PILOT TESTING OF A BIOLOGICAL TREATMENT PROCESS (BIOTITA™) FOR THE REMOVAL OF TCE, TCP, DBCP AND NITRATE

FINAL REPORT

January 2016
1.0 EXECUTIVE SUMMARY

The overall goal of this project was to evaluate the effectiveness of using a two-stage, fixed-bed biotreatment system (biottta™) to remove nitrate and various volatile organic compounds (VOCs) from groundwater within the Chino and Cucamonga Basins. Two parallel pilot studies were performed, one at the Chino Basin Desalter Authority’s (CDA) Chino Creek Well 18, and one at Cucamonga Valley Water District’s (CVWD's) Well 12. Pilot testing showed that the biottta™ system can effectively remove nitrate and VOCs over sustained periods, and the system is robust with respect to various changes in water quality and operating conditions. Both projects remained on budget and the CVWD project remained on schedule. Several unanticipated site challenges delayed the completion of the CDA pilot by almost 10 months. The final step in the project is to apply for and receive conditional approval from State Water Resources Control Board Division of Drinking Water (DDW) for using two-stage, fixed-bed biotreatment to treat VOCs in potable applications. To that end, a VOC treatment performance report, which combines four projects including the projects at Site 1 and Site 2, will be submitted in January 2016 to DDW's Treatment Technology Unit. In general, the simultaneous removal of multiple groundwater contaminants and the elimination of a concentrated waste stream make two-stage, fixed-bed biotreatment particularly suited for the California water industry.

2.0 INTRODUCTION

The biottta™ system uses two fixed bed (FXB) biological processes in series and includes specialized monitoring and chemical dosing algorithms, tailored media selection and configuration, and multiple biomass control tools (Figure 1). The FXB uses a stationary bed of granular activated carbon (GAC) media on which a bacterial biofilm develops. Water is drawn from a well, amended with an electron donor (acetic acid) and phosphorus, and then pumped across the media bed. Under appropriate conditions, these bacteria will convert nitrate and volatile organic compounds (VOCs) to innocuous by-products such as N₂ gas, water, and carbon dioxide.

![Figure 1: biottta™ System Process Flow.](image-url)
The biottta™ system was pilot tested within two groundwater basins: the Chino Basin (Site 1) and the Cucamonga Basin (Site 2).

- **Site 1:** CDA Chino Creek Well 18 is contaminated with nitrate and VOCs that include TCE and TCP at concentrations exceeding federal and California drinking water limits. Multiple additional VOCs are also present in the raw water. A 6 to 25 gallon per minute (gpm) biottta™ pilot testing skid was used. This skid is contained in a trailer that includes three 24-inch diameter by 8-feet tall epoxy-coated steel pressure vessels, consisting of a bioreactor, an aeration/degas tank, and a biofilter.

- **Site 2:** Five wells can feed CVWD's Reservoir 2A: Wells 8, 10, 12, 20, and 22. Presently, none of these wells are pumping due to nitrate and dibromochloropropane (DBCP) limits. Removing nitrate from any of the five wells will increase CVWD’s blending and overall production capacity. However, conventional nitrate treatment technologies like ion exchange (IX) and reverse osmosis (RO) are not feasible, as they create a concentrated waste stream for which CVWD does not have an easy treatment/handling option. A 0.3 to 3.5 gpm biottta™ pilot testing skid was used at Site 2. This skid is contained in a trailer that includes three 8"diameter columns in series configured similarly to the pilot used at Site 1.

The overall goal of this project was to evaluate the effectiveness of using the biottta™ system to remove nitrate and various VOCs from groundwater within the Chino and Cucamonga Basins. Specific objectives of the project were to:

- Develop full-scale design criteria for the biottta™ system targeting TCE, TCP, DBCP and nitrate;
- Identify any process limitations and potential failure scenarios;
- Confirm anticipate design criteria over multiple successive backwash cycles;
- Demonstrate the stability of the process under forced system disturbances;
- Apply for and receive conditional approval from the State Water Resources Control Board Division of Drinking Water (DDW) for using the biottta™ system to remove various VOCs (TCE, TCP, DBCP, etc.) from drinking water.

Several entities were involved with this project. The Chino Basin Desalter Authority and Cucamonga Valley Water District hosted the two pilot studies and provided input on project results and direction. In addition to MWD funds, CDA funded Site 1 costs and CVWD funded Site 2 costs. Carollo Engineers performed both pilot studies. The Inland Empire Utilities Agency managed the Metropolitan Water District of Southern California (Metropolitan) grant and served as the liaison between Metropolitan and Carollo. Western Municipal Water District (WMWD) also was involved as a recipient of a USEPA grant that secured additional funding for CDA for the biottta™ pilot testing at Site 1. The USEPA funded a portion of the Site 1 project costs and provided review throughout the project. DDW provided review throughout the project.

### 3.0 COST SUMMARY

The total project budget for Site 1 was $700,287, and the total project budget for Site 2 was $128,145. The Metropolitan grant covered up to 50% of the total project cost, not to exceed $350,143 of the Site 1 costs and $64,073 of the Site 2 costs. The estimated total grant eligible costs for Site 1 and Site 2 were $325,816.22 and $81,293.89 respectively, and are subject to change upon Metropolitan’s review. Site 1 costs were invoiced at 45% grant share for 2014 Quarters 1 and 2 and 48.79% grant share onwards. Site 1 Project Costs were also funded by a State and Tribal Assistant
Grant (STAG) from the U.S. Environmental Protection Agency (EPA). Site 2 costs have been invoiced at 50% grant share and were paid by CVWD. Due to the remaining grant award balance for Site 1, the costs that exceeded the Site 2 grant award were claimed as eligible project adjustments on the Final Invoice #139821-09. Through the Final Invoice #139821-09, the estimated total grant eligible Site 1 costs are $325,816.22 and $81,454.07 held for retention. Site 2 has been invoiced for $64,073, $16,018.25 held for retention, and $17,220.89 invoiced as eligible project adjustments on the Final Invoice #139821-09. The Site 1 project did not use up the entire grant award because of unallowable costs during the invoice review process and was also funded by the EPA Grant. The majority of the budget covered labor associated with site mobilization, pilot testing, site demobilization, reporting, meetings, and communication. Direct costs included well modification work, consumables (media and chemicals), piping/connectors/valves, analytical work, pilot rental, and air quality permit filing.

4.0 SCHEDULE SUMMARY

A summary of the tasks accomplished at each site is provided below.

Site 1
- Pilot Testing Protocol Development
- Permitting
- Pilot Mobilization
- Phase 1 – Pilot Testing – Biological Acclimation
- Phase 2 – Pilot Testing – Optimization
- Phase 3 – Pilot Testing – Optimal Operation
- Phase 4 – Pilot Testing – Robustness Characterization
- Demobilization
- Progress Reporting
- Technical Memoranda Preparation
- Kick-off, Interim, and Final Workshop Meetings
- Project Management

Site 2
- Pilot Testing Protocol Development
- Pilot Mobilization
- Phase 1 – Pilot Testing – Biological Acclimation
- Phase 2 – Pilot Testing – Steady State Operation
- Phase 3 – Pilot Testing – Robustness Demonstration
- Conceptual Design and Cost Estimates
- Demobilization
- Progress Reporting
- Technical Memoranda Preparation
- Kick-off, Interim, and Final Workshop Meetings
- Project Management
The Site 2 project schedule remained fairly close to the anticipated schedule. However, considerable project delays were encountered at Site 1. The Site 1 project was initially scheduled for completion by March 31, 2015, but was pushed back almost ten months for various reasons, including:

- Extensive work required to get the submersible pump installed in the well;
- Extended period required to procure AQMD permit;
- Electrical storm/lightening damaged piloting equipment, which had to be repaired or replaced;

5.0 PROJECT RESULTS AND ANALYSIS

The two-stage, fixed-bed biotreatment system (biotta™) effectively removed nitrate and VOCs over a sustained period at both testing sites. A summary of results is provided below.

**Site 1:**

- The system can efficiently remove high concentrations of raw water nitrate;
- With ~200 mg/L as NO$_3^-$ in the raw water, nitrate removal to < 5 mg/L as NO$_3^-$ can be achieved in the bioreactor at an EBCT as low as 12 minutes;
- Nitrite does not accumulate in the system;
- Sustained and consistent VOC removal can be achieved across the system;
- The occurrence of a biological VOC removal mechanism is supported by 1) VOC removal across a sand-based, bench-scale system and 2) minimal to no breakthrough of VOCs over the entire testing period (>40,000 bed volumes treated);
- Backwashing the bioreactor does not affect contaminant removal performance;
- Chemical feed failure has minimal effect on nitrate removal performance, which can be immediately re-established after the resumption of the chemical feed;
- Chemical feed failure does affect VOC removal;
- System shut-downs (tested up to one month) have little impact on system performance;
- DBP formation potential can increase across the system, likely caused by the excess acetic acid dosing requirement;
- Disinfection CTs as low as 4 mg-min/L can result in complete microbial inactivation; and
- The backwash wastewater contains low levels of COD, TSS, and TDS, allowing disposal of the wastewater in regular sewer, if available.

**Site 2:**
• Sustained nitrate removal can be achieved using biottta™ system;

• Nitrate removal to less than 5 mg/L can be achieved in the bioreactor at EBCTs as low as 3 minutes;

• Nitrite does not accumulate in the system;

• Throughout pilot testing, the biottta™ system consistently removed DBCP to below detection (<0.01 µg/L) while removing nitrate to below 5 mg/L. GAC leaching procedure tests can often be used as a tool to differentiate between VOC adsorption and biodegradation. However, leaching tests showed no DBCP desorption from any GAC sample, including GAC from the District’s full-scale contactor, which had been showing aqueous phase DBCP breakthrough at 25% and 50% bed depths. Therefore, other DBCP removal mechanism tests had to be performed. Pilot testing with preloaded GAC from the top of the District’s full-scale contactor showed no DBCP breakthrough. Bench-scale column tests using sand media showed partial DBCP removal. Two batch tests using biomass from the pilot-scale bioreactor showed DBCP removal during the first few hours of incubation. Taken together, these results suggest that bacteria indigenous to the District’s groundwater are capable of degrading DBCP, thus allowing for the efficient, simultaneous removal of nitrate and DBCP across the biottta™ system.

• Backwashing the bioreactor does not affect contaminant removal performance;

• Chemical feed failure has minimal effect on system performance and contaminant removal can be immediately re-established after the resumption of the chemical feed;

• System shut-down for up to 5 days does not affect the system performance. The microbial community was robust and microbial activity was re-established immediately after restarting the system; and

• The backwash wastewater contains low levels of COD, TSS, and TDS, allowing disposal of the wastewater in regular sewer, if available.

A VOC treatment performance report, which combines four projects including the projects at Site 1 and Site 2, will be submitted in January 2016 to DDW's Treatment Technology Unit. The goal is to receive conditional approval for using two-stage, fixed-bed biotreatment for removing VOCs from drinking water. Receiving conditional DDW approval is the only project objective that has not yet been achieved. DDW staff has indicated that they are still in the review process. It is not expected that this process will be complete until at least midsummer.

Numerous bench- and pilot-scale studies have demonstrated that two-stage, fixed-bed biotreatment (biottta™) is an effective and robust system for removing multiple contaminants simultaneously from
groundwater. Target contaminants include nitrate, perchlorate, and VOCs, which are fairly widespread across California. On-going biottta™ testing is targeting hexavalent chromium and 1,4-dioxane removal. From an operations perspective, the biottta™ system is comprised of familiar unit processes (akin to lead-lag IX or GAC adsorbers) and is designed to be fully automated. Thus, it is anticipated that full-scale implementation will not require a high degree of operator attention. However, the critical path to accurately assessing the feasibility of regional implementation requires full-scale operations, cost, and performance data.

6.0 CONCLUSION

Overall, biottta™ can provide an effective treatment option for the simultaneous removal of nitrate and VOCs. When considering other treatment alternatives to address both nitrate and VOCs, multiple unit processes would likely have to be considered (e.g., ion exchange (IX) + air stripping and/or GAC). Reverse osmosis can also treat multiple contaminants in a single step, but percent removal can vary depending on the target contaminant. In addition to multi-contaminant removal, the other critical aspect of biottta™ is that many contaminants are converted to harmless byproducts, thus eliminating the generation of a contaminant-laden, highly saline waste stream. Options for discharging these concentrated waste streams are dwindling, driving up costs. Avoiding these waste streams from the beginning eliminates these concerns and avoids shifting contaminants and salt around the environment.

Next steps required to broaden the applicability and impact of two-stage, fixed-bed biotreatment include:

1. Applying for and receiving conditional approval from DDW for using two-stage, fixed-bed biotreatment to treat VOCs in potable applications;
2. Gathering operations, cost, and performance data from the two upcoming full-scale biottta™ facilities;
3. Biottta™ system pilot testing to optimize the removal of hexavalent chromium (Cr(VI)) is on-going at the City of Delano, CA. A second biottta Cr(VI) pilot study is starting up in Norman, OK in May 2016.

Attached to this Final Report are the individual Final Pilot Performance Reports for Site 1 (CDA) and Site 2 (CVWD).
EVALUATION OF THE BIOTTTA™ SYSTEM AT CHINO CREEK WELL 18 FOR THE REMOVAL OF TCE, 1,2,3-TCP, AND NITRATE

FINAL REPORT

November 2015
TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY ....................................................................................................... 1
2.0 BACKGROUND ..................................................................................................................... 1
3.0 PROJECT OBJECTIVES ....................................................................................................... 2
4.0 TREATMENT OBJECTIVES ............................................................................................... 2
5.0 MATERIALS AND METHODS ............................................................................................ 3
  5.1 Process Flow and Pilot System Configuration ................................................................ 3
  5.2 Experimental Design ..................................................................................................... 4
    5.2.1 Phase I - Biological Acclimation ....................................................................... 4
    5.2.2 Phase II - System Optimization .................................................................. 4
    5.2.3 Phase III - Steady-State Operation ......................................................... 5
    5.2.4 Phase IV - Challenge Testing .................................................................. 6
    5.2.5 Additional Testing ............................................................................... 7
    5.2.6 Water Quality Monitoring .................................................................. 8
6.0 RESULTS ............................................................................................................................ 11
  6.1 Biological Acclimation and Optimization ................................................................. 11
  6.2 Steady State Operation ............................................................................................. 16
    6.2.1 Piloting Results .................................................................................. 16
    6.2.2 Additional Testing Performed During Steady-State Operation .................... 28
  6.3 Challenge Testing ...................................................................................................... 32
    6.3.1 Effects of Bioreactor Backwash ............................................................. 33
  6.4 VOC Leaching Test .................................................................................................... 44
  6.5 Microbial Community ............................................................................................... 44
  6.6 Bench-scale Testing ................................................................................................. 46
7.0 PILOT SITE DEMOBILIZATION .................................................................................... 49
8.0 CONCLUSIONS ............................................................................................................... 50
LIST OF TABLES

Table 1: Water Quality Monitoring Plan ................................................................. 9
Table 2: Analytical Methods.................................................................................... 10
Table 3: Treatment Chemicals.................................................................................. 10
Table 4: Optimized Process Parameters for biottta™ Pilot System Treating the Groundwater from Well 18 .................................................................................................................. 20
Table 5: Water Quality Profiles across the biottta™ Pilot System during Steady-State Operation ........................................................ 22
Table 6: BDOC Concentrations in the Raw and the Final Effluent ......................... 23
Table 7: Backwash Wastewater Characteristics ..................................................... 29
Table 8: Results of Batch CT Tests ........................................................................ 30
Table 9: Results of VOC Leaching Test .................................................................. 44

