individually shut down for approximately 24 hours to determine the impacts on Cr(VI) removal after the feed is resumed. The in-line analyzer failed during this testing, and the evaluation relied on lab analysis of grab samples.

5.4.2.1. *ACA Feed Failure Simulation*

The ACA feed was shut down on May 14 for approximately 26 hours (Figure 25). Cr(VI) data shown for the period prior to ACA feed resumption were collected from the in-line analyzer. The in-line analyzer failed during the testing. Grab samples for Cr(VI) and total chromium were collected after resuming the ACA feed on May 15.

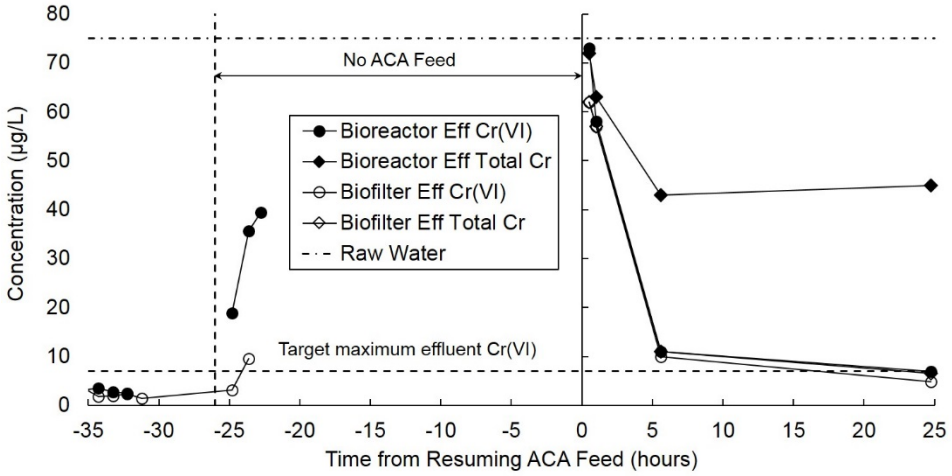

Figure 25. Results from the ACA feed failure simulation testing.

Based on Figure 25, the following observations can be made:

- As expected, the absence of ACA resulted in considerable increase in bioreactor and biofilter effluent Cr(VI). The bioreactor effluent Cr(VI) likely reached the raw water level during the 24 hours of ACA feed shut down.

- After the ACA feed was resumed, rapid recovery was observed, lowering the biofilter effluent Cr(VI) and total chromium concentrations to approximately 11 µg/L within 6 hours.

5.4.2.2. *PHA Feed Failure Simulation*

The PHA feed was shut down on May 16 for approximately 24 hours. Grab samples were collected after resuming the PHA feed. Shutting off the PHA feed minimally impacted Cr(VI) removal (Figure 26). Within 30 minutes of resuming the phosphoric feed, Cr(VI) concentrations in the biofilter effluent were less than 7 µg/L. This also suggests that PHA may not need to be fed at the full-scale system. This warrants further evaluation.

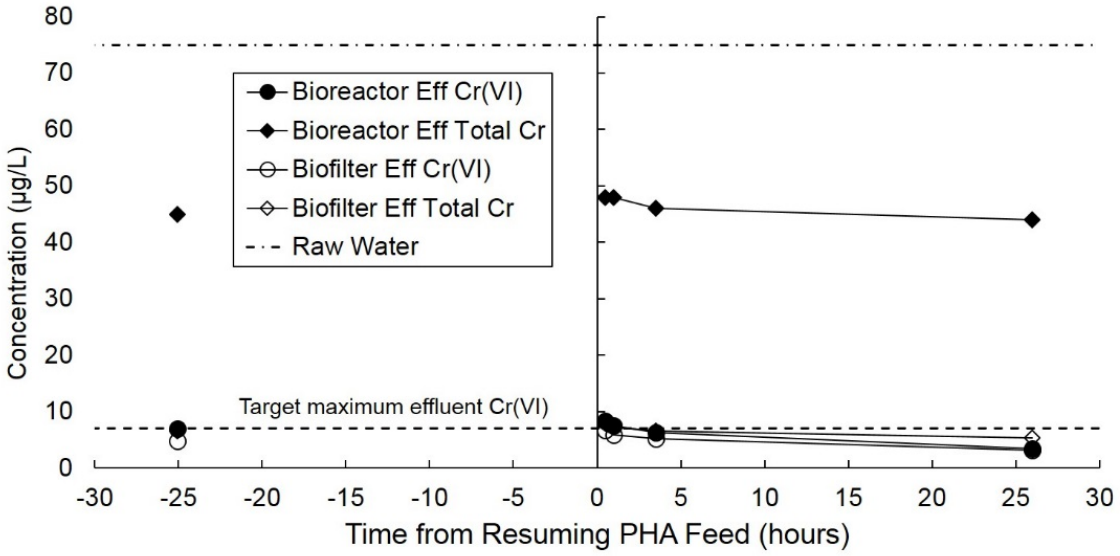

Figure 26. Results from the PHA feed failure simulation experiment.

5.4.3. System Shut-down Simulation

A 3-day system shutdown was tested from May 18 through May 21, 2017. Cr(VI) was closely monitored in the bioreactor and biofilter effluents upon restarting the system on May 21. After the 3-day system shutdown, the biofilter effluent Cr(VI) and total chromium increased (Figure 27), but the system rapidly recovered, lowering the biofilter effluent Cr(VI) to approximately 10 µg/L within 1 hour of restarting the system.

