FINAL REPORT

Direct Fixed-Bed Biological Perchlorate Destruction Demonstration

ESTCP Project ER-0544

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List of Acronyms

[D/A]	Electron Donor to Electron Acceptor Ratio
AA	Acetic acid
BAC	Biologically Active Carbon
BDOC	Biodegradable Dissolved Organic Carbon
BOD	Biochemical Oxygen Demand
BW	Backwash
CA DHS	California Department of Health Services
CDPH	California Department of Public Health
CFU	Colony Forming unit
COD	Chemical Oxygen Demand
CT	Concentration x Contact Time
DBP	Disinfection By Product
DBPFP	Disinfection By Product Formation Potential
DNA	Deoxyribonucleic Acid
DO	dissolved oxygen
DOC	Dissolved Organic Carbon
DoD	Department of Defense
DPH	Department of Public Health
DWEL	Drinking Water Equivalent Level
EBCT	Empty Bed Contact Time
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FXB	Fixed-Bed
GAC	Granular Activated Carbon
gpm	Gallons per minute
HAA	Haloacetic acids
HAc	Acetic acid
HPC	Heterotrophic Plate Count
IX	Ion Exchange
LB	Lysogeny broth
LOD	Limit of Detection
MCL	Maximum Contaminant Level
MSDS	Material Safety Data Sheets
MWH	Montgomery Watson Harza
NPDES	National Pollution Discharge Elimination System
NTU	Nephelometric Turbidity Unit
O&M	Operation and Maintenance
PCB	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
PRB	Perchlorate Reducing Bacteria
PVC	Poly Vinyl Chloride
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Program Plan
RDP	Ribosomal Database Project

RL	Reporting Limit
SCADA	Supervisory Control and Data Acquisition
SIC	Standard Industrial Classification
STLC	Soluble Threshold Limits Concentration
TCLP	Toxicity Characteristic Leaching Procedure
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TSS	Total Suspended Solids
TTHM	Total Trihalomethanes
TTLC	Total Threshold Limit Concentration
UM	University of Michigan
VOC	Volatile Organic Compounds
VSS	Volatile Suspended Solids
WET	Waste Extraction Tests

EXECUTIVE SUMMARY

In February 2007, a 10-month demonstration study was initiated in Rialto, California to treat perchlorate-contaminated groundwater using fixed-bed (FXB) bioreactor technology. Two first-stage, parallel FXB bioreactors (F120 with a 3.9-ft bed depth and a 2-ft diameter, and F130 with a 4.7-ft bed depth and a 2-ft diameter) treated groundwater to remove perchlorate. Effluent from these reactors was dosed with hydrogen peroxide (i.e., reoxygenate + oxidize residual organics and hydrogen sulfide). The reoxygenated water was then passed through a FXB biofilter (F150) to oxidize any remaining organics and sulfide and to remove turbidity. Chlorine was then dosed to the effluent of the biofilter as a final disinfection step. In parallel with the pilot testing, a mathematical model was developed and calibrated, which can be used to elucidate observed phenomena during pilot testing and to predict the perchlorate removal performance of a FXB bioreactor system at other sites. Additionally, molecular microbiological analyses were performed to quantify the relative abundance of specific bacteria within the mixed microbial community in the bioreactor bed. A bench-scale FXB bioreactor was also constructed to test how nutrient addition and intermittent electron donor addition patterns affect the performance and microbial community of a bioreactor. Tests were run using the bench-scale bioreactor that could not be easily conducted using the demonstration-scale system. The bench-scale system also provided "replicates" for the tests that were performed with both systems.

The overall objective of this work was to evaluate the efficacy of using FXB bioreactors and post-treatment to remove perchlorate from drinking water and to produce water that meets all regulations. Specific project emphases included the demonstration of sustained perchlorate removal capabilities, the identification and evaluation of process limitations and potential failure scenarios, and the development of realistic designs and cost estimates for full-scale, potable FXB biological perchlorate treatment.

The results of this study showed that 1) as FXB bioreactor treatment systems scale up, process efficiencies also go up (i.e., required contact times to achieve sustained, robust perchlorate removal decreased substantially relative to contact time requirements established during previous, smaller scale studies), 2) hydrogen peroxide reoxygenation, polishing filtration, and chlorination provide effective post-treatment, 3) system operation is straightforward, requiring no specialized training or extraordinary maintenance procedures, 4) the bacterial communities in these systems are largely gram-negative Proteobacteria, 5) site-specific performance of these systems can be predicted using a mathematical model developed as part of this demonstration, 6) costs for FXB biological perchlorate treatment systems can be low.