LIST OF FIGURES

Figure 1: biottta™ System Process Flow ................................................................. 2
Figure 2: biottta™ 3 Pilot Testing Skid ................................................................. 3
Figure 3: Pilot Process Flow Diagram ................................................................... 4
Figure 4: Nitrate Concentrations across the Pilot System during the Biological Acclimation .......... 11
Figure 5: Nitrite Concentrations across the Pilot System during the Biological Acclimation .... 12
Figure 6: PCE Concentrations across the Pilot System during the Biological Acclimation .... 12
Figure 7: TCE Concentrations across the Pilot System during the Biological Acclimation .... 13
Figure 8: 1,1-DCE Concentrations across the Pilot System during the Biological Acclimation .. 13
Figure 9: cis-1,2-DCE Concentrations across the Pilot System during the Biological Acclimation 14
Figure 10: 1,2,3-TCP Concentrations across the Pilot System during the Biological Acclimation Period ............................................................................................................................... 14
Figure 11: Chloroform Concentrations across the Pilot System during the Biological Acclimation 15
Figure 12: Effects of EBCT on Nitrate Removal ..................................................... 16
Figure 13: Effects of EBCT on PCE Removal .......................................................... 17
Figure 14: Effects of EBCT on TCE Removal .......................................................... 17
Figure 15: Effects of EBCT on 1,1-DCE Removal ................................................. 18
Figure 16: Effects of EBCT on cis-1,2-DCE Removal ............................................ 18
Figure 17: Effects of EBCT on trans-1,2-DCE Removal ......................................... 19
Figure 18: Effects of EBCT on 1,2,3-TCP Removal .............................................. 19
Figure 19: Nitrate Concentrations in the Raw Water and Final Effluent during Steady-State Operation .......................................................................................................................... 21
Figure 20: Nitrite Concentrations in the Raw Water and Final Effluent during Steady-State Operation .......................................................................................................................... 21
Figure 21: Final Effluent Turbidity during Steady-state Operation ......................... 22
Figure 22: PCE Concentrations across the Pilot System during Steady-State Operation ................................................................................................................................. 24
Figure 23: TCE Concentrations across the Pilot System during Steady-State Operation ................................................................................................................................. 24
Figure 24: 1,1-DCE Concentrations across the Pilot System during Steady-State Operation ................................................................................................................................. 25
Figure 25: cis-1,2-DCE Concentrations across the Pilot System during Steady-State Operation .... 25
Figure 26: trans-1,2-DCE Concentrations across the Pilot System during Steady-State Operation ........................................................................................................................ 26
Figure 27: Vinyl Chloride Concentrations across the Pilot System during Steady-State Operation ........................................................................................................................ 26
Figure 28: 1,2,3-TCP Concentrations across the Pilot System during Steady-State Operation ........................................................................................................................ 27
Figure 29: Chloroform Concentrations across the Pilot System during Steady-State Operation ........................................................................................................................ 27
Figure 30: Trichlorofluoromethane Concentrations across the Pilot System during Steady-State Operation ........................................................................................................................ 28
Figure 31: HAA5 Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples ................................................................. 30
Figure 32: TTHM Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples ................................................................. 31
Figure 33: Residual Chlorine in the Chlorinated Water Samples ...................................................... 31
Figure 34: pH in the Chlorinated Water Samples .............................................................................. 32
Figure 35: Nitrate + Nitrite Concentrations before and after Backwashing the Bioreactor ............... 33
Figure 36: PCE Concentrations before and after Backwashing the Bioreactor ................................. 34
Figure 37: TCE Concentrations before and after Backwashing the Bioreactor ............................................ 34
Figure 38: 1,1-DCE Concentrations before and after Backwashing the Bioreactor .......................... 35
Figure 39: trans-1,2-DCE Concentrations before and after Backwashing the Bioreactor ...................... 35
Figure 40: cis-1,2-DCE Concentrations before and after Backwashing the Bioreactor ....................... 36
Figure 41: 1,2,3-TCP Concentrations before and after Backwashing the Bioreactor .......................... 36
Figure 42: Chloroform Concentrations before and after Backwashing the Bioreactor ......................... 37
Figure 43: Trichlorofluoromethane Concentrations before and after Backwashing the Bioreactor ........................ 37
Figure 44: Effects of Chemical Feed Failure on Nitrate Removal ................................................... 38
Figure 45: Three-Day System Shutdown Test. The Average Raw Water Nitrate Concentration was 190 mg/L as NO₃⁻ .............................................................................................................. 39
Figure 46: Nitrate Concentrations in the Final Effluent before and after a 35-day System Shutdown. The Average Raw Water Nitrate Concentration was 190 mg/L as NO₃⁻ ........................................ 39
Figure 47: PCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 40
Figure 48: TCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 40
Figure 49: 1,1-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 41
Figure 50: cis-1,2-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 41
Figure 51: trans-1,2-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 42
Figure 52: 1,2,3-TCP Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 42
Figure 53: Chloroform Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 43
Figure 54: Trichlorofluoromethane Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown ........................................................................................................... 43
Figure 55: Phylum-level distribution of the bacteria identified in the bioreactor and biofilter ....... 45
Figure 56: Most Abundant Bacterial Genera Identified in the System .............................................. 45
Figure 57: Influent and Effluent PCE Concentrations in the Sand-based Bench-scale Bioreactor ... 47
Figure 58: Influent and Effluent TCE Concentrations in the Sand-based Bench-scale Bioreactor .. 47
Figure 59: Influent and Effluent 1,1-DCE Concentrations in the Sand-based Bench-scale Bioreactor .......................................................................................................................... 48
Figure 60: Influent and Effluent cis-1,2-DCE Concentrations in the Sand-based Bench-scale Bioreactor .......................................................................................................................... 48
Figure 61: Influent and Effluent 1,2,3-TCP Concentrations in the Sand-based Bench-scale Bioreactor .......................................................................................................................... 49
1.0 EXECUTIVE SUMMARY

The Chino Basin Desalter Authority’s (CDA) Chino Creek Well 18 is contaminated with nitrate and volatile organic compounds (VOCs), trichloroethylene (TCE) and 1,2,3-trichloropropane (1,2,3-TCP) at concentrations exceeding the federal and/or California drinking water limits. While the common conventional processes used for the removal of these compounds, such as reverse osmosis (RO), ion exchange (IX), granular activated carbon (GAC), air stripping, and advanced oxidation processes (AOPs) may remove some of these contaminants, biological treatment may allow the removal of all the compounds in a single step without generating a concentrated waste stream. To evaluate this, a one-year pilot study was conducted using a two-stage, fixed-bed biotreatment system (biottta™), which was approved by the California State Water Resources Control Board Division of Drinking Water (DDW) for nitrate and perchlorate removal.

The United States Environmental Protection Agency (EPA) has established maximum contaminant levels (MCL) for nitrate and TCE at 45 mg/L nitrate (10 mg/L nitrate as N) and 5 µg/L TCE, respectively. While there is no federal limit established for 1,2,3-TCP, the SWRCB has established a 5 µg/L MCL for the constituent. The targeted treatment goals for the study were to lower the raw water concentrations of approximately 200 mg/L nitrate as NO₃⁻, 200 µg/L TCE, and 8 µg/L 1,2,3-TCP to < 5 mg/L NO₃⁻, < 5 µg/L TCE, and < 5 µg/L 1,2,3-TCP, respectively. Sustained contaminant removals to less than the target levels were achieved. The optimum empty bed contact times (EBCTs) for nitrate removal and VOC removal were identified as 12 min and 7 min, respectively. Biological VOC removal was confirmed through multiple experiments, including the operation of a bench-scale, sand-based bioreactor. Multiple challenge tests demonstrated the robustness of the treatment system over a range of operating conditions and water quality characteristics. This pilot study demonstrated that the biottta™ system can be used to effectively, efficiently, and simultaneously remove nitrate and VOCs from contaminated water.

2.0 BACKGROUND

The CDA’s Chino Creek Well 18 is contaminated with nitrate and the VOCs, TCE, and 1,2,3-TCP at concentrations exceeding the federal and/or California drinking water limits. Conventional processes for treating nitrate include IX and RO, while VOCs are typically treated using GAC, air stripping, RO, or AOPs. Of these processes, only RO can remove all these contaminants in a single step. However, percent removal by RO membranes can be limited, RO is energy intensive, and handling RO concentrate waste streams can be complicated and costly.

Biological processes offer a different approach for removing nitrate, and VOCs, including dibromochloropropane (DBCP), TCE, and 1,2,3-TCP, from groundwater in a single step. Fixed-bed biological processes use a stationary bed of media such as sand, plastic, or GAC on which biofilms develop. Water is drawn from a well, amended with an electron donor (acetic acid) and phosphorus, and then pumped across the media bed. Under the appropriate conditions, these bacteria will convert nitrate and DBCP to innocuous by-products, such as chloride, N₂ gas, water, and carbon dioxide.

The biottta™ system offers an efficient and robust approach for degrading nitrate and VOCs to harmless end-products using bacteria indigenous to the local groundwater. biottta™ uses two fixed-
bed biological reactors in series and includes specialized monitoring and chemical dosing algorithms, tailored media selection and configuration, and multiple biomass control tools (Figure 1).

**Figure 1: biottta™ System Process Flow**

### 3.0 PROJECT OBJECTIVES

The overall objective of this project was to develop final design criteria and obtain permits for the biottta™ system to remove nitrate, TCE, and 1,2,3-TCP from Chino Creek Well 18 and to produce water that meets all regulatory standards. Specific objectives were to:

- Develop full-scale design criteria for the biottta™ system targeting nitrate, TCE, and 1,2,3-TCP
- Identify any process limitations and potential failure scenarios; and
- Apply for and receive conditional CDPH approval for using the biottta™ system to remove TCE and 1,2,3-TCP from drinking water.

### 4.0 TREATMENT OBJECTIVES

The following water quality goals were targeted for system effluent throughout the pilot study:

- Nitrate as NO₃⁻ ≤ 5 mg/L;
- Nitrite as NO₂⁻ ≤ 0.02 mg/L;
- VOCs ≤ respective MCLs;
- Turbidity < 0.3 Nephelometric turbidity units (NTU), 95% of samples monitored continuously.
- Dissolved oxygen (DO) ≥ 3 mg/L;
- Heterotrophic plate count (HPC) < 500 cfu/mL with the application of 4-log virus disinfection CT; and
- Total coliforms below detection with the application of 4-log virus disinfection CT.

5.0 MATERIALS AND METHODS

5.1 Process Flow and Pilot System Configuration

Figures 2 and 3 show a 3-D model and process flow diagram of the biottta™ pilot skid, respectively. The pilot-skid operates between 10 to 25 gpm with three, 2 feet (ft) diameter columns that are 8 ft tall and contained in a 40 ft x 8 ft x 8 ft trailer. The first column serves as the bioreactor.

Effluent from the bioreactor can travel directly to the biofilter or to an inter-stage aeration/degasification column upstream of the biofilter that removes turbidity and any residual organic carbon in the effluent of the bioreactor. Effluent from the biofilter is pumped into backwash tanks (two flat-bottom cylindrical 500-gallon polyethylene tanks) and then a chlorine contact tank (one flat-bottom cylindrical, 500-gallon polyethylene tank).

The skid is equipped with automatic backwash capabilities, chemical feed systems for electron donor, nutrient, polymer, and hydrogen peroxide. The skid also includes in-line monitoring and data logging capabilities for flow rate, headloss, DO, nitrate, and turbidity. The system also consists of a human-machine interface (HMI) that can be used onsite or accessed remotely to monitor and control pilot operation.

Raw water was pumped to the pilot feed/overflow line, then through the pilot plant. The bioreactor effluent was pumped into a backwash tank, which overflowed into an onsite catch basin. Backwash wastewater was discharged directly into the catch basin. The bioreactor and biofilter were packed with GAC-1 (effective size 1.4 mm; uniformity coefficient of 1.3) and GAC-2 (effective size 1 mm; uniformity coefficient of 1.7), respectively, to attain a bed depth of 48 inches (in) of GAC in the bioreactor and 36 in of GAC plus 12 in of sand in the biofilter.

Figure 2: biottta™ 3 Pilot Testing Skid
5.2 Experimental Design

5.2.1 Phase I- Biological Acclimation

The objectives of this phase were to establish the necessary biological activity in the system using organisms indigenous to Well 18 groundwater. The pilot testing was delayed due to some technical issues, including the installation of a submersible pump in the well, and testing officially commenced on June 16, 2014. Initially, a flow rate of 12.1 gpm was used, resulting in an empty bed contact time (EBCT) of 7 minutes. The influent water was supplemented with acetic acid and phosphoric acid to enhance biological activity for nitrate and VOC removal. The system was operated under bypass mode and hydrogen peroxide was dosed upstream of the polishing biofilter to reoxygenate the water. To meet effluent turbidity targets, a low molecular weight cationic polymer was dosed downstream of the hydrogen peroxide feed location, but upstream of the polishing biofilter. Typical run times were 12–16 hours for the bioreactor and 48 hours for the biofilter. Water samples were collected throughout the study, based on the schedule presented in the Water Quality Monitoring Plan (Table 1).

5.2.2 Phase II- System Optimization

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

5.2.2.1 System Backwash

To minimize stress on developing biofilms, backwashes were based initially only on headloss accumulation (approximately 5 ft terminal headloss). The system effluent was used to backwash the columns. The bioreactor was backwashed at an interval of 12 hours, while the biofilter was
backwashed every 48 hours. The backwash sequence included a drain step followed by air scour (5 standard cubic feet per minute (SCFM)/ft² for 5 minutes), followed by a short combined air scour/water fluidization (4 SCFM/ft², 12 gallons per minute (gpm)/ft²), and finishing with a 10-minute fluidization (10 gpm/ft²).

### 5.2.2.2 Acetic Acid and Phosphoric Acid Dose Optimization

Initially, acetic acid dose was determined based on stoichiometric requirement considering a net biomass yield of 0.4 mg biomass chemical oxygen demand COD/mg COD of acetate. A molar ratio of 1 (C):100 (P) was used to determine the phosphoric acid dose. Acetic and phosphoric acid doses were adjusted to determine the minimum concentrations required without compromising nitrate removal performance. However, real-time raw water nitrate concentrations could not be determined due to the limitation of the in-line nitrate analyzer and acetic acid was used in excess of the stoichiometric requirement (discussed below).

### 5.2.2.3 EBCT Optimization

Initially, the system was operated with an EBCT of 7 min. Using the optimal acetic acid and phosphoric acid doses, the flow rate was adjusted to determine the minimum EBCT required to achieve an effluent nitrate concentration of ≤ 5 mg/L as NO₃⁻. The minimum EBCT required for the removal of nitrate allowed for complete removal of the VOCs as well.

### 5.2.2.4 Hydrogen Peroxide and Polymer Dose Optimization

While the first stage bioreactor was being optimized for nitrate removal, hydrogen peroxide and polymer doses were also adjusted to meet the final effluent DO and turbidity requirements. However, due to excess dosing of acetic acid, the final effluent DO was < 3 mg/L (discussed below) and further optimization of hydrogen peroxide was not conducted.

### 5.2.3 Phase III- Steady-State Operation

The purpose of this phase was to demonstrate sustained nitrate removal for approximately one month using the optimum operating conditions determined during Phase II. Based on optimization testing, the biotta™ pilot was operated for approximately 30 days under the following conditions:

- EBCT: 12 min;
- Typical acetic acid dose: 220 mg/L (used in excess of the stoichiometric requirement);
- Peroxide dose: 14 to 16 mg/L;
- Polymer dose: 1.5 mg/L;
- Phosphoric acid dose: 2.0 mg/L;
- Bioreactor run time: 12 to 16 hours;
- Biofilter run time: 48 hours; and
- Pilot operation mode: bypass (i.e., no degas step).

The following additional tests were also performed during this phase:

5.2.3.1 Backwash Wastewater Characterization

Two sets of backwash wastewater composite samples were collected for both the bioreactor and biofilter. The samples were analyzed for total dissolved solids (TDS), total suspended solids (TSS), COD, and nitrate.

5.2.3.2 Disinfection Testing

Disinfection tests for 4-log virus inactivation requirements were performed with the biofilter effluent at run start and run end. For comparison, a disinfection test was also conducted with the raw water. The testing was expected to generate a CT curve. Tracer tests were not conducted to determine the t₁₀ for the continuous-flow disinfection tests and a ratio of t₁₀ to chlorine contact tank hydraulic retention time (HRT) of 0.4 was used based on a tracer study conducted in 2007 in the same pilot skid. Before starting the test, the chlorine feed system was evaluated for the targeted chlorine concentration in the chlorine contact tank. The results of the continuous-flow disinfection testing were inconsistent and suggested possible contamination of the samples. Therefore, two separate batch disinfection tests were conducted targeting a CT of 4 mg/L-min and 8.5 mg/L-min. Instantaneous free chlorine demand was also evaluated for the CT tests. HPC, total coliform, and *Escherichia coli* (*E. coli*) were determined in the duplicate treated (chlorine dosed) and untreated (unchlorinated) raw water and biofilter effluent samples.