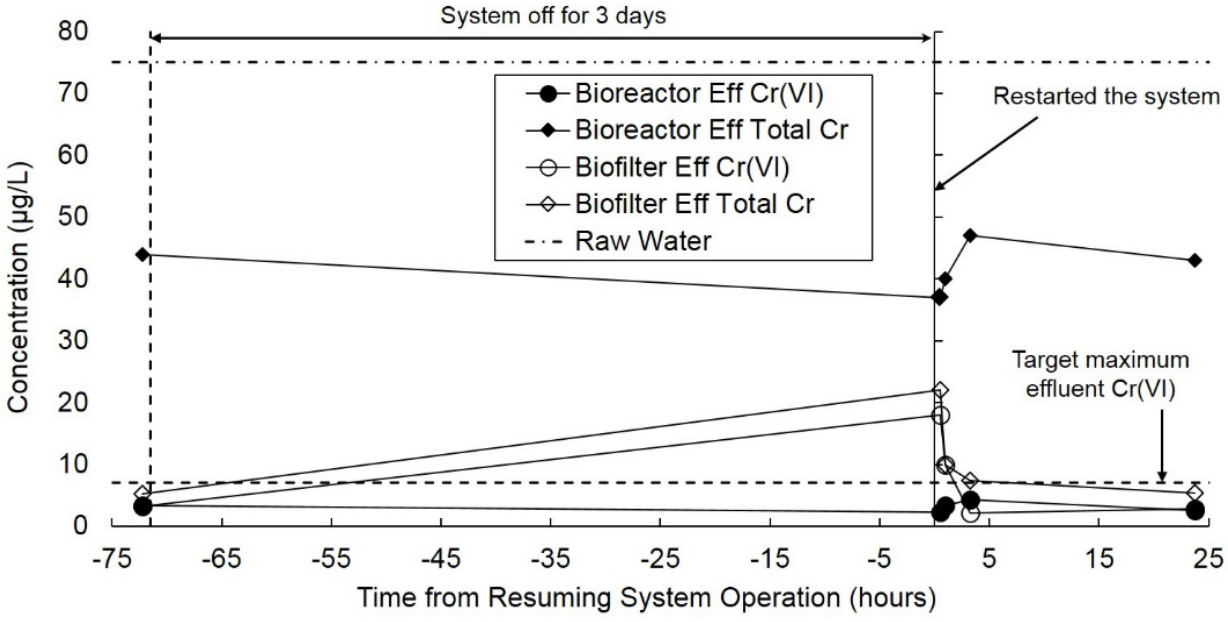

Figure 27. Effluent Cr(VI) Concentrations when the system operation was resumed after a 3-day shutdown

Figure 28 presents biofilter effluent turbidity after restarting the system on May 21, 2017. Immediately after restarting the system, turbidity remained higher, which could be due to sloughing off of dead biomass. However, within 32 minutes, turbidity was well below the treatment goal of 0.3 NTU.

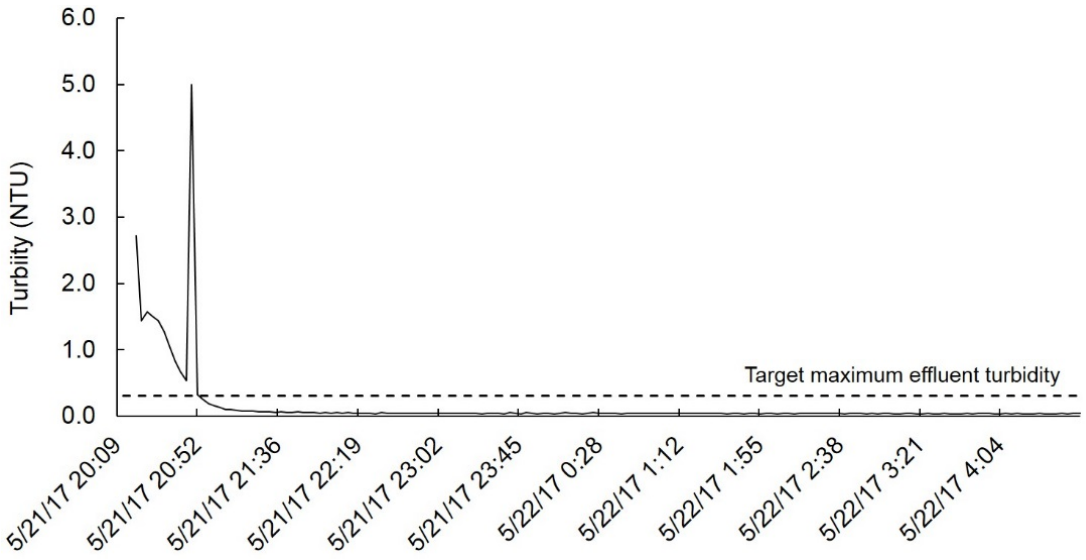

Figure 28. Biofilter effluent turbidity after the 3-day system shutdown.

Grab samples for sulfide were not collected after restarting the system. However, sulfide was not smelled in the effluent samples, suggesting that sulfide was not generated when the system was shut down for 3 days.

5.4.4. Raw Water Quality Fluctuations

A solution of potassium dichromate was prepared and injected at the top of the bioreactor from May 22 to May 25, 2017 to simulate increased Cr(VI) concentrations in the raw water. No changes were made to the chemical feed doses or the EBCT. The in-line analyzer reported 104 ± 13.9 (average ± standard deviation) µg/L Cr(VI)at the top of the bioreactor during this period (Figure 5.25). A grab sample collected from the top of the bioreactor showed 89 µg /L Cr(VI).