5.2.3.3 DBPFP Tests

Two separate disinfection by-product formation potential (DBPFP) tests were conducted with the raw water and biofilter effluents. Instantaneous free chlorine demand was determined and chlorine was added to achieve a target free chlorine dose of 5 mg/L after satisfying the instantaneous demand. Total trihalomethane (TTHM) and haloacetic acid (HAA5) samples were collected at 15 minutes, 1 day, 3 days, and 7 days after chlorine addition. Residual chlorine was also measured at these sampling time points.

5.2.4 Phase IV- Challenge Testing

The purpose of this phase was to evaluate the process response to forced system disturbances. Optimal operating conditions determined during Phase II were used throughout these tests. The following operational disturbances were tested as discussed in the following sections

- Impact of bioreactor backwashing;
- Acetic acid and phosphoric acid feed failure simulation; and
• System shutdown simulation.

5.2.4.1 Impact of Bioreactor Backwashing

Since portions of established microbial populations are removed from the bioreactor during a backwash, it is important to determine if the system performance is affected by backwashing events. This type of evaluation also aids in the determination of whether a re-acclimation (ripening) or filter-to-waste period is required following a backwash. To evaluate the effects of backwashing, high-resolution samples were collected for VOCs immediately following a bioreactor backwash. The inline nitrate data were used to evaluate the impacts of backwashing on nitrate removal performance.

5.2.4.2 Acetic Acid and Phosphoric Acid Feed Failure Simulation

The acetic acid feed system was turned off for a 24-hour period to simulate a full-scale chemical dosing system failure. In-line nitrate + nitrite results were used to evaluate the effect on nitrate removal. The effect of the absence of phosphoric acid was also evaluated by turning off the phosphoric acid feeding pump for 24 hours. Inline nitrate + nitrite results were closely monitored to determine the effects.

5.2.4.3 System Shutdown Simulation

The pilot system was shut down completely for a 72-hour period. Inline nitrate + nitrite and turbidity were monitored closely upon system restart to evaluate the system performance. High resolution VOC samples were also collected to evaluate the effect on VOC removal.

5.2.5 Additional Testing

5.2.5.1 Microbial Community Characterization

Media samples were collected to characterize the microbial community in the bioreactor and biofilter. The samples were sent to the University of Texas, Austin overnight on ice. The media samples were subjected to DNA extraction and 16S rRNA genes were sequenced through multiplexed 16S amplicon sequencing on MiSeq (Illumina, San Diego, CA). Phylogenetic analyses were conducted by comparing the DNA sequences against DNA sequences in Greengenes reference database.

5.2.5.2 VOC Leaching Test

Towards the end of the project, media core samples were collected from the bioreactor and the biofilter using a wheat-thief filter coring device. The samples were loaded in 40 mL amber vials, sealed with Teflon coated caps, and shipped to Eurofins Eaton Analytical for the determination of VOCs adsorbed onto the GAC. The samples were analyzed after methanol extraction. EPA methods SW-846 5030B and SW-846 8260B were used for sample preparation and sample analysis, respectively.
5.2.5.3 Operation of a Bench-scale Sand Bioreactor

To isolate the VOC removal mechanisms in the pilot-scale system, a bench-scale bioreactor, packed with sand, was operated in parallel with the pilot system. The bioreactor consisted of a 50 cm (internal diameter) glass column, which was packed with 6” of sand. The sand was thoroughly cleaned before packing into the column to remove any organic matter present in the sand. Raw water was pumped through the pressurized bioreactor using a peristaltic pump and the EBCT was maintained at 30 min. The system was fed with acetic acid and phosphoric acid using a peristaltic pump. Influent and effluent samples were collected on a regular basis and analyzed for VOCs.

5.2.6 Water Quality Monitoring

5.2.6.1 Sampling Schedule

Table 1 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. Duplicate grab samples were collected per Table 1 to verify the in-line nitrate + nitrite and turbidity data. Grab samples were also collected to determine VOC concentrations in the raw water, bioreactor effluent, and biofilter effluent.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling Location</th>
<th>Sampling Frequency</th>
<th>Lab/Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phases I, II, III, and IV Testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate + Nitrite</td>
<td>Raw, BRE, BFE</td>
<td>1/30 minutes</td>
<td>in-Line Probe</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Raw, BRE, BFE</td>
<td>2/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Raw, BRE, BFE</td>
<td>2/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>DO</td>
<td>Raw, BRE, BFE</td>
<td>1/30 minutes</td>
<td>in-Line Probe</td>
</tr>
<tr>
<td>DO</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Field probe</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>DOC</td>
<td>Raw, BRE, BFE</td>
<td>2/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>TOC</td>
<td>Raw, BRE, BFE</td>
<td>2/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>DOCs</td>
<td>Raw, BRE, BFE</td>
<td>3/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Raw, BRE, BFE</td>
<td>1/30 minutes</td>
<td>in-Line Probe</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Raw, BRE, BFE</td>
<td>3/week</td>
<td>Field Probe</td>
</tr>
<tr>
<td>pH</td>
<td>Raw, BRE, BFE</td>
<td>3/week</td>
<td>Field Probe</td>
</tr>
<tr>
<td>Temperature</td>
<td>Raw</td>
<td>2/week</td>
<td>Field Probe</td>
</tr>
<tr>
<td>Headloss</td>
<td>Bioreactor and Biofilter</td>
<td>Continuous</td>
<td>in-Line Gauge</td>
</tr>
<tr>
<td>Flow</td>
<td>Bioreactor and Biofilter</td>
<td>Continuous</td>
<td>in-Line Flow Meter</td>
</tr>
<tr>
<td><strong>Phase III Testing: Steady-State Operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDOC</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td><strong>Disinfection Testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPC</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Total Coliform</td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Raw, BRE, BFE</td>
<td>1/week</td>
<td>Eurofins</td>
</tr>
<tr>
<td><strong>DBP formation potential testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTHMs</td>
<td>Raw, BRE, BFE</td>
<td>2 tests, at 15 min, 1 day, 3 days, and 7 days</td>
<td>Eurofins</td>
</tr>
<tr>
<td>HAA₅</td>
<td>Raw, BRE, BFE</td>
<td>2 tests, at 15 min, 1 day, 3 days, and 7 days</td>
<td>Eurofins</td>
</tr>
<tr>
<td><strong>Backwash Wastewater Characterization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSS</td>
<td>Bioreactor</td>
<td>2X</td>
<td>Eurofins</td>
</tr>
<tr>
<td>COD</td>
<td>Bioreactor</td>
<td>2X</td>
<td>Eurofins</td>
</tr>
<tr>
<td>VSS</td>
<td>Bioreactor</td>
<td>2X</td>
<td>Eurofins</td>
</tr>
<tr>
<td><strong>Phase IV Testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effects of Bioreactor Backwashing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOCs</td>
<td>Raw, BRE, BFE</td>
<td>at -1 hr, 15 min, 30 min, 1 hr, 2 hr, and 4 hr</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Nitrate + nitrite</td>
<td>Raw, BRE, BFE</td>
<td>Continuous</td>
<td>in-line Probe</td>
</tr>
<tr>
<td><strong>Acetic Acid Feed Failure Testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOCs</td>
<td>Raw, BRE, BFE</td>
<td>at 30 min, 1 hr, 2 hr, 4 hr, 24 hr, and 48 hour</td>
<td>Eurofins</td>
</tr>
<tr>
<td>Nitrate + nitrite</td>
<td>Raw, BRE, BFE</td>
<td>Continuous</td>
<td>in-line Probe</td>
</tr>
<tr>
<td>Notes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw = Bioreactor feed; BRE= Bioreactor Effluent; and BFE= Biofilter Effluent</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2.6.2 Analytical Methods

Analyses of water quality parameters were conducted using EPA or AWWA-approved standard methods. Table 2 lists the analytical methods used during this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analytical Method</th>
<th>Parameter</th>
<th>Analytical Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (as NO₃⁻)</td>
<td>EPA 300.0</td>
<td>HPC</td>
<td>SM 9215</td>
</tr>
<tr>
<td>Nitrite (as N)</td>
<td>EPA 300.0</td>
<td>Total Coliform</td>
<td>SM 9221 B</td>
</tr>
<tr>
<td>DO</td>
<td>SM 4500-O</td>
<td>E. coli</td>
<td>EPA 1603</td>
</tr>
<tr>
<td>Sulfate</td>
<td>EPA 300.0</td>
<td>TTHMs</td>
<td>EPA 502.2</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>EPA 300.0</td>
<td>HAA₅</td>
<td>EPA 552.3</td>
</tr>
<tr>
<td>DOC</td>
<td>SM 5310 C</td>
<td>TSS</td>
<td>SM 2540 D</td>
</tr>
<tr>
<td>TOC</td>
<td>SM 5310C</td>
<td>VSS</td>
<td>SM 2540 E</td>
</tr>
<tr>
<td>Turbidity</td>
<td>SM 2130 B</td>
<td>VOCs</td>
<td>EPA 524.2</td>
</tr>
<tr>
<td>COD</td>
<td>EPA 410.4/ SM 5220D</td>
<td>pH</td>
<td>4500-H+ B</td>
</tr>
<tr>
<td>BDOC</td>
<td>Servais Method</td>
<td>Temperature</td>
<td>SM 2550 B</td>
</tr>
</tbody>
</table>

5.2.6.3 Treatment Chemicals

Chemicals used in this pilot study are presented in Table 3.

<table>
<thead>
<tr>
<th>Name</th>
<th>Specific Gravity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid, Glacial (100%)</td>
<td>1.04</td>
<td>2.4</td>
</tr>
<tr>
<td>Phosphoric Acid (85%)</td>
<td>1.69</td>
<td>1.0</td>
</tr>
<tr>
<td>Hydrogen Peroxide (20%)</td>
<td>1.07</td>
<td>2.0</td>
</tr>
<tr>
<td>Cationic Polymer (30-45%)</td>
<td>1.10</td>
<td>5.0 – 7.0</td>
</tr>
</tbody>
</table>
6.0 RESULTS

6.1 Biological Acclimation and Optimization

Pilot testing officially started on June 16, 2014 and the testing lasted for 12 months. During the study, a total of 41,340 bed volumes of water were treated through the system. Figures 4 and 5 show nitrate and nitrite concentrations, respectively, during the biological acclimation and a part of the process optimization phase.

Figure 4: Nitrate Concentrations across the Pilot System during the Biological Acclimation
**Figure 5:** Nitrite Concentrations across the Pilot System during the Biological Acclimation

Figures 6 through 11 show VOC removal across the pilot system during the biological acclimation period.

**Figure 6:** PCE Concentrations across the Pilot System during the Biological Acclimation
**Figure 7:** TCE Concentrations across the Pilot System during the Biological Acclimation

**Figure 8:** 1,1-DCE Concentrations across the Pilot System during the Biological Acclimation
Figure 9: cis-1,2-DCE Concentrations across the Pilot System during the Biological Acclimation Period.

Figure 10: 1,2,3-TCP Concentrations across the Pilot System during the Biological Acclimation Period.
The following observations can be made:

- Biological acclimation for nitrate removal occurred within 30 days, resulting in complete removal of 200 mg/L nitrate as NO₃⁻ to less than the method detection limit (MDL) of 0.02 mg/L NO₃⁻. The acclimation period was longer than initially anticipated, and was likely caused by (1) the inability to monitor real-time raw water concentrations and adjust the acetic acid dose accordingly, and (2) frequent acetic acid feed failures during the start-up of the project;

- Complete VOC removal was observed in the bioreactor from the beginning, possibly due to the adsorption onto the GAC or combined adsorption/biodegradation once biological activity developed;

- Breakthrough of some of the VOCs from the bioreactor was observed after 16 days of reactor operation. However, the VOC concentrations in the bioreactor effluent did not continue to increase, suggesting that adsorption was not the dominant removal mechanism; and

- The biofilter provided a polishing step for 1,1-DCE, cis-1,2-DCE, and chloroform, which may have been due to the aerobic condition of the biofilter.
6.2 Steady State Operation

6.2.1 Piloting Results

The process optimization phase of the study was initiated once stable nitrate and VOC removal performance was established. In general, an acetic acid (mg/L) to nitrate (mg/L and NO₃⁻) ratio of 1.2 was sufficient in maintaining < 5 mg/L NO₃⁻ in the final effluent. However, due to the limitation of the inline nitrate analyzer, real-time raw water concentrations higher than 100 mg/L nitrate as NO₃⁻ could not be measured. The system relied on the acetic acid dose to influent nitrate ratio for nitrate removal but, without knowing the raw water nitrate concentrations real time, it was difficult to achieve < 5 mg/L NO₃⁻ while maintaining the minimum level of acetic acid in the final effluent. To avoid potential higher effluent nitrate levels caused by insufficient acetic acid dosing, the acetic acid dose was adjusted to a higher than optimal dose. This resulted in system effluent DO levels < 3 mg/L due to the excess acetic acid reaching the biofilter. Given the challenges in determining the raw water nitrate concentrations, the team focused on contaminant removal only, and did not focus on achieving a particular DO level in the final effluent. Process optimization also included the optimization of EBCT for two scenarios: (1) the removal of nitrate and VOCs and (2) the removal of VOCs only. The polymer dose was also optimized to achieve final effluent turbidity < 0.3 NTU.

Figure 12 presents the nitrate removal across the system during the periods with 12-min and 7-min EBCTs. Figures 13 through 18 present the VOC concentrations across the system during the 12-min and 7-min EBCT periods.

![Figure 12: Effects of EBCT on Nitrate Removal](image)

Figure 12: Effects of EBCT on Nitrate Removal
**Figure 13:** Effects of EBCT on PCE Removal

**Figure 14:** Effects of EBCT on TCE Removal
Figure 15: Effects of EBCT on 1,1-DCE Removal

Figure 16: Effects of EBCT on cis-1,2-DCE Removal
The following observations can be made based on Figures 12 through 18:

- Complete nitrate removal was achieved with an EBCT as low as 7 min. However, with the 7-min EBCT, the bioreactor effluent had significant nitrate concentrations, raising the concerns of excessive biomass growth in the biofilter. Therefore, an EBCT of 12 min was considered the optimum EBCT for nitrate removal; and

- VOC removal across the system was not impacted by EBCTs as low as 7 min.
The resulting optimum process parameters are summarized in Table 4.

<table>
<thead>
<tr>
<th>Table 4: Optimized Process Parameters for biottta™ Pilot System Treating the Groundwater from Well 18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Operation mode</td>
</tr>
<tr>
<td>EBCT</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Chemical Doses</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Backwash</td>
</tr>
<tr>
<td>Bioreactor</td>
</tr>
<tr>
<td>Biofilter</td>
</tr>
<tr>
<td>Notes:</td>
</tr>
<tr>
<td>(1) Due to the inability to determine the real-time raw water nitrate, a constant dose of 200 mg/L as NO₃⁻ acetic acid was used.</td>
</tr>
<tr>
<td>(2) Hydrogen peroxide was added to the backwash water for cleaning the underdrain and media bed during a backwash.</td>
</tr>
</tbody>
</table>

Using the optimized process parameters, the pilot system was operated continuously for approximately one month. Figures 19 and 20 present nitrate + nitrite across the system during the steady-state testing period. Figure 21 shows the turbidity in the final effluent.
Figure 19: Nitrate Concentrations in the Raw Water and Final Effluent during Steady-State Operation

Figure 20: Nitrite Concentrations in the Raw Water and Final Effluent during Steady-State Operation
Table 5 presents other water quality parameters monitored during the steady-state operating period and Table 6 presents the biodegradable dissolved organic carbon (BDOC) concentrations in the raw water and the biofilter effluent.

**Table 5: Water Quality Profiles across the biotta™ Pilot System during Steady-State Operation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>Minimum (mg/L)</th>
<th>Maximum (mg/L)</th>
<th>Average (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC (mg/L) (n=7)</td>
<td>Raw Water</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Bioreactor Eff</td>
<td>7.8</td>
<td>35.0</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>1.2</td>
<td>15.0</td>
<td>8.0</td>
</tr>
<tr>
<td>DOC (mg/L) (n=7)</td>
<td>Raw Water</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Bioreactor Eff</td>
<td>6.6</td>
<td>30.0</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>0.9</td>
<td>13.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Orthophosphate as PO₄³⁻ (mg/L) (n=7)</td>
<td>Raw Water</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Bioreactor Eff</td>
<td>0.4</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>0.3</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Sulfate (mg/L) (n=7)</td>
<td>Raw Water</td>
<td>130.0</td>
<td>130.0</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>Bioreactor Eff</td>
<td>130.0</td>
<td>130.0</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>120.0</td>
<td>130.0</td>
<td>128.0</td>
</tr>
</tbody>
</table>
**Table 6: BDOC Concentrations in the Raw and the Final Effluent**

<table>
<thead>
<tr>
<th>Date</th>
<th>Initial DOC (mg/L)</th>
<th>Final DOC (mg/L)</th>
<th>BDOC (mg/L)</th>
<th>Initial DOC (mg/L)</th>
<th>Final DOC (mg/L)</th>
<th>BDOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/6/2015</td>
<td>1.1</td>
<td>0.78</td>
<td>0.3</td>
<td>20</td>
<td>16</td>
<td>4.0</td>
</tr>
<tr>
<td>5/14/2015</td>
<td>1.3</td>
<td>1.0</td>
<td>0.3</td>
<td>9.2</td>
<td>6.3</td>
<td>2.9</td>
</tr>
<tr>
<td>5/18/2015</td>
<td>1.4</td>
<td>1.1</td>
<td>0.3</td>
<td>9.2</td>
<td>6.8</td>
<td>2.4</td>
</tr>
<tr>
<td>5/21/2015</td>
<td>1.4</td>
<td>1.1</td>
<td>0.3</td>
<td>6.9</td>
<td>3.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Based on Figures 19 –21 and Tables 5 and 6, the following observations can be made:

- Consistent and complete nitrate removal to < 5 mg/L NO₃⁻ was demonstrated throughout the steady state operation testing phase;
- Nitrite did not accumulate in the system;
- Phosphate concentrations in the final effluent were low;
- Sulfate reduction across the system was negligible; and
- Organic carbon concentrations increased across the system, but this was expected due to the need to dose excess acetic acid (i.e., inability to monitor raw water nitrate concentrations real time).

Figures 22 through 30 present VOC concentrations across the system during the steady state operation testing phase.
Figure 22: PCE Concentrations across the Pilot System during Steady-State Operation

Figure 23: TCE Concentrations across the Pilot System during Steady-State Operation
Figure 24: 1,1-DCE Concentrations across the Pilot System during Steady-State Operation

Figure 25: cis-1,2-DCE Concentrations across the Pilot System during Steady-State Operation
Figure 26: trans-1,2-DCE Concentrations across the Pilot System during Steady-State Operation

Figure 27: Vinyl Chloride Concentrations across the Pilot System during Steady-State Operation
Figure 28: 1,2,3-TCP Concentrations across the Pilot System during Steady-State Operation

Figure 29: Chloroform Concentrations across the Pilot System during Steady-State Operation
The following observations can be made:

- Complete and sustained PCE, TCE, 1,1-DCE, and 1,2,3-TCP removal was achieved across the system;
- The biofilter provided additional polishing for VOC removal; and
- Low-level breakthrough of cis-DCE, chloroform, and trichlorofluoromethane was observed, but effluent concentrations did not continue to increase, suggesting that adsorption was not the dominant removal mechanism for these compounds.

6.2.2  Additional Testing Performed During Steady-State Operation

Additional testing was performed during the steady state operation period, including backwash wastewater characterization, disinfection (CT) testing, DBPFP testing, and effects of bioreactor backwashing. The results of the testing are summarized below.

6.2.2.1  Backwash Wastewater Characterization

Two sets of composite backwash wastewater samples were collected from the bioreactor and biofilter, respectively. The wastewater samples were analyzed for COD, nitrate, TSS, VSS, and VOCs. The
results are presented in Table 7. In general, backwash wastewater exhibited typical municipal wastewater characteristics. COD ranged from 120-270 mg/L; TSS ranged from 110-160 mg/L; VSS ranged from 110-150 mg/L; nitrate was not detected, and except for chloroform, the VOCs were below detection or at very low levels.

### Table 7: Backwash Wastewater Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Biofilter BW Wastewater</th>
<th>Bioreactor BW Wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/19/2015</td>
<td>5/28/2015</td>
</tr>
<tr>
<td></td>
<td>5/1/2015</td>
<td>5/26/2015</td>
</tr>
<tr>
<td>PCE</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TCE</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>1,1-DCE</td>
<td>0.52</td>
<td>0.56</td>
</tr>
<tr>
<td>cis-1,2-DCE</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>trans-1,2-DCE</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1,2,3-TCP</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloroform</td>
<td>5.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Trichlorofluoromethane</td>
<td>0.61</td>
<td>0.77</td>
</tr>
<tr>
<td>COD</td>
<td>240</td>
<td>120</td>
</tr>
<tr>
<td>Nitrate</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>VSS</td>
<td>130</td>
<td>110</td>
</tr>
<tr>
<td>TSS</td>
<td>140</td>
<td>110</td>
</tr>
</tbody>
</table>

#### 6.2.2.2 Disinfection Testing

Initially, continuous-flow disinfection tests were performed, which were expected to generate a CT curve. Tracer tests were not conducted to determine $t_{10}$, and a $t_{10}$ to chlorine tank hydraulic retention time (HRT) value of 0.4 was used based on the tracer study conducted with the system in 2007. Before starting the test, the chlorine feed system was evaluated for the targeted chlorine concentration in the chlorine contact tank. Biofilter effluent samples collected at the start and end of a run were used for the testing. For comparison, the raw water was also included in the testing. Inconsistent inactivation results were observed with different CTs, suggesting the possibility of contamination during sampling periods. Therefore, disinfection testing was performed again in batch mode. Two separate batch tests were performed targeting a CT of 4 mg/L-min and 8.5 mg/L-min. Each sample was taken in duplicate (A and B). The results showed that effective disinfection was achieved with both CTs (Table 8).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Chlorinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Water</td>
<td>Biofilter Eff</td>
</tr>
<tr>
<td>Sample A</td>
<td>Sample B</td>
<td>Sample A</td>
</tr>
<tr>
<td>HPC</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>E. coli (MPN/100 mL)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total coliform (MPN/100 mL)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

6.2.2.3 Disinfection Byproduct Formation Potential Testing

Disinfection byproduct formation potential tests were performed with the final effluent collected at the start and end of a bioreactor run. The samples were incubated with a target chlorine residual of 5 mg/L. The chlorine dose was adjusted based on the initial chlorine demand of the samples, which ranged from 0.2 to 0.25 mg/L. Figures 31 and 32 show HAA5 and TTHMs formation, respectively, over the incubation period. Figures 33 and 34 show residual chlorine concentrations and pH of the samples at different time points.

![Figure 31: HAA5 Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples](image-url)
**Figure 32:** TTHM Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples.

**Figure 33:** Residual Chlorine in the Chlorinated Water Samples.
The following observations can be made:

- Stable pH was observed throughout the DBPFP incubation period;
- Effluent HAA formation potential was $< 40 \, \mu g/L$;
- THM formation potential of the raw water was significant, and increased across the system. This result was not surprising, as excess acetic acid was dosed to the bioreactor feed to ensure that it was not limiting (i.e., real-time monitoring of the raw water nitrate was not possible due to the maximum detection limit of the in-line analyzer).
- The DBPFP tests were conservative, as the final residual chlorine in the incubated raw water was $> 3.5 \, mg/L$, while the biofilter effluent samples had approximately 2 mg/L residual chlorine.

6.3 Challenge Testing

To simulate some of the challenges a full-scale system may encounter and to evaluate the system responses to those challenges, a series of tests was performed after steady-state performance demonstration. The results of these tests are summarized below.
6.3.1 Effects of Bioreactor Backwash

During backwashing, portions of biomass are removed from the bioreactor. To evaluate whether the biotta™ system requires a re-acclimation or filter-to-waste period, high resolution samples were collected for VOCs. The inline nitrate data were used to evaluate any effect of the backwash on nitrate removal performance.

Figure 35 shows nitrate plus nitrite before and after the bioreactor backwash. Figures 36 through 43 present VOC concentrations immediately before and after the bioreactor backwash. Nitrate and VOC removal performance was not affected by the bioreactor backwash, suggesting that a re-acclimation/filter-to-waste is not required after a backwash.

Figure 35: Nitrate + Nitrite Concentrations before and after Backwashing the Bioreactor
Figure 36: PCE Concentrations before and after Backwashing the Bioreactor

Figure 37: TCE Concentrations before and after Backwashing the Bioreactor
Figure 38: 1,1-DCE Concentrations before and after Backwashing the Bioreactor

Figure 39: trans-1,2-DCE Concentrations before and after Backwashing the Bioreactor
Figure 40: cis-1,2-DCE Concentrations before and after Backwashing the Bioreactor

Figure 41: 1,2,3-TCP Concentrations before and after Backwashing the Bioreactor
6.3.1.1 Chemical Feed Failure

To simulate a full-scale chemical dosing failure, the acetic acid feed was switched off for a 24-hour period. Once the system performance was re-established after the resumption of the acetic acid feed system, the phosphoric acid feed was switched off for a 24-hour period. Other design criteria remained identical with steady-state operation conditions during the feed failure testing.
Figure 44 shows the final effluent nitrate concentrations during this challenge test. As seen in the Figure, the acetic acid feed failure affects nitrate removal, but the system recovers quickly after the resumption of chemical feed operation. The impact of the absence of phosphoric acid feed failure on nitrate removal performance was minimal.

<table>
<thead>
<tr>
<th></th>
<th>H₃PO₄</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 44: Effects of Chemical Feed Failure on Nitrate Removal

VOCs were not monitored during the chemical feed failure testing, but results from the periods of accidental acetic acid feed failure suggest that VOC removal across the system is not affected by the short absence of acetic acid.

6.3.1.2 Process Shutdown

The pilot system was completely shut down for a 72-hour period to evaluate the effect of short-term system failure conditions. In addition, the system was completely shut down for approximately one month due to mechanical issues. In both cases, the bioreactor was backwashed before restarting the system. Figures 45 and 46 show nitrate removal immediately after the system restart following the shutdowns.
Figure 45: Three-Day System Shutdown Test. The Average Raw Water Nitrate Concentration was 190 mg/L as NO$_3^-$

Figure 46: Nitrate Concentrations in the Final Effluent before and after a 35-day System Shutdown. The Average Raw Water Nitrate Concentration was 190 mg/L as NO$_3^-$
Figures 47 through 54 present the VOC concentrations across the system during the acetic acid feed failure system shutdown testing.

**Figure 47:** PCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown

**Figure 48:** TCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown
Figure 49: 1,1-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown

Figure 50: cis-1,2-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown
Figure 51: trans-1,2-DCE Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown

Figure 52: 1,2,3-TCP Concentrations across the System during the Resumption of System Operation after the 3-day Shutdown
Based on Figures 47 through 54, it can be concluded that nitrate and VOC removal were not affected by a short-term shutdown. Nitrate and VOC removal performance is immediately re-established even after 30 days of system shutdown.
6.4 VOC Leaching Test

Table 9 presents the results of the VOC leaching test. The results show that <1% of the VOC mass removed by the pilot system was measured on the surface of the GAC. However, subsequent leaching tests using exhausted, non-biological GAC from a contactor removing VOCs from groundwater, showed that <1% of the VOC mass removed was measured on the surface of the GAC. Therefore, it was determined that the standard VOC leaching test is not valid for quantifying the mass of adsorbed VOCs.

### Table 9: Results of VOC Leaching Test

<table>
<thead>
<tr>
<th></th>
<th>Total mass applied to system (µg)</th>
<th>Total mass removed across bioreactor (µg)</th>
<th>Percent removal across bioreactor</th>
<th>Total mass adsorbed on to the bioreactor media (µg)</th>
<th>Percent adsorbed (of mass removed)</th>
<th>Percent removed by non-adsorptive mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1-DCE</td>
<td>38,039,110</td>
<td>29,442,939</td>
<td>77.4%</td>
<td>105264.4</td>
<td>0.358%</td>
<td>99.6%</td>
</tr>
<tr>
<td>1,2,3-TCP</td>
<td>55,385,209</td>
<td>52,870,461</td>
<td>95.5%</td>
<td>46259.1</td>
<td>0.087%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>137,049,218</td>
<td>78,835,814</td>
<td>57.5%</td>
<td>600037.3</td>
<td>0.761%</td>
<td>99.2%</td>
</tr>
<tr>
<td>cis DCE</td>
<td>86,556,026</td>
<td>60,512,077</td>
<td>69.9%</td>
<td>258917.9</td>
<td>0.428%</td>
<td>99.6%</td>
</tr>
<tr>
<td>TCE</td>
<td>1,660,362,389</td>
<td>1,575,910,415</td>
<td>94.9%</td>
<td>1652348.9</td>
<td>0.105%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Freon-11</td>
<td>30,290,227</td>
<td>26,901,130</td>
<td>88.8%</td>
<td>142804.2</td>
<td>0.531%</td>
<td>99.5%</td>
</tr>
<tr>
<td>VC</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
<td>0.0</td>
<td>0.000%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

6.5 Microbial Community

Figure 55 shows the phylum-level distribution of the bacteria identified through the 16s rRNA gene sequencing. Figure 56 shows the most abundant (> 1 percent) bacterial genera present in the bioreactor and biofilter. In some cases, the genera could not be identified and the respective unclassified family names are presented.
The following observations can be made:

- **Proteobacteria** dominated the microbial community in both bioreactor and biofilter;

- The bioreactor had less bacterial diversity;

- Bacteria from the *Comamonadaceae* family dominated the bacterial community in the bioreactor;

- Bacteria from the *Zoogloea* genus and the *Rhodocyclaceae* family dominated the bacterial community in the biofilter; and
• Bacteria from Agrobacterium genus and unidentified bacteria from Rhizobiaceae family were present at considerable abundance in both bioreactor and biofilter.

Other bacterial genera from the Comamonadaceae family detected (relative abundance <1 percent) in the bioreactor included Acidovorax, Comamonas, Delfia, Diaphorobacter, Methylibium. These bacteria have been suggested to be able to reduce many environmental contaminants including nitrate. Given the very high level of nitrate present in the raw water and its removal in the bioreactor, it is not surprising that the bacteria related to Comamonadaceae family dominated the bioreactor.

Bacteria from Agrobacterium genus and Rhizobiaceae family were present in the bioreactor and biofilter. Similarly, bacteria related to Nocardiodaceae family were also identified in the bioreactor, even though in low abundance. Agrobacterium genus is one of the genera included in Rhizobiaceae family and members of this genus are capable of degrading chlorinated solvents, such as 2,3-Dichloro-1-Propanol and 2-chloropropionic acid (Effendi et al. 2000). Similarly, Nocardiodaceae family contains members that can grow on vinyl chloride (Coleman et al., 2002). Other bacteria related to previously described genera from VOC contaminated sites, including Geobacter, Desulfovibrio, Burkholderia, and Methyloceae family were also detected in the bioreactor, even though the relative abundances were low.

Bacteria related to Dehalococcoides genus, which includes bacterial species with the capability of complete PCE degradation to CO₂ were not identified in the system. However, It is to be noted that the description of VOC-degrading capacity of Dehalococcoides is based on its culturability (i.e., Dehalococcoides species have been successfully grown in laboratory cultures). Given that a diverse group of microorganisms exist in natural environments and less than one percent has been described through laboratory incubations, it is likely that many other bacterial genera are capable of degrading VOCs. The presence of bacteria related to genera and families reported to contain bacteria capable of VOC degradation suggest that biological VOC removal was taking place in the bioreactor and biofilter. It is also likely that many other bacterial groups present in the system were involved in VOC removal either through catabolic or co-metabolic pathways.