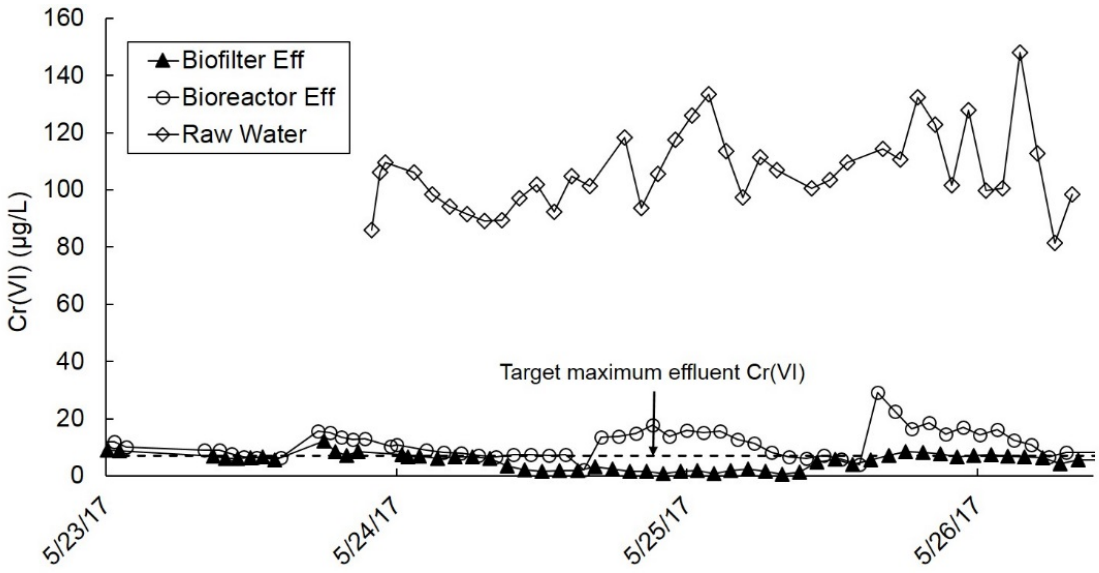

Figure 29. Cr(VI) spiking test results.

Spiking the raw water concentration to approximately 100 µg/L minimally affected Cr(VI) removal: the final effluent Cr(VI) remained equal to or less than 7 µg/L even with the increased influent concentration.

5.5. Intermittent Operation

During intermittent operation testing, three on-demand operating scenarios were tested from May 26 to June 9, 2017.

5.5.1. 7 Days On, 7 Days Off

During this testing, the system was completely shut down for seven days and then operated under normal operating conditions for the next seven days. This operational cycle was repeated with the last on-line period of only 4 days. Figure 30 presents the in-line Cr(VI) results for the bioreactor and biofilter effluent. Figure 31 shows the biofilter effluent DO during this period.

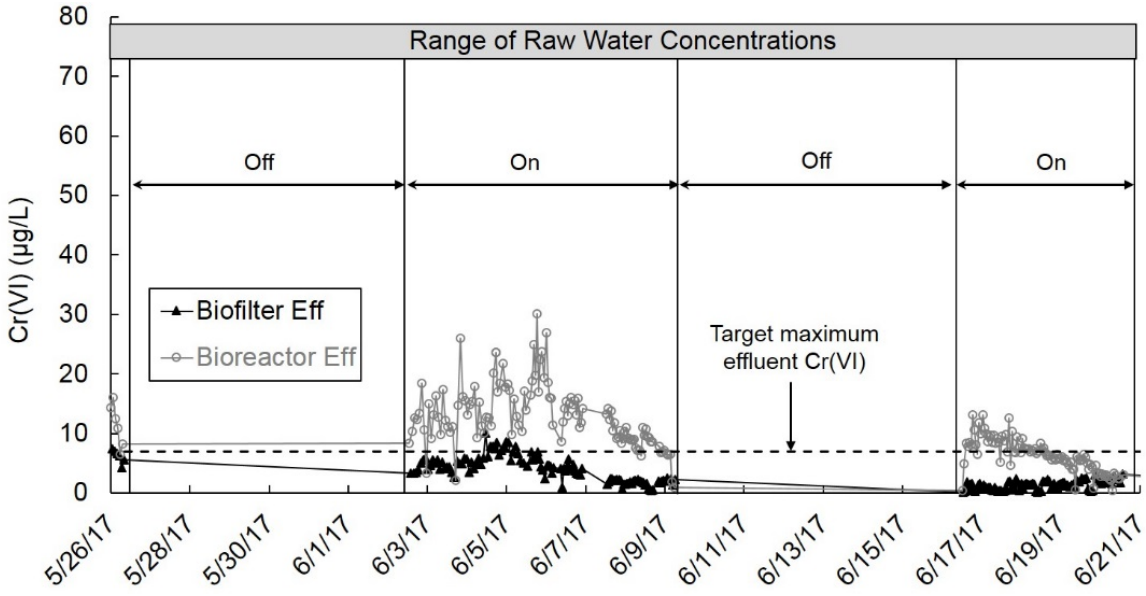

Figure 30. Bioreactor and biofilter effluent Cr(VI) concentrations during the 7-Day On/7-Day Off Testing.

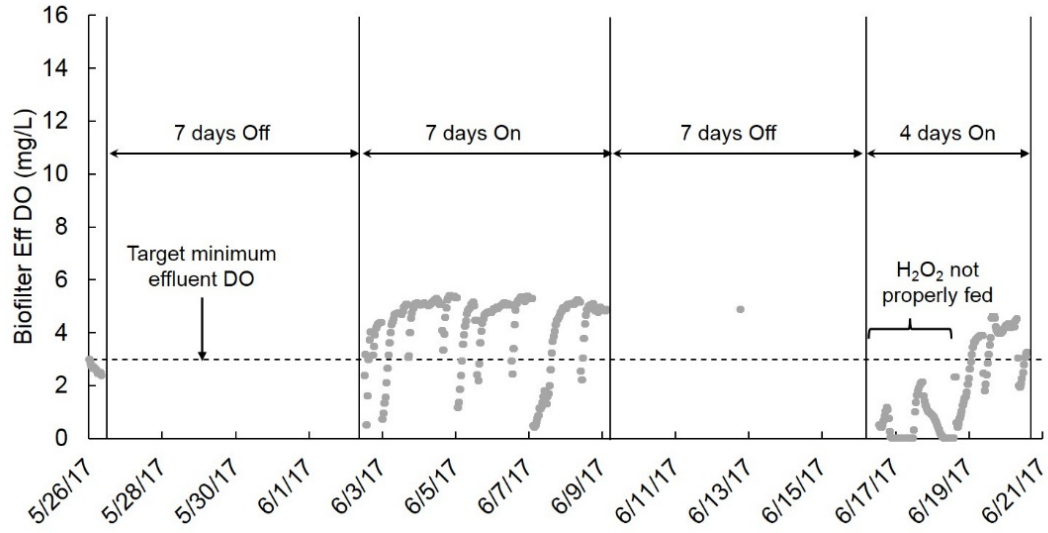

Figure 31. Biofilter effluent DO concentrations during the 7-Day On/7-Day Off Testing.