6.6 Bench-scale Testing

Clearly visible biomass growth was observed in the bench-scale, sand-based bioreactor. In addition, nitrogen bubbles were regularly released from the bed, suggesting significant denitrification occurring in the system. Figures 57 through 61 present VOC concentrations across the sand-based bench-scale bioreactor. The effluent VOC concentrations were appreciably lower than the influent VOC concentrations. Since sand is inert, these data strongly suggest the occurrence of a biological VOC removal mechanism.
Figure 57: Influent and Effluent PCE Concentrations in the Sand-based Bench-scale Bioreactor

Figure 58: Influent and Effluent TCE Concentrations in the Sand-based Bench-scale Bioreactor
Figure 59: Influent and Effluent 1,1-DCE Concentrations in the Sand-based Bench-scale Bioreactor

Figure 60: Influent and Effluent cis-1,2-DCE Concentrations in the Sand-based Bench-scale Bioreactor
7.0 PILOT SITE DEMOBILIZATION

Once pilot testing was complete, the system was taken offline, and the bioreactor, aeration tank, and biofilter were drained. The media was removed from the bioreactor and the biofilter, and discarded. The reactors and accessible pipes were flushed with water from Well 18 to remove biomass and media. All reactors, piping, analyzers, and tanks were drained and air-dried. The backwash water storage tanks and finished water tanks were flushed multiple times to remove any dirt and biomass settled in the tanks. All excess chemical solutions were disposed of in accordance with the EPA’s guidelines and the chemical tanks were cleaned thoroughly. The flexible tubing used for the chemical feed system was removed and discarded. Arrangements were made for the pick-up and disposal of the remaining bulk chemical stocks in accordance with EPA’s guidelines.

The external piping from the well to the trailer and from the trailer to the discharge basin was removed and discarded. The Well 18 video-tube that was used for the submersible pump was capped. The submersible pump and all accessory equipment were left in place for use by the Inland Empire Utilities Agency and the Chino Basin Desalter Authority. All the equipment manuals were provided to CDA staff. The piping and emergency shower were removed and secured within the pilot skid for future use. The utility water line was shut off, and the end of the line was capped above grade. Finally, the electrical supply to trailer was disconnected and arrangements were made for the transport of the pilot skid to Salt Lake City, Utah for storage and maintenance.

Figure 6.1: Influent and Effluent 1,2,3-TCP Concentrations in the Sand-based Bench-scale Bioreactor
8.0 CONCLUSIONS

The two-stage, fixed-bed biotreatment system (biotta™) effectively removed nitrate and multiple VOCs over a sustained period. The following overall conclusions can be made:

- The system can efficiently remove high concentrations of raw water nitrate;
- Nitrate removal to < 5 mg/L as NO₃⁻ can be achieved in the bioreactor at an EBCT as low as 12 minutes;
- Nitrite does not accumulate in the system;
- Sustained and consistent VOC removal was observed across the system;
- Bacteria related to previously described species from VOC contaminated environments were observed in the system;
- The occurrence of a biological VOC removal mechanism is supported by 1) VOC removal across the sand-based, bench-scale system and 2) minimal to no breakthrough of VOCs over the entire testing period (>40,000 bed volumes treated);
- Backwashing the bioreactor did not affect the contaminant removal performance;
- Chemical feed failure has minimal effect on nitrate removal performance, which can be immediately re-established after the resumption of the chemical feed;
- Chemical feed failure did not affect VOC removal;
- System shut-downs (tested up to one month) did not affect system performance;
- DBP formation potential increased across the system, likely caused by the excess acetic acid dosing requirement;
- CTs as low as 4 mg-min/L resulted in complete microbial inactivation;
- The backwash wastewater contains low levels of COD, TSS, and TDS, allowing disposal of the wastewater in regular sewer, if available.

Overall, biotta™ can provide an effective treatment option for the simultaneous removal of nitrate and VOCs. A VOC treatment performance report, which combines four projects including the CDA Well 18 project, is being prepared for submission to DDW's Treatment Technology Unit. The goal is to receive approval for using two-stage, fixed-bed biotreatment for removing VOCs from drinking water. Submission of the report is scheduled for December 2015.
CUCAMONGA VALLEY WATER DISTRICT

biotta™ Pilot Testing For Nitrate and DBCP Removal

FINAL REPORT

March 2015
CUCAMONGA VALLEY WATER DISTRICT

*biotita™* Pilot Testing For Nitrate and DBCP Removal

FINAL REPORT

**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>EXECUTIVE SUMMARY</td>
<td>1</td>
</tr>
<tr>
<td>2.0</td>
<td>BACKGROUND</td>
<td>1</td>
</tr>
<tr>
<td>3.0</td>
<td>PROJECT OBJECTIVES</td>
<td>2</td>
</tr>
<tr>
<td>4.0</td>
<td>RAW WATER QUALITY</td>
<td>2</td>
</tr>
<tr>
<td>5.0</td>
<td>TREATMENT OBJECTIVES</td>
<td>3</td>
</tr>
<tr>
<td>6.0</td>
<td>PILOT TESTING</td>
<td>3</td>
</tr>
<tr>
<td>6.1</td>
<td>Process Flow and Pilot System Configuration</td>
<td>3</td>
</tr>
<tr>
<td>6.2</td>
<td>Experimental Design</td>
<td>5</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Biological Acclimation and Optimization</td>
<td>5</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Steady-state Operation</td>
<td>5</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Robustness Characterization</td>
<td>6</td>
</tr>
<tr>
<td>7.0</td>
<td>RESULTS</td>
<td>7</td>
</tr>
<tr>
<td>7.1</td>
<td>NITRATE REMOVAL</td>
<td>7</td>
</tr>
<tr>
<td>7.1.1</td>
<td>Biological Acclimation and Optimization</td>
<td>7</td>
</tr>
<tr>
<td>7.1.2</td>
<td>Steady-State Operation</td>
<td>14</td>
</tr>
<tr>
<td>7.1.3</td>
<td>Robustness Demonstration</td>
<td>19</td>
</tr>
<tr>
<td>7.1.4</td>
<td>Microbial Community Characterization</td>
<td>22</td>
</tr>
<tr>
<td>7.1.5</td>
<td>Additional Testing</td>
<td>23</td>
</tr>
<tr>
<td>7.2</td>
<td>DBCP REMOVAL</td>
<td>28</td>
</tr>
<tr>
<td>7.2.1</td>
<td>Experimental Details</td>
<td>28</td>
</tr>
<tr>
<td>7.3</td>
<td>DBCP Removal in the Pilot System</td>
<td>28</td>
</tr>
<tr>
<td>7.3.1</td>
<td>DBCP Removal with Fresh GAC-1</td>
<td>28</td>
</tr>
<tr>
<td>7.3.2</td>
<td>DBCP Removal with Fresh GAC-2</td>
<td>29</td>
</tr>
<tr>
<td>7.3.3</td>
<td>DBCP Removal with Pre-Loaded GAC (GAC-3)</td>
<td>29</td>
</tr>
<tr>
<td>7.3.4</td>
<td>DBCP Removal with GAC-4</td>
<td>29</td>
</tr>
<tr>
<td>7.4</td>
<td>Bench-Scale Column Testing</td>
<td>30</td>
</tr>
<tr>
<td>7.4.1</td>
<td>Testing with Virgin GAC-1</td>
<td>30</td>
</tr>
<tr>
<td>7.4.2</td>
<td>Testing with Sand</td>
<td>30</td>
</tr>
<tr>
<td>7.5</td>
<td>Leaching Procedure Tests</td>
<td>30</td>
</tr>
<tr>
<td>7.6</td>
<td>Batch Tests</td>
<td>31</td>
</tr>
<tr>
<td>7.6.1</td>
<td>Batch Test 1</td>
<td>31</td>
</tr>
<tr>
<td>7.6.2</td>
<td>Batch Test 2</td>
<td>32</td>
</tr>
</tbody>
</table>
8.0 RESULTS .................................................................................................................. 34
8.1 DBCP Removal in the Pilot System ............................................................................. 34
  8.1.1 DBCP Removal with Fresh GAC-1 and GAC-2 .................................................. 34
  8.1.2 DBCP Removal with Pre-Loaded GAC ............................................................... 34
  8.1.3 DBCP Removal with Fresh GAC-4 ..................................................................... 35
8.2 Bench-Scale Column Testing .................................................................................... 38
  8.2.1 Testing with Virgin GAC-1 ................................................................................. 38
  8.2.2 Testing with Sand ............................................................................................... 39
8.3 Leaching Procedure Tests ......................................................................................... 40
8.4 Batch Tests .............................................................................................................. 41
  8.4.1 Batch Test 1 ....................................................................................................... 41
  6.4.2 Batch Test 2 ..................................................................................................... 43
9.0 CONCLUSIONS ......................................................................................................... 45
LIST OF TABLES

Table 1: 2000-2012 Water Quality – Reservoir 2A Well field .................................................. 3
Table 2: Details of GACs used in the Study .............................................................................. 7
Table 3: Water Quality Profiles across the biottta™ Pilot System during Steady-State Operation.. 19
Table 4: Microbial Community Present in the Bioreactor .......................................................... 22
Table 5: Microbial Community Present in the Biofilter .............................................................. 23
Table 6: Bioreactor Backwash Wastewater Characteristics ...................................................... 24
Table 7: Biofilter Backwash Wastewater Characteristics ........................................................... 24
Table 8: Disinfection Tests with the Raw Water Samples ............................................................ 24
Table 9: Disinfection Tests with Biofilter Effluent Samples ......................................................... 25
Table 10: Second Batch Test: Sampling Matrix ......................................................................... 33

LIST OF FIGURES

Figure 1: biottta™ System Process Flow .................................................................................. 2
Figure 2: biottta™ Pilot Testing Skid ......................................................................................... 4
Figure 3: Pilot Process Flow Diagram ....................................................................................... 5
Figure 4: Initial Biological Acclimation of the biottta™ System (grab sample data shown since the in-line nitrate analyzer was not working properly during some of this period) .............. 8
Figure 5: Biological Acclimation of the biottta™ Pilot System when GAC-1 Was Replaced with GAC-2. ....................................................................................................................... 8
Figure 6: Biological Acclimation of the biottta™ Pilot System when GAC-2 Was Replaced with GAC-3 (Pre-exhausted GAC from Well 19 contactor). ................................................................. 9
Figure 7: Biological Acclimation of the biottta™ Pilot System when GAC-3 Was Replaced with GAC-2. ............................................................................................................................ 9
Figure 8: Biological Acclimation of the biottta™ Pilot System when GAC-2 Was Replaced with GAC-4. .......................................................................................................................... 10
Figure 9: Nitrate Removal during EBCT Optimization ................................................................. 11
Figure 10: Nitrate Concentration Profile along the Depth of the Bioreactor .............................. 11
Figure 11: Nitrate Removal during Phosphoric Acid Dose Optimization .................................. 12
Figure 12: Headloss in the Bioreactor and the Biofilter during Phosphoric Acid Dose Optimization. ........................................................................................................................................ 13
Figure 13: Nitrate Removal across the System during Steady-State Operation. ....................... 14
Figure 14: Nitrite Concentrations across the System during Steady-State Operation............... 15
Figure 15: Comparison of Raw Water Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab. .................................................................................................................. 15
Figure 16: Comparison of the Bioreactor Effluent Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab. .................................................................................................. 16
Figure 17: Comparison of the Biofilter Effluent Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab. ............................................................................................. 16
Figure 18: DO Concentrations across the System during Steady-state Operation. .................... 17
Figure 19: Final Effluent Turbidity during Steady-state Operation. .......................................... 18
Figure 20: Nitrate Concentrations before and after Backwashing the Bioreactor. Time 0 represents the time of the backwashing event. Results are average values for duplicate samples. .................................................................................................................................................. 20
Figure 21: Nitrite Concentrations before and after Backwashing the Bioreactor. Time 0 represents the time of the backwashing event. Results are average values for duplicate samples. ......................................................................................................................................... 21
Figure 22: Effects of Chemical Feed Failure on Nitrate Removal. ........................................... 21
Figure 23: Five-Day System Shutdown Test. ................................................................. 22
Figure 24: TTHM Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples. ................................................................. 26
Figure 25: HAA5 Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples. ................................................................. 26
Figure 26: Residual Chlorine in the Chlorinated Water Samples. .................................... 27
Figure 27: pH in the Chlorinated Water Samples. .......................................................... 27
Figure 28: DBCP Removal in the Pilot System with GAC-1 and GAC-2 ............................. 34
Figure 29: DBCP Concentrations in the Raw Water, Bioreactor Effluent, and Biofilter Effluent Using Pre-Loaded GAC ................................................................. 35
Figure 31: DBCP Removal along the Depth of the Beds. ............................................. 36
Figure 32: Effects of Backwashing the Bioreactor: DBCP Concentrations before and after Backwashing the Bioreactor. Time 0 represents the backwashing event. Results are average values for duplicate samples. ................................................................. 37
Figure 34: DBCP Concentrations in the Raw Water, Bench-scale Bioreactor Influent, and Bench-Scale Bioreactor Effluent. ................................................................. 39
Figure 35: DBCP Removal in the Bench-Scale, Sand-Based Bioreactor ............................. 40
Figure 36: Batch Test 1 Nitrate Removal Performance. The error bars represent one standard deviation. ........................................................................................................... 41
Figure 37: Batch Test 1 DBCP Removal Performance. The error bars represent one standard deviation. ........................................................................................................... 42
Figure 38: Nitrate Concentrations in the Control and Bioreactor Bottles (Batch Test 2). ........ 43
Figure 39: DBCP Concentrations in the Control and Bioreactor Bottles (Batch Test 2). ........ 44
1.0 EXECUTIVE SUMMARY

Cucamonga Valley Water District’s (District’s) Reservoir 2A well field is contaminated with nitrate and 1,2-dibromo-3-chloropropane (DBCP) above their respective maximum contaminant levels (MCLs). A 17-month pilot study was conducted at Well 12 using a two-stage, fixed-bed biological treatment system (biotta™) to evaluate the simultaneous removal of nitrate and DBCP. Sustained removal of both contaminants was achieved at empty bed contact times (EBCT) as low as 5 minutes and the system was robust with respect to multiple fluctuations in raw water quality and operating conditions. Multiple DBCP removal mechanism tests were performed, which taken together, suggest that bacteria indigenous to the District’s Well 12 groundwater are capable of degrading DBCP, thus allowing for the efficient, simultaneous removal of nitrate and DBCP across the two-stage fixed-bed biotreatment system.

2.0 BACKGROUND

The District’s Reservoir 2A well field is contaminated with nitrate and DBCP above their respective maximum contaminant levels (MCLs). Removing nitrate and DBCP from the groundwater will increase the District’s blending and overall production capacity. Conventional processes for treating nitrate include ion exchange (IX) and reverse osmosis (RO), while VOCs, including DBCP, are typically treated using granular activated carbon (GAC), air stripping, RO, or advanced oxidation processes (AOPs). Of these processes, only RO can remove both nitrate and DBCP in a single step. However, the use of an RO process is not feasible, as RO generates a high strength brine waste, for which the District does not have an easy treatment/handling option.

Biological processes offer a different approach for removing nitrate and VOCs, including DBCP from groundwater in a single step. Fixed-bed biological processes use a stationary bed of media such as sand, plastic, or GAC on which biofilms develop. Water is drawn from a well, amended with an electron donor (acetic acid) and phosphorus, and then pumped across the media bed. Under the appropriate conditions, these bacteria will convert nitrate and DBCP to innocuous by-products, such as chloride, N₂ gas, water, and carbon dioxide.

The biotta™ system offers an efficient and robust approach for degrading nitrate and DBCP to harmless end-products using bacteria indigenous to the local groundwater. biotta™ uses two fixed-bed biological reactors in series and includes specialized monitoring and chemical dosing algorithms, tailored media selection and configuration, and multiple biomass control tools (Figure 1).
3.0 PROJECT OBJECTIVES

The Division of Drinking Water (DDW) has conditionally approved the two-stage fixed-bed system for nitrate and perchlorate treatment. The approval requires that the system undergo site-specific pilot testing, and the approval does not yet apply to DBCP. Therefore, the objectives of this project were to:

- Confirm anticipated full-scale biotta™ design criteria for the removal of nitrate;
- Apply for and receive conditional DDW approval for using biotta™ to remove DBCP from drinking water;
- Demonstrate the stability of the system under forced operating disturbances; and
- Familiarize District staff with system equipment and operation.

4.0 RAW WATER QUALITY

Pilot testing was performed at Well 12 within the District’s Reservoir 2A well field. Historical water quality data (2000-2012) for all five Reservoir 2A wells are provided in Table 1 below.
Table 1: 2000-2012 Water Quality – Reservoir 2A Well field

<table>
<thead>
<tr>
<th></th>
<th>Well 8 NO₃⁻ (mg/L)</th>
<th>Well 8 DBCP (µg/L)</th>
<th>Well 10 NO₃⁻ (mg/L)</th>
<th>Well 10 DBCP (µg/L)</th>
<th>Well 12 NO₃⁻ (mg/L)</th>
<th>Well 12 DBCP (µg/L)</th>
<th>Well 20 NO₃⁻ (mg/L)</th>
<th>Well 20 DBCP (µg/L)</th>
<th>Well 22 NO₃⁻ (mg/L)</th>
<th>Well 22 DBCP (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ave</td>
<td>38</td>
<td>0.076</td>
<td>45</td>
<td>0.155</td>
<td>40</td>
<td>0.234</td>
<td>70</td>
<td>0.841</td>
<td>33</td>
<td>0.205</td>
</tr>
<tr>
<td>Min</td>
<td>11</td>
<td>0.000</td>
<td>25</td>
<td>0.000</td>
<td>28</td>
<td>0.000</td>
<td>26</td>
<td>0.000</td>
<td>16</td>
<td>0.000</td>
</tr>
<tr>
<td>Max</td>
<td>89</td>
<td>0.350</td>
<td>82</td>
<td>0.580</td>
<td>71</td>
<td>0.830</td>
<td>90</td>
<td>1.400</td>
<td>55</td>
<td>0.690</td>
</tr>
<tr>
<td>50th %</td>
<td>34</td>
<td>0.031</td>
<td>42</td>
<td>0.110</td>
<td>37</td>
<td>0.200</td>
<td>71</td>
<td>0.849</td>
<td>32</td>
<td>0.170</td>
</tr>
<tr>
<td>98th %</td>
<td>88</td>
<td>0.260</td>
<td>77</td>
<td>0.459</td>
<td>65</td>
<td>0.652</td>
<td>86</td>
<td>1.284</td>
<td>49</td>
<td>0.570</td>
</tr>
</tbody>
</table>

5.0 TREATMENT OBJECTIVES

The following water quality goals were targeted for system effluent throughout the pilot study:

- Nitrate as NO₃⁻ ≤ 5 mg/L;
- Nitrite as NO₂⁻ ≤ 0.02 mg/L;
- DBCP ≤ 0.01 µg/L;
- Turbidity < 0.3 NTU, 95% of samples monitored continuously;
- Dissolved oxygen (DO) ≥ 4 mg/L;
- HPCs < 500 cfu/mL with the application of 4-log virus disinfection CT.
- Total coliforms = not-detected with the application of 4-log virus disinfection CT.

6.0 PILOT TESTING

6.1 Process Flow and Pilot System Configuration

Figures 2 and 3 show a 3-D model and process flow diagram of the biotta™ pilot skid, respectively. The pilot-skid operates between 0.3-3.5 gpm and contains three 8” diameter columns that are 8’ tall and contained in a 20’x8’x9.5’ trailer. The first column serves as the bioreactor. The second column serves as an open basin, which could be used for flocculation or degasification. The third column is a polishing biofilter that removes turbidity and any
residual organic carbon in the effluent of the bioreactor. The skid is equipped with automatic backwash capabilities, chemical feed systems for electron donor, nutrient, polymer, and hydrogen peroxide. The skid also includes in-line monitoring and data logging for flow rate, headloss, DO, nitrate, and turbidity. A human-machine interface can be used on site or remotely to monitor and control pilot operations.

Raw water was pumped to the pilot feed/overflow line, which was then pumped through the pilot plant. Effluent from the bioreactor can travel directly to the biofilter or to an interstage degasification column ahead of the biofilter. Effluent from the biofilter was pumped into a backwash tank, which overflows to the storm drain canal discharge point. Backwash wastewater was discharged to a 1,500-gallon tank on site, which was periodically vacuumed out by a water vactor truck for sewer discharge. Initially, the bioreactor and biofilter were packed with GAC-1 (effective size 1.3 - 1.5 mm) and GAC-2 (effective size 1 mm), respectively. Different GACs were tested during the study, including DBCP-exhausted GAC from the District’s Well 19 GAC contactor. The majority of the testing was conducted with 48” of GAC in the bioreactor and 36” of GAC plus 12” of sand in the biofilter.

Figure 2: biottta™ Pilot Testing Skid.
6.2 Experimental Design

6.2.1 Biological Acclimation and Optimization

The objectives of this phase were to (1) establish the necessary biological activity in the system using organisms indigenous to Well 12 groundwater, and (2) determine optimal operating conditions for achieving all water quality goals. Initially, an EBCT of 10 minutes was used. The influent water was supplemented with acetic acid and phosphoric acid to enhance biological activity for nitrate and DBCP removal. Typical run times were 12 to 24 hours for the bioreactor and 48 hours for the biofilter. Stored system effluent was used to backwash the bioreactor and the biofilter. Process optimization included EBCT, chemical dosages, and backwash protocol.

6.2.2 Steady-state Operation

The objective of this phase was to demonstrate system stability and sustained contaminant removal using the optimum process parameters identified during the acclimation/optimization phase, except phosphoric acid dose (discussed below). The system was operated under steady state conditions for approximately four weeks. The following additional tests were also conducted during this phase.

Disinfection Testing. Disinfection tests were performed using biofilter effluent. Since biofilter effluent turbidity is highest shortly after a bioreactor backwash, one disinfection test
was run at the beginning of a bioreactor run. Another disinfection test was run near the end of a bioreactor run. In total, three disinfection tests were performed with bioreactor run start and bioreactor run end samples. For comparison, the disinfection demand of the raw water samples was also determined using two sets of raw water samples. Heterotrophic plate counts (HPCs), total Coliform, and \textit{E. coli} were measured in the chlorinated and non-chlorinated duplicate samples. A CT (i.e., concentration * contact time) of 2.5 mg-min/L was used.

\textit{Disinfection Byproduct Formation Potential Testing.} Similar to CT testing, disinfection byproduct formation potential (DBPFP) testing was conducted with the raw water and the final effluent. Final effluent samples were collected at the start and end of a bioreactor run. In total, three sets of run start samples and two sets of run end samples were tested. For comparison, two sets of raw water samples were tested to determine the background DBPFP.

DBPFP testing were conducted with free chlorine dose that would result in 3 to 5 mg/L of free chlorine after 7 days of incubation. The free chlorine dose also considered the initial chlorine demand of the samples. Duplicate samples for TTHMs and HAA5 were collected at 15 min, 30 min, 1 day, 3 days, and 7 days after the chlorine addition. Residual chlorine and pH were also determined in the samples at these time points.

\textit{Backwash Wastewater Characterization.} Four sets of bioreactor backwash wastewater composite samples and three sets of biofilter backwash wastewater composite samples were analyzed for total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), nitrate, nitrite, and DBCP.

\textit{Microbial Community Characterization.} Media samples were collected to characterize the microbial community in the bioreactor and biofilter. The samples were sent overnight on ice to The University of Texas, Austin. The media samples were subjected to DNA extraction and the 16S rRNA genes were sequenced using Miseq. All Miseq data were analyzed in QIIME (Caporaso et al., 2010) and the sequences were compared with RDP data base for taxonomic classification of the bacteria.

\textbf{6.2.3 Robustness Characterization}

The objective of this phase was to evaluate system performance during and after forced system disturbances. The optimum process parameters identified during the acclimation/optimization phase were used during this phase. Four disturbances were tested:

\textit{Backwashing.} To evaluate the effects of periodic biomass removal, high-resolution nitrate, nitrite, and DBCP samples were taken from 2 hours prior to 4 hours after a bioreactor backwash event. Samples were collected from the bioreactor and biofilter effluent.

\textit{Chemical Feed Failure.} The phosphoric acid feed was shut off for 24 hours and the acetic acid feed was shut off for 5 days. The system was allowed to recover after the phosphoric acid feed failure before starting the acetic acid feed failure test.
**Temporary System Shutdown.** The pilot system was shut down completely for 24-, 48-, and 120-hour periods.

**Higher Raw Water Contaminant Concentrations.** Since the highest background nitrate levels observed (87 mg/L) were close to the 2000-2012 max for the entire Reservoir 2A well field (90 mg/L), nitrate spiking tests were not performed. A one-week DBCP spiking test was performed using the 2000-2012 maximum DBCP concentration observed in Reservoir 2A wells (i.e., 1.4 µg/L).

### 7.0 RESULTS

#### 7.1 NITRATE REMOVAL

##### 7.1.1 Biological Acclimation and Optimization

The biottta™ pilot skid was mobilized in September 2013. After some initial mechanical troubleshooting, the pilot skid began formal operation on November 8, 2013. Figures 4 through 7 show different biological acclimation periods associated with the various virgin GACs evaluated during the study. The system was initially operated with GAC-1 as the media bed in the bioreactor. GAC-1 was replaced with GAC-2 on March 5, 2014 to evaluate performance with smaller GAC media. Since complete DBCP removal occurred throughout the system operating with both GAC-1 and GAC-2 (discussed in Section 7.2), GAC-2 was replaced with GAC-3 (DBCP-exhausted GAC from the District’s Well 19 GAC contactor) on April 9, 2014. GAC-3 was replaced with GAC-2 on May 16, 2014, which was replaced with GAC-4 on June 27, 2014. **Table 2** presents the details of the GACs used in the study.

<table>
<thead>
<tr>
<th>Table 2: Details of GACs used in the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
</tr>
<tr>
<td>GAC-1</td>
</tr>
<tr>
<td>GAC-2</td>
</tr>
<tr>
<td>GAC-3</td>
</tr>
<tr>
<td>GAC-4</td>
</tr>
</tbody>
</table>
Figure 4: Initial Biological Acclimation of the biotta™ System (grab sample data shown since the in-line nitrate analyzer was not working properly during some of this period).

Figure 5: Biological Acclimation of the biotta™ Pilot System when GAC-1 Was Replaced with GAC-2.
Figure 6: Biological Acclimation of the biottta™ Pilot System when GAC-2 Was Replaced with GAC-3 (Pre-exhausted GAC from Well 19 contactor).

Figure 7: Biological Acclimation of the biottta™ Pilot System when GAC-3 Was Replaced with GAC-2.
The following biological acclimation observations can be made:

- Complete biological acclimation typically occurred within 10 days of start-up with a new GAC; and
- GAC type did not affect the rate of biological acclimation.

Figure 9 shows nitrate removal across the pilot system during EBCT optimization. From March 22 through March 28, the system was shut down. From April 5 through 7, acetic acid feed limitation was experienced while optimizing the acetic acid feed dose. Figure 10 shows a nitrate profile along the depth of the bioreactor taken on July 7, 2014.
**Figure 9:** Nitrate Removal during EBCT Optimization

**Figure 10:** Nitrate Concentration Profile along the Depth of the Bioreactor.
The following observations can be made from the EBCT optimization tests:

- Sustained nitrate removal to below 5 mg/L was observed at an EBCT as low as 3 minutes;
- System performance was not affected by the 5-day system shutdown; and
- Nitrate removal performance correlated well with the acetic acid feed dose.

Nitrate removal during acetic acid optimization is presented in Figure 9. Figure 11 shows nitrate removal across the system during the optimization of phosphoric acid. Figure 12 shows headloss trends during phosphoric acid optimization.

![Figure 11: Nitrate Removal during Phosphoric Acid Dose Optimization.](image-url)
The following observations can be made:

- The acetic acid dose requirement was approximately equal to 0.9 to 1.2 mg/L for each mg/L of raw water nitrate;
- A phosphoric acid dose as low as 0.35 mg/L supported complete nitrate removal; and
- Phosphoric acid dose appeared to impact headloss across the bioreactor. This may be related to the production of extracellular polymeric substances (EPS), which are sticky and can be produced in high amounts under nutrient-limited conditions.

Based on these observations, a ratio of acetic acid to nitrate between 0.9 to 1.3 was used throughout the study. Because headloss accumulation can be a challenge in small-scale columns (8 inch diameter in this case), phosphoric acid was dosed at 1 mg/L throughout the study to ensure no nutrient limitation. A hydrogen peroxide dose of 10-13 mg/L resulted in >4 mg/L dissolved oxygen (DO) in the final effluent, and a polymer dose of 0.75 mg/L polymer successfully maintained turbidity well below 0.3 NTU in the final effluent.

Figure 12: Headloss in the Bioreactor and the Biofilter during Phosphoric Acid Dose Optimization.
7.1.2 Steady-State Operation

7.1.2.1 Nitrate/Nitrite

Based on optimization testing, the biottta™ pilot was operated for approximately 30 days under the following conditions:

- EBCT: 7 min
- Typical acetic acid to raw water nitrate ratio: 0.9 to 1.3
- Peroxide dose: 10-13 mg/L
- Polymer dose: 0.75 mg/L
- Phosphoric acid dose: 1.0 mg/L
- Bioreactor run time: 12-16 hours
- Biofilter run time: 48 hours
- Pilot operation mode: bypass (i.e., no degas step)

Figure 13 shows the nitrate concentrations in the raw water and the final effluent during steady-state operation. Nitrite measured in the grab samples during this period are shown in Figure 14. Figures 15, 16, and 17 compare the in-line and grab sample nitrate values for the raw water, bioreactor effluent, and biofilter effluent, respectively.

![Figure 13: Nitrate Removal across the System during Steady-State Operation.](image-url)
Figure 14: Nitrite Concentrations across the System during Steady-State Operation.

Figure 15: Comparison of Raw Water Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab.
Figure 16: Comparison of the Bioreactor Effluent Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab.

Figure 17: Comparison of the Biofilter Effluent Nitrate Concentrations Measured by the In-line Analyzer and the Local Lab.
Based on Figures 13 through 17, the following observations can be made:

- Sustained nitrate removal to well below the 5 mg/L target can be achieved using a 7-minute EBCT;
- Nitrite did not accumulate in the system; and
- In-line and grab sample nitrate/nitrite data matched well.

### 7.1.2.2 Dissolved Oxygen

Figure 18 shows the DO concentrations in the raw water and the final effluent. From November 4 through 12, mechanical problems with hydrogen peroxide feed system resulted in lower DO levels in the final effluent. Once the mechanical problems were fixed, the target effluent DO level was successfully maintained.

**Figure 18: DO Concentrations across the System during Steady-state Operation.**
7.1.2.3 Turbidity

Figure 19 shows the final effluent turbidity during steady-state operation. Turbidity remained well below the 0.3 NTU target. The brief turbidity peaks (pegged at 3.0 NTU) are associated with biofilter backwashes. A Hach 2100Q handheld turbidimeter was used to verify turbidity immediately after a backwash. The maximum turbidity recorded was 1.41 NTU, which dropped to <0.1 NTU within 8 minutes of operation. The difference between the in-line and grab sample turbidity values may be caused by air bubbles in the pilot sample lines, which can get trapped in the sample tubing during a backwash and are flushed out once the pilot is put back in service.

Figure 19: Final Effluent Turbidity during Steady-state Operation.
7.1.2.4 Other Water Quality Parameters

Table 3 shows other water quality parameters monitored during the steady-state operation phase.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Location</th>
<th>Average (mg/L)</th>
<th>Minimum (mg/L)</th>
<th>Maximum (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>Raw Water</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(n= 3)</td>
<td>Bioreactor Eff</td>
<td>1.0</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>DOC</td>
<td>Raw Water</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(n= 3)</td>
<td>Bioreactor Eff</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>Raw Water</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>(PO₄³⁻) (n= 3)</td>
<td>Bioreactor Eff</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Raw Water</td>
<td>47.7</td>
<td>46.0</td>
<td>49.0</td>
</tr>
<tr>
<td>(n= 3)</td>
<td>Bioreactor Eff</td>
<td>47.7</td>
<td>46.0</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>47.3</td>
<td>46.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Raw Water</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(n= 3)</td>
<td>Bioreactor Eff</td>
<td>0.004</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Biofilter Eff</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The following observations can be made:

- Total and dissolved organic carbon concentrations in the system effluent were low;
- As expected with a 1 mg/L phosphoric acid dose, phosphate increased slightly across the system; and
- Sulfate reduction was not observed, and no sulfide was detected in the effluent of the system.