5.5.2. 100 Hours On, 68 Hours Off

During this testing, the system was continuously operated for 100 hours and then turned off for the next 68 hours. This simulated an on-demand scenario in which the system would be operational from 8 am on Monday through noon on Friday and then offline through the weekend. This testing was completed from June 16 through July 2, 2017, and the first 100-hour on period overlapped with the last on-line period of the 7-day on/7-day off testing. Figure 32 and Figure 33 show bioreactor and biofilter effluent Cr(VI) and biofilter effluent DO during this testing, respectively.

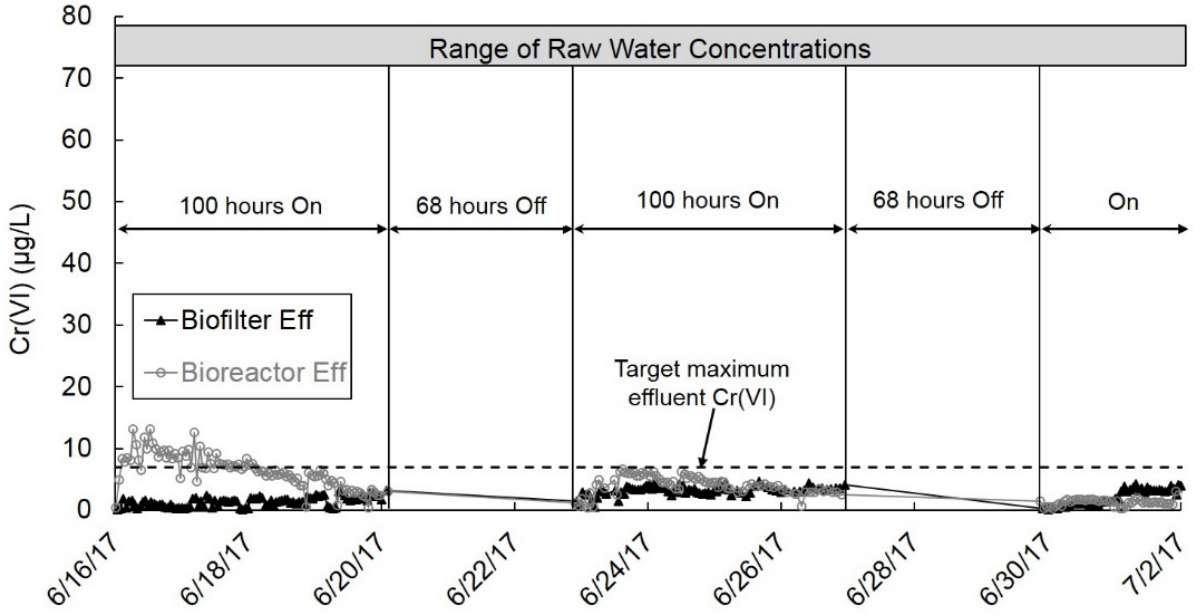

Figure 32. Bioreactor and biofilter effluent Cr(VI) concentrations during the 100-Hour On/68-Hour Off Testing.

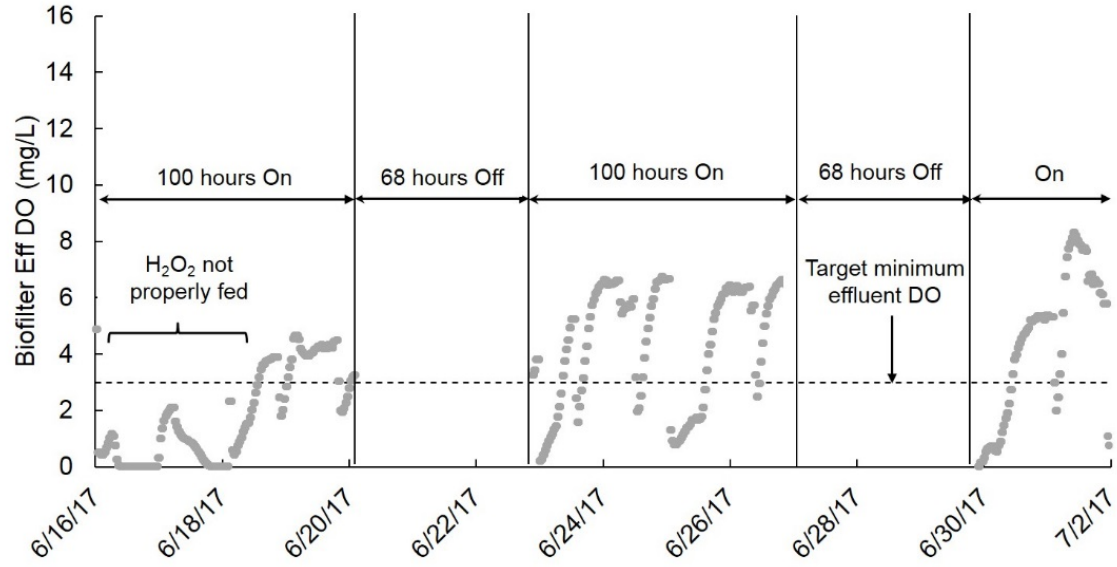

Figure 33. Biofilter effluent DO during the 100-Hour On/68-Hour Off Testing.

5.5.3. 1-Hour On/1-Hour Off for the First 12 Hours, then Off for the Next 12 Hours

This testing was conducted from July 12 through July 15, 2017. To avoid complications of low DO due to more frequent off-line periods, the H_2O_2 was continuously fed (16 mg/L H_2O_2) during this testing. c show the biofilter effluent Cr(VI) and DO, respectively.

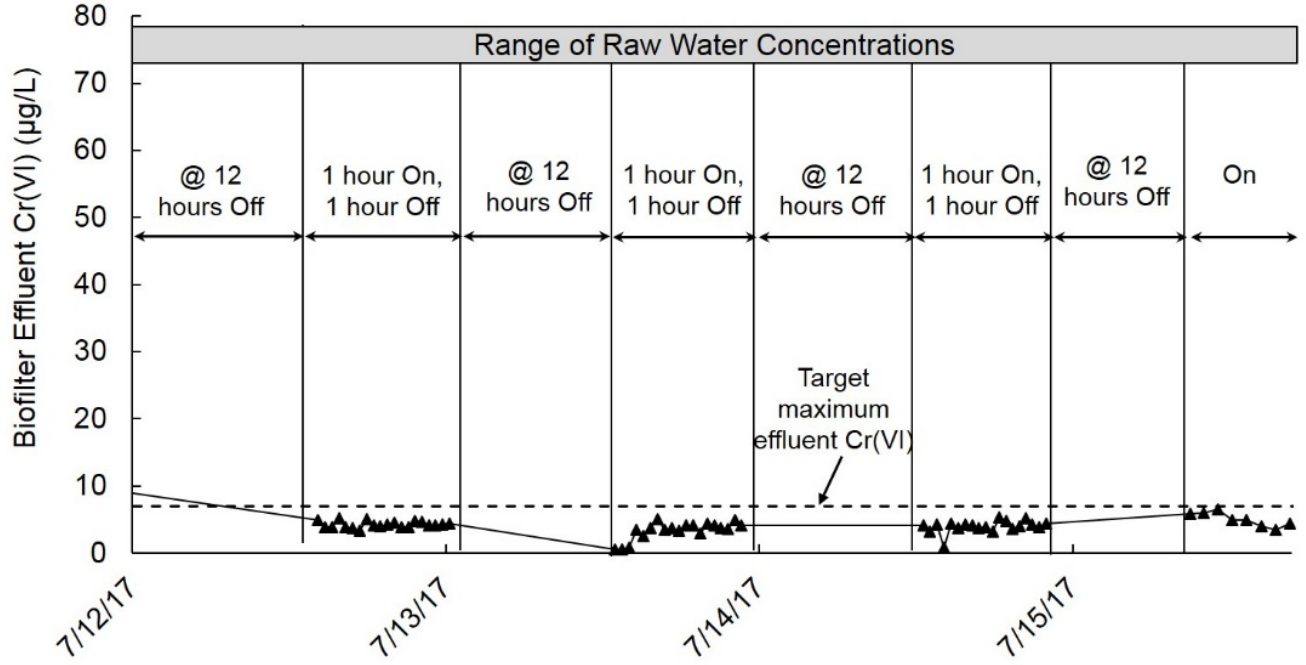

Figure 34. Biofilter effluent Cr(VI) concentrations during the 1-Hour On/1-Hour Off for the First 12 Hours and Off the Next 12 Hours Testing.

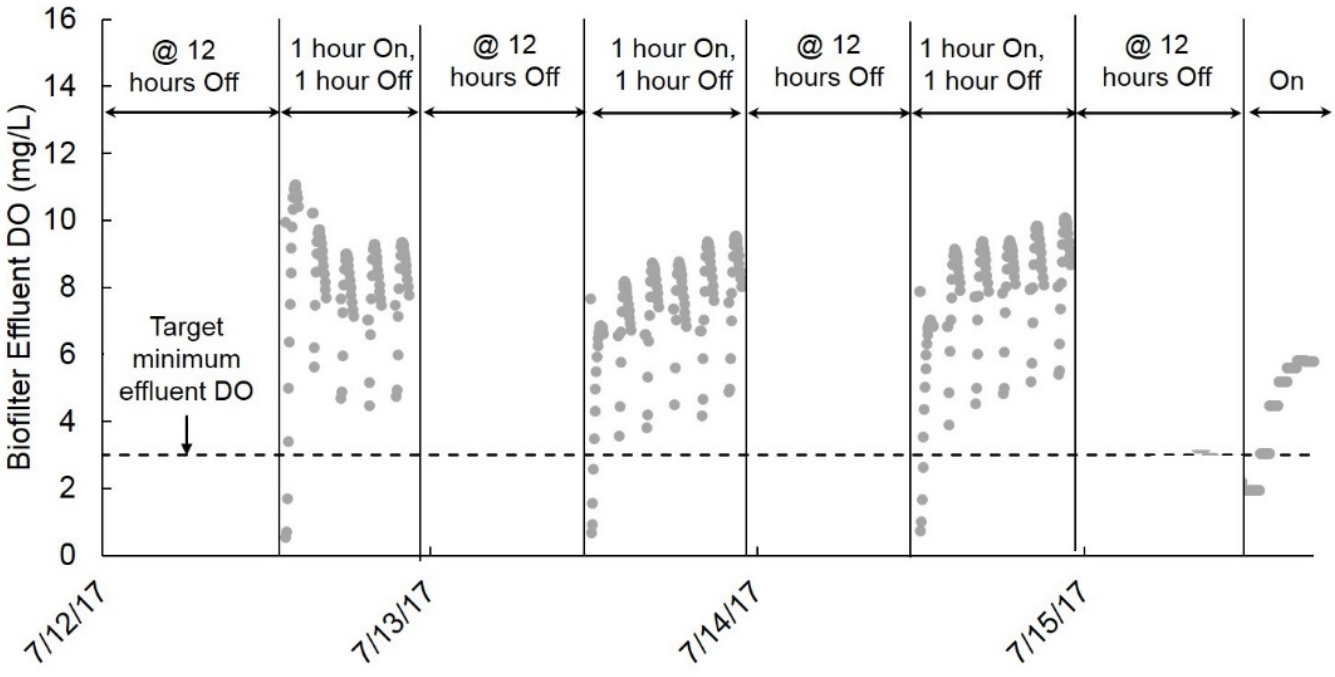

Figure 35. Biofilter effluent DO during the 1-Hour On/1-Hour Off for the First 12 Hours and Off the Next 12 Hours Testing.