7.1.3 Robustness Demonstration

7.1.3.1 Effect of Backwashing

The bioreactor and the biofilter were backwashed typically at 12-16 hours and 48 hours of run time, respectively. To evaluate the effect of backwashing, high resolution samples were
collected for nitrate and nitrite. Figures 20 and 21 show nitrate and nitrite, respectively, before and after the bioreactor backwash.

**Figure 20:** Nitrate Concentrations before and after Backwashing the Bioreactor. Time 0 represents the time of the backwashing event. Results are average values for duplicate samples.

**Figure 21:** Nitrite Concentrations before and after Backwashing the Bioreactor. Time 0 represents the time of the backwashing event. Results are average values for duplicate samples.
The data show that backwashing the bioreactor does not impact nitrate and nitrite removal performance.

### 7.1.3.2 Chemical Feed Failure

Figures 22 presents the results of the phosphoric acid and acetic acid feed failure tests.

#### Figure 22: Effects of Chemical Feed Failure on Nitrate Removal.

The following observations can be made:

- The phosphoric acid feed failure impacted nitrate removal performance but to a lesser extent than the acetic acid feed failure;
- The system recovers quickly after the resumption of chemical feed operation.

### 7.1.3.3 Process Shutdown

The pilot system was completely shut down on November 26, 2014 and restarted on December 1, 2014 (5-day shutdown). The bioreactor was backwashed before restarting the system. Figure 23 shows the nitrate concentrations in the raw water and the biofilter effluent when the system was restarted. The system shutdown did not affect the nitrate removal performance. From December 2 through 4, final effluent nitrate was closer to 5 mg/L due to an error in the preparation of the acetic acid stock solution. Nitrate removal improved immediately after restarting the system with the correct acetic acid stock.
Figure 23: Five-Day System Shutdown Test.

7.1.4 Microbial Community Characterization

Microbial community structure was determined through DNA extraction and Miseq 16S rRNA sequencing. Tables 4 and 5 present the microbial communities in the bioreactor and biofilter, respectively.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus*</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>Burkholderiales</td>
<td>Comamonadaceae</td>
<td>-</td>
<td>19.11</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.59</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>Burkholderiales</td>
<td>Comamonadaceae</td>
<td>others</td>
<td>12.45</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhizobiales</td>
<td>Rhizobiales</td>
<td>-</td>
<td>10.00</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>Rhodocyclales</td>
<td>Rhodocyclaceae</td>
<td>Rhodocyclus</td>
<td>8.24</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Xanthomonadales</td>
<td>Xanthomonadaceae</td>
<td>Lysobacter</td>
<td>6.58</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Flavobacteriia</td>
<td>Flavobacteriales</td>
<td>Flavobacteriaceae</td>
<td>Flavobacterium</td>
<td>6.51</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhizobiales</td>
<td>-</td>
<td>-</td>
<td>4.16</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Moraxellaceae</td>
<td>Acinetobacter</td>
<td>4.12</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>SBla14</td>
<td>-</td>
<td>-</td>
<td>2.51</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Sphingobacteriia</td>
<td>Sphingobacteriales</td>
<td>-</td>
<td>-</td>
<td>1.92</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhodobacterales</td>
<td>Rhodobacteraceae</td>
<td>Rhodobacter</td>
<td>1.69</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhizobiales</td>
<td>Hyphomicrobiaceae</td>
<td>-</td>
<td>1.15</td>
</tr>
</tbody>
</table>
As seen in Tables 4 and 5, more diverse bacterial populations were present in the biofilter compared to the bioreactor. Genera previously described from nitrate contaminated environments and identified in this system included *Dechloromonas, Azospirillum, Pseudomonas, Geobacter, Rhodocyclus, and Zoogloea*. Similarly, bacteria from the *Comamonadaceae* and *Rhodocyclaceae* have been reported to biodegrade nitrate. While bacterial isolates have not been described for the degradation of DBCP, it is likely that many of the bacteria present in the bioreactor and biofilter mediated DBCP-removal through co-metabolic or reductive dehalogenation.

### 7.1.5 Additional Testing

#### 7.1.5.1 Backwash Wastewater Characterization

Three and four sets of composite backwash wastewater samples were collected from the bioreactor and biofilter, respectively. The wastewater samples were analyzed for COD, nitrate, nitrite, TDS, and TSS. Each sample was taken in duplicate (A and B). The results are provided in Tables 6 and 7. In general, backwash wastewater exhibited typical municipal wastewater characteristics. COD ranged from 72-400 mg/L; TSS ranged from 48-290 mg/L; minimal nitrate, nitrite, and DBCP were detected.
### Table 6: Bioreactor Backwash Wastewater Characteristics

<table>
<thead>
<tr>
<th>Conc. (mg/L)</th>
<th>Samples from 10/10/2014</th>
<th>Samples from 10/15/2014</th>
<th>Samples from 10/29/2014</th>
<th>Avg. of all sets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sam A</td>
<td>Sam B</td>
<td>Avg</td>
<td>Sam A</td>
</tr>
<tr>
<td>COD</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>380</td>
</tr>
<tr>
<td>DBCP</td>
<td>0.05</td>
<td>0.049</td>
<td>0.050</td>
<td>0.081</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>2.1</td>
<td>2.3</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0.79</td>
<td>0.77</td>
<td>0.78</td>
<td>0</td>
</tr>
<tr>
<td>TSS</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>160</td>
</tr>
<tr>
<td>TDS</td>
<td>370</td>
<td>360</td>
<td>365</td>
<td>380</td>
</tr>
</tbody>
</table>

### Table 7: Biofilter Backwash Wastewater Characteristics

<table>
<thead>
<tr>
<th>Conc. (mg/L)</th>
<th>Samples from 10/16/2014</th>
<th>Samples from 10/30/2014</th>
<th>Samples from 12/05/2014</th>
<th>Samples from 12/12/2014</th>
<th>Avg. of all sets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sam A</td>
<td>Sam B</td>
<td>Avg</td>
<td>Sam A</td>
<td>Sam B</td>
</tr>
<tr>
<td>COD</td>
<td>340</td>
<td>380</td>
<td>360</td>
<td>68</td>
<td>76</td>
</tr>
<tr>
<td>DBCP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻-N</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TSS</td>
<td>140</td>
<td>350</td>
<td>245</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>TDS</td>
<td>350</td>
<td>350</td>
<td>350</td>
<td>340</td>
<td>340</td>
</tr>
</tbody>
</table>

### 7.1.5.2 Disinfection Testing

Disinfection tests were performed with the final effluent collected at the start and end of a bioreactor run. For comparison, disinfection tests were also performed with raw water samples. Tables 8 and 9 present the disinfection results for the raw water and biofilter effluent samples, respectively. Each sample was taken in duplicate (A and B). As shown in the tables, a CT of 2.5 mg-min/L was sufficient to achieve complete inactivation of HPCs and any coliform bacteria.

### Table 8: Disinfection Tests with the Raw Water Samples

<table>
<thead>
<tr>
<th></th>
<th>Raw Water</th>
<th>Raw Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples from 12/19/2014</td>
<td>Samples from 12/22/2014</td>
</tr>
<tr>
<td></td>
<td>Not Treated</td>
<td>Treated</td>
</tr>
<tr>
<td>Tot coli.</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>E. coli</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>HPC</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

Note: A – Absent, P – Present, ND – Not detected
7.1.5.3 Disinfection Byproduct Formation Potential

Disinfection byproduct formation potential tests were performed with the final effluent collected at the start and end of a bioreactor run. The samples were incubated with a target chlorine residual of 3 to 5 mg/L after 7 days of testing. The chlorine dose was adjusted based on the initial chlorine demand of the samples, which ranged from 0.25 to 0.75 mg/L. Two separate DBPFP tests were conducted, one on September 9, 2014 and one on December 19, 2014. Figures 24 and 25 show TTHMs and HAA5 formation, respectively, over the incubation period. Figures 26 and 27 show residual chlorine concentrations and pH of the samples at different time points.
Figure 24: TTHM Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples.

Figure 25: HAA5 Concentrations in the Chlorinated Water Samples. Each data point represents the average of duplicate samples.
Figure 26: Residual Chlorine in the Chlorinated Water Samples.

Figure 27: pH in the Chlorinated Water Samples.
The following observations can be made:

- Stable pH was observed throughout the incubation period;
- The final residual chlorine was more than 3.5 mg/L; and
- DBP formation potential in the system effluent was low.

7.2 DBCP REMOVAL

One of the primary objectives of pilot testing was to acquire conditional approval from DDW for the removal of DBCP.

7.2.1 Experimental Details

Multiple tests were performed to evaluate DBCP removal efficiency and to isolate the mechanism of DBCP removal:

1. Pilot testing with virgin GAC-1, virgin GAC-2, and virgin GAC-4;
2. Pilot testing with pre-exhausted coconut shell-based GAC (GAC-3) taken from the District’s Well 19 GAC contactor);
3. Bench-scale column testing with GAC-1 and sand, with and without acetic acid addition;
4. Leaching procedure tests with used GAC;
5. Batch studies using biomass collected from the pilot-scale bioreactor.

7.3 DBCP Removal in the Pilot System

7.3.1 DBCP Removal with Fresh GAC-1

The pilot operation officially started on November 8, 2013. The bioreactor (F210) was packed with virgin GAC-1 to achieve a bed depth of 5 ft. The biofilter (F220) was packed with 4 ft of virgin GAC-2 and 1 ft of sand to attain a total bed depth of 5 ft. Initially, the pilot system was operated with an EBCT of 10 min. Glacial acetic acid and phosphoric acid were dosed from dedicated chemical feed pumps. The pilot system was operated in ‘bypass mode’ and the bioreactor effluent was supplemented with hydrogen peroxide to re-oxygenate the water and enhance the growth of aerobic heterotrophs in the biofilter. Cationic polymer was also
dosed to the bioreactor effluent to facilitate turbidity removal across the biofilter. The bioreactor and biofilter were backwashed with treated effluent every 24 and 48 hours, respectively. Grab samples of raw water, bioreactor effluent, and biofilter effluent were collected approximately three times per week.

### 7.3.2 DBCP Removal with Fresh GAC-2

After operating the system for approximately five months using GAC-1 in the bioreactor, the GAC-1 was removed and replaced with GAC-2 (March 5, 2014). The purpose of this change-out was to evaluate hydraulics and nitrate/DBCP removal with a smaller GAC and a shallower bed depth (42” instead of 60”). Throughout the five months of pilot testing with GAC-1, no DBCP breakthrough was observed from the bioreactor or biofilter (discussed in the Results section). EBCT with the GAC-2 was set at 10 minutes, and the system was operated for approximately one month.

### 7.3.3 DBCP Removal with Pre-Loaded GAC (GAC-3)

DBCP breakthrough from the bioreactor using GAC was not observed (discussed in the Results section). In an effort to “find” DBCP breakthrough from the system, the GAC-2 in the bioreactor was replaced with “pre-loaded” Aquacarb 1230C GAC (i.e., GAC-3) that was collected on April 9, 2014 from the District’s full-scale contactor, which treats water from Well 19 (Reservoir 3A well field). The full-scale contactor had been loaded with virgin GAC-3 and operation began in early 2014 treating Well 19 raw water. The GAC was collected from the top of the contactor, and the District reported that water samples taken depthwise from the contactor on March 24, 2014 showed 0.14 µg/L DBCP at 25% bed depth and 0.018 µg/L at 50% bed depth. Therefore, it was concluded that GAC taken from the top of the contactor would contain a reasonable mass of adsorbed DBCP. The pilot system was operated for approximately one month with GAC-3.

### 7.3.4 DBCP Removal with GAC-4

GAC-3 was replaced with GAC-2 on May 16, 2014. After re-evaluating the GAC-2 for approximately 40 days, GAC-2 was replaced with GAC-4 on June 27, 2014. The system was operated with GAC-4 for approximately 7 months.
7.4 Bench-Scale Column Testing

7.4.1 Testing with Virgin GAC-1
A single-stage bench-scale system, consisting of a 2-inch glass column and packed with GAC-1, was operated in parallel with the pilot system from the beginning of the pilot-scale operation. The bed depth was maintained at 6 inches. Raw water was diverted into the bench-scale system using a peristaltic pump and the flow rate was adjusted so that the bench- and pilot-scale system EBCTs matched. Initially, no acetic acid or phosphoric acid was supplemented to the bench-scale system. Therefore, it was expected that biological growth and DBCP-removing bioactivity would not be established in the system, resulting in a higher mass of DBCP adsorbed to the GAC-1 than that adsorbed onto the pilot-scale GAC-1. It was also expected that DBCP might breakthrough from the bench-scale system after some time.

7.4.2 Testing with Sand
DBCP breakthrough across the bench-scale system was not observed during four months of operation without dosing acetic acid or phosphoric acid (discussed in the Results section). The GAC in the bench-scale system was replaced with sand to eliminate adsorption as a potential removal mechanism. The sand was washed thoroughly and loaded into the column to a 6-inch bed depth. Initially, the sand system was operated without any chemical addition. After demonstrating that no DBCP removal was taking place across the sand bed, acetic acid and phosphoric acid were fed to the bench-scale system using a peristaltic pump starting April 9, 2014. Doses matched those used for the pilot-scale system.

7.5 Leaching Procedure Tests
To determine the total mass of any adsorbed DBCP, GAC leaching procedure tests were performed. When a given GAC was changed out in the bioreactor (pilot- or bench-scale), the entire GAC bed was removed from the column, placed in a tub, and manually mixed to homogenize the media. Grab samples were then taken from two different areas of the mixed media, placed in 100 mL glass bottles with no headspace, and shipped on ice overnight to Eurofins Eaton Analytical, Inc., California. Leaching tests were performed to quantify any VOCs adsorbed on the GAC. Specifically, the mass of adsorbed DBCP was quantified in the samples using EPA method SW846 8260B. Purge-and-trap technique as per EPA method 5035 was used to introduce the samples into a GC/MS system.

Since no DBCP was detected from any of the GAC samples during the leaching procedure tests (discussed in the Results section), a second set of GAC samples, which had been in
storage at 4 °C since the GAC had been removed from a given column, was analyzed by Eurofins Eaton Analytical. This round of leaching tests included an additional methanol extraction step to enhance the release of any DBCP from the carbon. However, no DBCP was detected from any of the GAC samples even with the methanol extraction step. For laboratory comparison, another set of GAC samples (previously collected and stored at 4 °C) was shipped to a second laboratory (Advanced Environmental Laboratories, Florida) for leaching tests using the same method.

In summary, the GAC samples tested for DBCP leaching included:

1. GAC-1 from the biological pilot-scale system after approximately four months of operation;
2. GAC-1 from the bench-scale column after operating without acetic acid or phosphoric acid for approximately five months;
3. GAC-2 from the biological pilot-scale system after approximately one month of operation;
4. GAC-3 (Aquacarb 1230C) from the biological pilot-scale system after approximately one month of operation;
5. GAC-3 (Aquacarb 1230C) from the full-scale GAC contactor that showed DBCP breakthrough at a 25% bed depth.

### 7.6 Batch Tests

As another method to isolate the occurrence of biological DBCP degradation, two separate batch tests were conducted using biomass collected from the bioreactor during a backwash.

#### 7.6.1 Batch Test 1

On April 15, 2014, 2 L of bioreactor backwash waste was collected in a graduated cylinder and allowed to settle for approximately 2 hours. In a 2-L volumetric flask, approximately 1 L of raw water was collected, and acetic acid and phosphoric acid were added to achieve a final concentration of 70 mg/L and 1 mg/L, respectively. The flask was filled with additional raw water to adjust the volume to 2 L. After thoroughly mixing, the ‘prepared’ raw water was transferred to a 1-gallon HDPE jar (with an air-tight lid). To the ‘prepared’ water, 2 mL of
DBCP (Sigma Aldrich) were added using DBCP ampules targeting a final concentration of 200 µg/L. The 1-gallon jar was capped and shaken vigorously for thorough mixing. Using a funnel, the DBCP-spiked water was immediately transferred into eight 65-mL serum bottles labeled ‘control’. The serum bottles were sealed immediately with Teflon-coated butyl rubber septa. No headspace was left in the serum bottles. Approximately 100 mL of the concentrated biomass (after settling) was mixed with the remaining DBCP-spiked raw water in the HDPE jar. The biomass-added DBCP-spiked raw water was mixed thoroughly to keep the biomass in suspension, and the liquid was then poured into twelve 65-mL serum bottles labeled ‘bioreactor’ without any headspace and sealed using Teflon-coated butyl rubber septa. The bottles were incubated at room temperature.