The following observations can be made based on these figures:

- Intermittent operation did not affect system performance. Cr(VI) in the biofilter effluent remained well below the target level of 7 µg/L.\
- In general, biofilter effluent DO remained greater than the target level of 3 mg/L. Exceptions were observed when the H_2O_2 feed was not adequate, including the period immediately after a backwash when the chemical feed pump was ramping up.

5.6. Arsenic Removal Testing

A week-long test evaluated whether the operating conditions could be modified to achieve simultaneous removal of Cr(VI) and arsenic. The raw water contained 4.9±1.5 µg/L arsenic. Since ferric (Fe[III]) hydroxides can remove arsenic through adsorption or coprecipitation, a solution of ferrous chloride ($FeCl_2$) was prepared and injected at the bioreactor effluent from July 21 to July 28, 2017 with a target dose of 2 mg/L ferrous iron (Fe[II]). Fe(II) can be chemically oxidized to Fe(III) by hydrogen peroxide, thus removing arsenic. The arsenic containing Fe(III) hydroxides is then removed in the biofilter through filtration.

During this testing, both H_2O_2 and Fe(II) were continuously fed. The chemical feed doses optimized for Cr(VI) removal were used and the EBCT was maintained at 10 minutes. Since the iron(II) chloride was prepared in distilled water at near-neutral pH, some iron(III) precipitation was observed in the chemical feed tank, resulting in an Fe(II) dose less than 2 mg/L. Figure 36 shows arsenic results before and after spiking the Fe(II) in the bioreactor effluent. Bioreactor effluent arsenic was monitored only during this experiment. Figure 37 and Figure 38 present Cr(VI) and total chromium in the raw water and effluents during this testing.

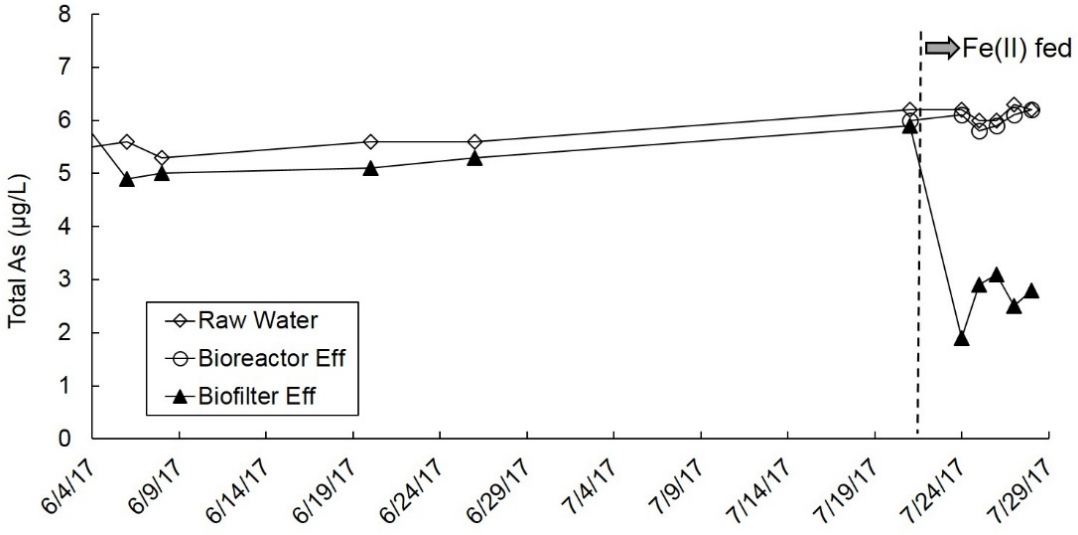

Figure 36. Arsenic in the raw water, and bioreactor and biofilter effluents before and after feeding Fe(II).

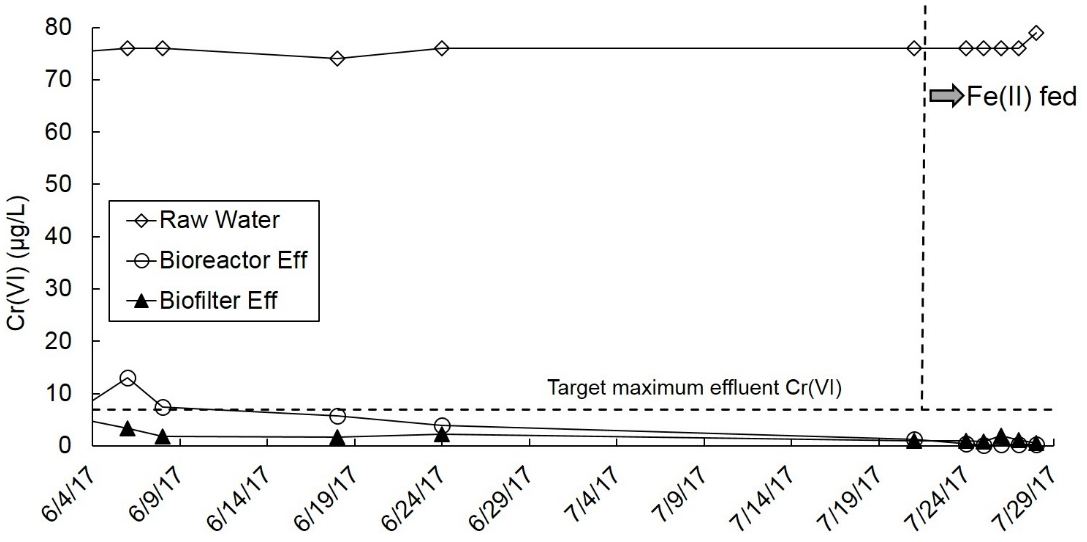

Figure 37. Cr(VI) in the raw water, and bioreactor and biofilter effluents before and after feeding Fe(II).