Eight control serum bottles and 12 bioreactor serum bottles allowed for four sampling events, as two control bottles and three bioreactor bottles were sacrificed per sampling event. Samples were taken at 1.5, 211, 355, and 715 hours. For a given sampling event, the set of bottles was stored at 4 °C until shipped overnight on ice to Eurofins Eaton Analytical, Inc.; for nitrate and DBCP analysis. Analysis for nitrate was conducted only if the holding time of a particular sample did not exceed 48 hours. EPA Method 504 was used for aqueous phase DBCP determination. Nitrate concentrations were determined using ion chromatography (EPA method 300). Based on the results from three time points (discussed in the Results section), the fourth set of samples was not analyzed.

### 7.6.2 Batch Test 2

Results from the first batch test indicated significant removal of DBCP at 1.5 hours but no appreciable removal after that (discussed in the Results section). In a biological system, DO and nitrate reduction reactions typically occur first. Given that many volatile organic compounds (VOCs) are degraded through co-metabolic pathways as secondary substrates, it was hypothesized that DBCP was removed as a co-substrate in the first batch test. DO and nitrate were consumed within the first 1.5 hours of incubation thereby facilitating DBCP degradation. Once the DO and nitrate were gone, no further DBCP removal would occur. To evaluate this hypothesis, a second batch test was started on June 27, which included nitrate and hydrogen peroxide (a potential source of DO) spiking part-way through the incubation period. The intent was to initiate a second DBCP co-metabolic degradation period by initiating a second DO and nitrate reduction phase in the serum bottle reactors.

A total of 3.5 L of raw water was prepared by adding acetic acid and phosphoric acid, targeting 70 mg/L and 1 mg/L, respectively. Graduated cylinders were used for volume measurements. Three ampules (3 mL) DBCP were added to the prepared raw water, targeting a final concentration of approximately 140 µg/L DBCP. The DBCP-spiked raw water was
immediately transferred into twelve 100 mL serum bottles: eight bottles labeled Control, two bottles labeled Nitrate-Spiked Control, and two bottles labeled H₂O₂-Spiked Control. Approximately 1 mL of headspace was left in the Nitrate- or H₂O₂-spiked control bottles. All the control bottles were immediately sealed with Teflon-coated butyl rubber septa and incubated at room temperature. Approximately 100 mL of concentrated biomass (collected using the same method as was used during the first batch test) was added to the remaining DBCP-spiked water. After suspending the biomass thoroughly by shaking, the biomass-added DBCP-spiked water was transferred into twelve serum bottles: eight bottles labeled Bioreactor, two bottles labeled Nitrate-Spiked Bioreactor, and two bottles labeled H₂O₂-Spiked Bioreactor. Approximately 1 mL headspace was left in the Nitrate- or H₂O₂-spiked bioreactors. The serum bottles were sealed immediately with Teflon-coated butyl rubber septa. All the serum bottles were stored at room temperature.

Thus, in addition to the regular ‘controls’ and ‘bioreactors’ described in Batch Test 1 above, nitrate- or H₂O₂-spiked controls were included to compare with the performance of nitrate- or H₂O₂-spiked bioreactors (see Table 10). Performance of nitrate- or H₂O₂-spiked bioreactors was compared with the regular controls and with nitrate- or H₂O₂-spiked controls.

Sampling events occurred at 0.5, 4, 72, and 126 hours. Immediately after collecting the samples at 72 hours, nitrate or hydrogen peroxide was injected into the respective spiked-controls and spiked-bioreactors targeting final concentrations of 25 mg/L nitrate-N and 10 mg/L peroxide. For each sampling event, two controls and two bioreactors were transferred to a 4 °C refrigerator until the samples were shipped overnight on ice to Eurofins Eaton Analytical. Nitrate samples that passed the 48 hours holding time were not analyzed.

### Table 10: Second Batch Test: Sampling Matrix

<table>
<thead>
<tr>
<th>Sampling Time (hours)</th>
<th>Control</th>
<th>Bioreactor</th>
<th>Nit-spiked Con</th>
<th>Nit-spiked Bio</th>
<th>H₂O₂-spiked Con</th>
<th>H₂O₂-spiked Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>126</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
8.0 RESULTS

8.1 DBCP Removal in the Pilot System

8.1.1 DBCP Removal with Fresh GAC-1 and GAC-2

Figure 28 presents the DBCP concentrations in the raw water, the bioreactor effluent, and the biofilter effluent. The following observations can be made from the data:

- The raw water DBCP ranged from 0.12 to 0.4 µg/L;
- Regardless of the raw water DBCP concentration, consistent and complete DBCP removal (i.e., <0.010 µg/L DBCP in the effluent) was observed across the bioreactor with both GAC-1 and GAC-2;
- The replacement of GAC-1 by GAC-2 did not affect DBCP removal performance.

![Figure 28: DBCP Removal in the Pilot System with GAC-1 and GAC-2](image)

8.1.2 DBCP Removal with Pre-Loaded GAC

DBCP removal observed in the bioreactor might have been due to adsorption, biodegradation, or a combination of adsorption and biodegradation. In an effort to minimize any occurrence of adsorption, GAC-2 in the bioreactor was replaced with pre-loaded GAC taken from the top of the District’s Well 19 contactor (i.e., GAC-3). The contactor had experienced DBCP breakthrough at the 25% bed depth six weeks prior to the GAC sampling event. Therefore, it was concluded that the extent of DBCP removal observed in the pilot system would require
some degree of biodegradation. Otherwise, breakthrough would be observed. Figure 29 presents the DBCP concentrations in the raw water, the bioreactor effluent, and the biofilter effluent using the pre-loaded Aquacarb 1230C (GAC-3). The following observations can be made from the data:

- The raw water DBCP ranged from 0.16 to 0.35 µg/L;
- The replacement of GAC-2 by pre-loaded coconut-shell-based GAC (i.e., GAC-3) did not affect DBCP removal performance;
- DBCP breakthrough from the pilot system was not detected even after operating for more than one month.

![Figure 29: DBCP Concentrations in the Raw Water, Bioreactor Effluent, and Biofilter Effluent Using Pre-Loaded GAC](image)

8.1.3 DBCP Removal with Fresh GAC-4

GAC-3 was replaced with GAC-2 again on May 16, 2014. After evaluating the effectiveness of GAC-2, the media in the bioreactor was replaced with GAC-4 on June 27, 2014. Consistent removal of DBCP below the MRL (i.e., 0.01 µg/L) was observed with GAC-4 also (Figure 30).
Figure 30: DBCP Concentrations in the raw water, bioreactor effluent, and biofilter effluent when the pre-exhausted GAC-2 and GAC-4 were used as the filter media.

Overall, the pilot-scale system consistently demonstrated complete removal of DBCP. The majority of the DBCP removal in the pilot system took place in the bioreactor (Figure 31).

Figure 31: DBCP Removal along the Depth of the Beds.
8.1.3.1 Effect of Backwashing on DBCP Removal

To evaluate the effect of bioreactor backwashing on DBCP removal, high resolution DBCP grab samples were collected at predetermined time intervals between 2 hours prior to and 4 hours after the backwash. Figure 32 shows the DBCP concentrations in the raw water, bioreactor effluent, and biofilter effluent. DBCP removal was not affected by the bioreactor backwash and DBCP concentrations in the bioreactor and biofilter effluents remained below the MRL of 0.1 µg/L.

![Figure 32: Effects of Backwashing the Bioreactor: DBCP Concentrations before and after Backwashing the Bioreactor. Time 0 represents the backwashing event. Results are average values for duplicate samples.](image)

8.1.3.2 DBCP Spiking Test

A DBCP spiking test was conducted from January 22 through February 4, 2015. The raw water was spiked with 1.4 µg/L DBCP (calculated). Figure 33 presents DBCP concentrations in the raw water, bioreactor effluent, and biofilter effluent during this test.
The following observations can be made:

- The raw water concentrations ranged from 0.1 to 0.25 µg/L (average 0.18 µg/L);
- The DBCP spiking resulted in an average DBCP concentration of 0.93 µg/L (range 0.53 to 1.5 µg/L) at sampling port A in the bioreactor (i.e., immediately above the bed); and
- The effluent DBCP concentrations were well below the MRL.

8.2 Bench-Scale Column Testing

8.2.1 Testing with Virgin GAC-1

A single-stage bench-scale bioreactor was operated in parallel with the pilot-scale system without the addition of chemicals. With no substrate or nutrient, appreciable biological activity was not expected, thereby allowing 1) a greater mass of DBCP to accumulate on the GAC, and possibly 2) DBCP breakthrough from the system. Figure 34 presents the DBCP removal across the bench-scale GAC system. The following observations can be made from the data:
• DBCP concentrations in the bench-scale system influent closely matched the raw water DBCP concentrations measured in the pilot system influent;

• No DBCP breakthrough was detected;

![Figure 34: DBCP Concentrations in the Raw Water, Bench-scale Bioreactor Influent, and Bench-Scale Bioreactor Effluent.](image)

**8.2.2 Testing with Sand**

It is possible that low-level biological activity in the bench-scale system accounted for some of the DBCP removal observed. In fact, biomass was observed in the bench-scale bioreactor even though no acetic- or phosphoric- acid was being dosed to the system. To eliminate the potential for DBCP removal through adsorption, the GAC-1 in the bench-scale system was replaced with sand. No acetic- or phosphoric- acid was dosed to the system initially. Once it was shown that influent DBCP concentrations matched the effluent DBCP concentrations, acetic- and phosphoric- acid dosing was initiated. With the chemical dosing, visible biomass was observed in the sand-based, bench-scale system. Figure 35 shows DBCP concentrations in the raw water, bench-scale bioreactor influent, and bench-scale bioreactor effluent.
The following observations can be made from the data:

- For most samples, DBCP concentrations in the bench-scale system influent closely matched the raw water DBCP concentrations measured in the pilot system influent;
- DBCP removal did not occur through adsorption when sand was used; and
- After the addition of acetic- and phosphoric- acid, partial DBCP removal occurred across the sand bed, suggesting the presence of DBCP-degrading biological activity. Lower percent removals relative to the pilot-scale GAC-based bioreactor system may have been due to lower biomass concentrations on sand compared with GAC. It is also possible that adsorption and biodegradation are synergistic in the GAC-based bioreactor (e.g., adsorption may concentrate DBCP and allow for higher concentrations of DBCP-degraders to grow on the carbon).

### 8.3 Leaching Procedure Tests

Leaching test results from both Eurofins Eaton Analytical (California) and Advanced Environmental Laboratory (Florida) showed no DBCP (or other VOCs) in/on any of the GAC even when using the additional methanol extraction method. This included the GAC samples from the bench-scale system that had operated with no acetic acid or phosphoric acid for four
months as well as the pre-loaded GAC samples from the District’s Well 19 contactor. Furthermore, the known mass loading to the various GAC media (4-9 mg/kg media) was well above the method reporting limit for the DBCP leaching procedure method (0.02 mg/kg media). Consequently, though the biologically active GAC samples may not have contained any adsorbed DBCP, it was expected that the preloaded GAC from the District’s full-scale contactor would leach DBCP. Therefore, the only conclusion that can be drawn from the leaching tests is that DBCP is difficult to extract from GAC once adsorbed.

8.4 Batch Tests

8.4.1 Batch Test 1

Figures 36 and 37 present the results of the first batch test. Since DBCP removal was not observed beyond 211 hours, the samples from fourth data point were not analyzed.

![Figure 36: Batch Test 1 Nitrate Removal Performance. The error bars represent one standard deviation.](image)

**Figure 36:** Batch Test 1 Nitrate Removal Performance. The error bars represent one standard deviation.
The following observations can be made from the data:

- Comparing the control bottles with the bioreactor bottles (both of which used the same batch of “prepared” water), significant nitrate and DBCP removal was observed in the bioreactors within the first 1.5 hours;

- Complete nitrate degradation occurred in the bioreactor bottles between 1.5 hours and 211 hours;

- Some nitrate removal was observed in the control bottles. The experiment was not conducted under sterile conditions and visible microbial growth was observed in the control bottles by 211 hours;

- No DBCP removal was observed in any of the bottles after 1.5 hours.

As discussed in Section 7.6.2, it was hypothesized that DBCP may have been removed as a co-substrate during DO and/or nitrate reduction during the first 1.5 hours. To evaluate this further, a second batch test was conducted, which included nitrate and hydrogen peroxide (a potential source of DO) spiking.
6.4.2 Batch Test 2

Nitrate was not measured in samples collected from 0 and 4 hours. The average nitrate concentrations in the two controls collected at 72 hours was 11 mg/L as N (Figure 38), while nitrate in the bioreactor bottles was below detection (<0.2 mg/L as N). At 126 hours (i.e., after spiking nitrate at 72 hours), nitrate was not detected in control or bioreactor bottles. Nitrate was also not detected in the \( \text{H}_2\text{O}_2 \)-Spiked Bioreactors. The \( \text{H}_2\text{O}_2 \)-Spiked Controls were broken during shipping. The Nitrate-Spiked Controls had an average nitrate concentration of 38.5 mg/L N, while the Nitrate-Spiked Bioreactors showed an average nitrate level of 15 mg/L, indicating a second phase of nitrate reduction after 72 hours. Visible biomass was seen in all the bottles, including the controls at 126 hours, likely because the test was not performed under sterile conditions.

![Figure 38: Nitrate Concentrations in the Control and Bioreactor Bottles (Batch Test 2).](image-url)
Figure 39 presents the DBCP concentration in the samples throughout incubation.

The following observations can be made:

- Similar to what was observed during Batch Test 1, significant DBCP removal was observed during the initial incubation period (0.5- and 4-hour data points comparing the Control bottles against the Bioreactor bottles);

- DBCP removal did not take place beyond the initial incubation period even after nitrate or peroxide was dosed to two sets of bottles. These results suggest that DO and nitrate reduction may not have been facilitating DBCP biodegradation during the initial incubation period. However, it is possible that peroxide was consumed through reactions with other constituents in the water (chemical or microbial), preventing it from forming DO. Because DO augmentation after peroxide spiking could not be measured, aerobic co-metabolic DBCP degradation cannot be ruled out.

*Figure 39: DBCP Concentrations in the Control and Bioreactor Bottles (Batch Test 2).*
CONCLUSIONS

The biottta™ pilot system effectively removed nitrate and DBCP, and the following overall conclusions can be made:

- Sustained nitrate removal can be achieved using biottta™ system;
- Nitrate removal to less than 5 mg/L can be achieved in the bioreactor at an EBCT as low as 3 minutes;
- Nitrite does not accumulate in the bed;
- Throughout pilot testing, the two-stage, fixed-bed biotreatment system (biottta™) has consistently removed DBCP to below detection (<0.01 µg/L) while removing nitrate to below 5 mg/L. GAC leaching procedure tests can often be used as a tool to differentiate between VOC adsorption and biodegradation. However, leaching tests showed no DBCP desorption from any GAC sample, including GAC from the District’s full-scale contactor, which had been showing aqueous phase DBCP breakthrough at 25% and 50% bed depths. Therefore, other DBCP removal mechanism tests had to be performed. Pilot testing with preloaded GAC from the top of the District’s full-scale contactor showed no DBCP breakthrough. Bench-scale column tests using sand media showed partial DBCP removal. Two batch tests using biomass from the pilot-scale bioreactor showed DBCP removal during the first few hours of incubation. Taken together, these results suggest that bacteria indigenous to the District’s Well 12 groundwater are capable of degrading DBCP, thus allowing for the efficient, simultaneous removal of nitrate and DBCP across the two-stage, fixed-bed biotreatment system.
- Backwashing the bioreactor does not affect the contaminant removal performance;
- Chemical feed failure has minimal effect on system performance and contaminant removal can be immediately re-established after the resumption of the chemical feed;
- System shut-down for up to 5 days does not affect the system performance. The microbial community was robust and microbial activity was re-established immediately after restarting the system; and
- The backwash wastewater contains low levels of COD, TSS, and TDS, allowing disposal of the wastewater in regular sewer, if available.

Overall, biottta™ can provide an effective treatment option for the simultaneous removal of nitrate and DBCP.