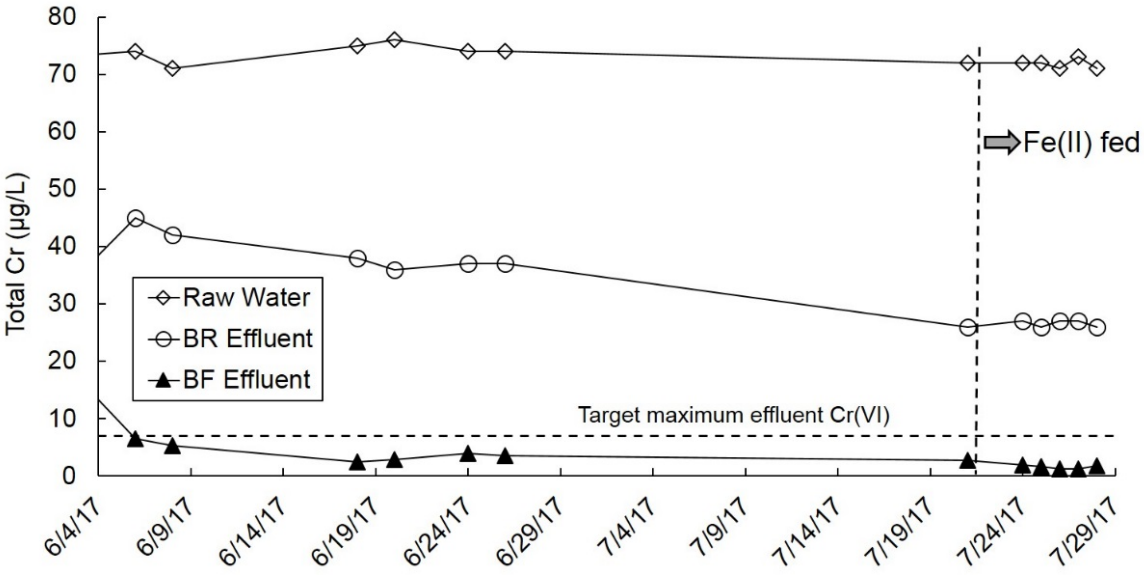

Figure 38. Total chromium in the raw water, and bioreactor and biofilter effluents before and after feeding Fe(II)

Based on these figures, the following observations can be made:

- With an EBCT of 10 minutes, adding iron resulted in removing approximately 3 to 4 µg/L of arsenic from the initial concentration of 6 µg/L. Complete arsenic removal could likely be achieved by optimizing the iron(II) feed dose.

- Cr(VI) removal was not affected by adding iron or removing arsenic.

5.7. Microbial Community Characterization

Microbial community analysis showed that *Proteobacteria* and *Bacteroidetes* were the dominant phyla with *Betaproteobacteria* and *Gammaproteobacteria* as the most abundant bacterial class in both bioreactor and biofilter (Table 11). *Dechloromonas, Acinetobacter, zoogloea* were the most dominant genera in the bioreactor. The dominant genera in the biofilter were *Acinetobacter, Zoogloea, and* A*zohydromonas* (from *Comamonadaceae* family).

Dechloromonas falls within *Rhodocyclaceae* family, which was previously identified as one of the dominant bacterial family in anaerobic Cr(VI)-removing batch reactors. Members from the *Dechloromonas* genus can also remove multiple inorganic contaminants including perchlorate (Coates and Achenbach 2004 and Li et al. 2010), nitrate (Li et al. 2010 and Upadhyaya et al. 2010), and selenium (Knight and Nijenhuis 2002). It has also been previously reported from chromium removing systems (Chung et al. 2006). Members of *Acinetobacter* are well described for intracellular reduction of Cr(VI) to Cr(III) under aerobic conditions (Ahmad et al. 2010, Bhattacharya et al. 2014, Lai et al. 2016, Dermou and Vayenas 2007). *Comamonadaceae* likely reduce Cr(VI) to Cr(III) under aerobic conditions. The presence of *Dechloromonas* as the most abundant genus in the bioreactor can be explained by the fact that the majority of the Cr(VI) was removed along with nitrate in the bioreactor. Given that the biofilter acted as a polishing biofilter, providing additional removal of Cr(VI), the dominance of bacteria related to genera *Acinetobacter* and *Azohydromonas* is not surprising.

Table 11. Community Structure of the Microorganisms Present in the Bioreactor and Biofilter

Taxonimic Level					Samples	
Phylum	**Class**	**Order**	**Family**	**Genus**	**Bioreactor**	**Biofilter**
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*	*Fluviicola*	2.60%	
			Flavobacteriaceae	*Flavobacterium*	5.60%	1.7%
			Weeksellaceae	*Cloacibacterium*	4.69%	
	Saprospirae	*[Saprospirales]*	*Chitinophagaceae*	Unclassified *Chitinophagaceae-*	1.95%	2.9%
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	Unclassified *Rhizobiales-*			3.5%
			Rhizobiaceae	Unclassified *Rhizobiaceae*	1.56%	4.6%
				Agrobacterium		1.0%
				Rhizobium		7.0%
		Rhodobacterales	*Rhodobacteraceae*	Unclassified *Rhodobacteraceae*		2.9%
			Rhodospirillaceae	Unclassified *Rhodospirillaceae*	1.56%	
		Sphingomonadales	Unclassified *Sphingomonadales-*			3.8%
	Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	Unclassified *Comamonadaceae*	7.7%	1.0%
			Comamonadaceae	*Azohydromonas*	1.2%	13.3%
			Comamonadaceae	*Hydrogenophaga*	4.2%	1.0%
			Oxalobacteraceae	Unclassified *Oxalobacteraceae*	3.4%	1.9%
		Rhodocyclales	*Rhodocyclaceae*	Unclassified *Rhodocylaceae*	2.9%	
			Rhodocyclaceae	*Dechloromonas*	25.0%	5.7%
			Rhodocyclaceae	*Zoogloea*	9.5%	14.5%
	Deltaproteobacteria	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*		1.2%
		Myxococcales	Unclassified *Myxococcales-*			2.46
	Gammaproteobacteria	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	23.6%	22.9%
		Xanthomonadales	*Xanthomonadaceae*	*Pseudoxanthomonas*	2.3%	

Reference List

Ahmad, W. A., Z. A. Zakaria, A. R. Khasim, M. A. Alias, S. M. Ismail, and M. S. H. Shaik, 2010. *Pilot-scale removal of chromium from industrial wastewater using the ChromeBac system.* Bioresource Technology, 101(12): 4371–4378.

Anderson R. A., 1995. *Chromium and Parenteral Nutrition*, Nutrition, 11:83–86.

Bartlett, R. J. 1991. *Chromium Cycling in Soils and Water: Links, Gaps, and Methods, Environmental Health Perspectives*, 92, 17–24.

Bhattacharya, A., A. Gupta, A. Kaur, and D. Malik, 2014. Efficacy of Acinetobacter sp . B9 for simultaneous removal of phenol and hexavalent chromium from co-contaminated system. Applied Microbiology and Biotechnology, 98: 9829–9841.

Blute, N., X. Wu, C. Cron, R. Abueg, D. Froelich, and L. Fong, 2014. Hexavalent chromium treatment implementation in Glendale , Calif . Journal - American Water Works Association, 106(3): 160–175.

Brandhuber, P., M. Frey, M. McGuire, P. F. Chao, C. Seidel, G. Amy, J. Yoon, L. McNeil, and K. Banerjee, 2005. Low-Level Hexavalent Chromium Treatment Options: Bench-Scale Evaluation, Project 2814, Water Research Foundation, Denver, Colorado.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. Gonzalez Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsk, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight, 2010. QIIME Allows Analysis of High-Throughput Community Sequencing Data. Nature Methods, 7(5): 335–336. Coates, J. D. and L. Achenbach, 2004. Microbial perchlorate reduction: rocket-fueled metabolism, Nature reviews. Microbiology, 2(7): 569–580.

Chung, J., R. Nerenberg, and B. E. Rittmann, 2006. Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor, Water Research, 40: 1634–1642.

Coates, J. D. and Achenbach, L. A. (2004). Microbial Perchlorate Reduction: Rocket-Fuelled Metabolism. Nature Reviews Microbiology, 2, 569-580. http://dx.doi.org/10.1038/nrmicro926.

Dermou, E. and D. V. Vayenas, 2007. A kinetic study of biological Cr(VI) reduction in trickling filters with different filter media types, 145(1–2): 252–262.

Edgar, R.C., 2010. Search and Clustering Orders of Magnitude Faster than BLAST. Bioinformatics, 26(19): 2460–2461.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight, 2011. UCHIME Improves Sensitivity and Speed of Chimera Detection. Bioinformatics, 27(16), 2194–2200.

IARC. 1990. Monographs on the Evaluation of Carcinogenic Risks to Humans: Chromium, Nickel and Welding, Vol 49, International Agency for Research on Cancer, Lyon, France.

Knight, V. K. and I. Nijenhuis, 2002. Degradation of Aromatic Compounds Coupled to Selenate Reduction, Geomicrobiology Journal, 19(1): 77–86.

Kozich, J.J., S.L. Westcott, N.T. Baxter, S.K. Highlander, and P.D. Schloss. 2013. Development of a Dual-index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform, Applied and Environmental Microbiology, 79(17): 5112–5120.

Lai, C. Y., L. Zhong, Y. Zhang, J. X. Chen, L. L. Wen, L. D. Shi, Y. P. Sun, F. Ma, B. E., Rittmann, C. Zhou, Y. Tang, P. Zheng, H. P. Zhao, 2016. Bioreduction of Chromate in a Methane-Based Membrane Biofilm Reactor, Environmental Science and Technology, 50: 5832–5839.

Li, X., G. Upadhyaya, W. Yuen, J. Brown, /E. Morgenroth, and L. Raskin, 2010. Changes in the structure and function of microbial communities in drinking water treatment bioreactors upon addition of phosphorus, Applied and Environmental Microbiology, 76(22): 7473–7481.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz, 2012. An Improved Greengenes Taxonomy with Explicit Ranks for Ecological and Evolutionary Analyses of Bacteria and Archaea, ISME, 6(3): 610–618.

National Toxicology Program (NTP). 2008. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Chromium Picolinate Monohydrate (Cas No 27882-76-4) in F344/N Rats and B6c3f1 Mice (Feed Studies), Report No. NTP TR 556. National Toxicology Program, Washington DC.

Ramírez-Díaz, M. I., C. Díaz-Pérez, E. Vargas, E., H. Riveros-Rosas, J. Campos-García, Carlos C. 2008. Mechanisms of bacterial resistance to chromium compounds, Biometals, 21: 321–332.

Upadhyaya G. and J. Brown, 2016. Biologically Mediated Simultaneous Removal of VOCs and Inorganic Contaminants from Drinking Water Sources, AWWA Annual Conference and Exposition, Chicago, Illinois, June 19–22.

Upadhyaya G., J. Jackson, T. M. Clancy, S. P. Hyun, J. Brown, K. F. Hayes, and L. Raskin, 2010. Simultaneous removal of nitrate and arsenic from drinking water sources utilizing a fixed-bed bioreactor system. Water Research, 44(17): 4958–4969.

Upadhyaya G., P. Ghosh, T. M. Clancy, Y. Bi, J. Carpenter, K. F. Hayes, and L. Raskin, 2012. Water Quality Technology Conference, Toronto, Canada, November 4–8.

Wang Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole, 2007. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, 73(16): 5261–5267.

SI Metric Conversion Table

From US Customary Unit	To SI Unit	Conversion Factor
ft	m	Divide by 3.28084
gpm	L/s	Multiply by 15.8508
gpm/ft^2	L/m^2-s	Multiply by 0.6791
scfm	L/s	Multiply by 0.47195
$scfm/ft^2$	L/m^2-s	Multiply by 5.0800

Appendices

The following Appendices are provided in a separate electronic file.

Appendix A: Nitrate, DO, and Turbidity Results Collected using In-line Analyzers
Appendix B: Cr(VI) Results Collected In-house with Hach DR900
Appendix C: Cr(VI) Results Collected using an AMA In-line Analyzer
Appendix D: Lab Results