Results Summary

FXB Bioreactor Performance

STEADY STATE OPERATION	
Operating Parameter/Effluent Water Quality	**Performance and Comments**
Perchlorate	Sustained perchlorate removal to below detection was achieved using organisms indigenous to the local groundwater. The detection limit was 0.5 μg/L during some of the testing, and 2 μg/L for most of the testing.
Empty-bed contact time	The shortest rmpty-bed contact time (EBCT) tested was 5 minutes (7 gpm/ft^2), and this contact time supported sustained perchlorate removal to below detection. The design EBCT was established as 10 minutes, which was used during the majority of demonstration testing.
Bed depth	Bed depths of 3.9 and 4.7 feet were tested in parallel to determine if loading rate impacted the degree of perchlorate removal and/or rates of headloss generation (i.e., EBCT was held constant between the two bioreactors, which allowed the comparison of different loading rates), and no significant performance differences between the two conditions were observed. Changing the EBCT did impact perchlorate removal performance. Thus, a full-scale system would be designed around a target EBCT while maintaining a bed depth of approximately 5 feet.
Acetic acid dose	With raw water dissolved oxygen (DO) and nitrate concentrations of 10 mg/L and 30 mg/L (as NO_3^-), respectively, the minimum acetic acid dose required to achieve perchlorate removal to below detection at a 10-minute EBCT was 18.7 mg/L as carbon. This represents a stoichiometric ratio of electron donor to electron acceptor ([D/A]) of 1.7 (i.e., 70% more acetic acid was added than the stoichiometric acetic acid demand exerted by raw water DO and nitrate concentrations). For simplicity only, the [D/A] calculation assumes that the fraction of electrons used for energy is 1 (i.e., $f_e = 1$). Bench-scale bioreactor tests showed that intermittent acetic acid dosing cannot sustain good perchlorate removal performance. Acetic acid must be dosed continuously.
Phosphorus dose	Approximately 40-60% perchlorate removal was achieved without the addition of phosphorus (water from Rialto Well #2 has a background phosphorus concentration of 30-40 mg/L as P). When phosphorus was added at 100-150 mg/L as P, perchlorate removal to below detection was achieved and sustained.
Run time	Typical run times (i.e., length of production periods between backwashes) were 17-24 hours.
Headloss	After a backwash, headloss was typically 0.5 psig (1.2 feet) and

	increased to just above 1 psi (2.3 feet) over the course of a 17-24 hour run.
Dissolved oxygen	<1 mg/L
Nitrate	<1.3 mg/L as NO_3^-
Chlorate	Non-detect (MRL = 10 μg/L)
Chlorite	Non-detect (MRL = 0.010 mg/L)
Dissolved organic carbon (DOC)	0.9-2 mg/L as carbon
Biodegradable organic carbon (BDOC)	Non-detect (<0.1 mg/L) to 1 mg/L as carbon
Hydrogen sulfide	Not detected analytically (detection limit = 10 μg/L), though it could be detected by smell during sampling.
Turbidity	0.4-0.5 NTU in the bioreactor effluent (raw water = 0.3 NTU).
pH	7.1 (raw water pH = 7.5).
Total/fecal coliforms	Not detected
Heterotrophic plate counts	Too numerous to count (>5700 CFU/mL)
HAA5 disinfection by-product formation potential	21-42 μg/L
TTHM disinfection by-product formation potential	22-34 μg/L
NON-STEADY STATE OPERATION (ROBUSTNESS TESTING)	
Backwashing	Backwashing with water containing 4-8 mg/L DO did not appreciably impact perchlorate removal performance in the bioreactor. Backwashing with chlorinated water (0.5 mg/L as Cl_2) did not appreciably impact perchlorate removal performance in the bioreactor.
Simulated acetic acid feed failure	These tests demonstrated that up to 10 hours are available after an acetic acid feed pump failure before perchlorate breakthrough occurs. The maximum perchlorate breakthrough after a 24-hour acetic acid feed pump shut-off was 11 μg/L. After the pump was restarted at Hour 24, perchlorate removal to below detection was again achieved after approximately 4 hours.
Perchlorate spiking	Step feed perchlorate spikes to 100, 400, 600, 800, and 930 μg/L were tested. Each dose was spiked for 1 to 4 days, and the EBCT and acetic acid dose were constant at 10 minutes and 18.7 mg/L as carbon, respectively. In each spiking test, sustained perchlorate removal to below detection was achieved.
Nitrate spiking	Step feed nitrate spikes to 38 mg/L and 45 mg/L (as NO_3^-) were tested. Each spike was tested for 1-2 days, and the EBCT

	remained unchanged at 10 minutes. The acetic acid dose was increased to account for the additional nitrate, but remained at a 1.7 [D/A]. In each spiking test, sustained perchlorate removal to below detection was achieved.
MICROBIOLOGICAL ANALYSES	
Clone library	Clone library microbial analyses revealed a diverse community of gram-negative bacteria in the bioreactor. The bench-scale microbial analyses showed increasing the phosphorus concentration in the feed water can increase the relative abundance of bacteria from two perchlorate-reducing genera: *Azospira* and *Dechloromonas*. Clone library analyses conducted with BAC samples from the pilot-scale bioreactors did not detect *Azospira* in any sample, and showed that the relative abundance of *Dechloromonas* decreased from the pre- to the post-phosphorus BAC samples.
MEDIA CHARACTERIZATION	
General	Toxicity Characteristic Leaching Procedure (TCLP) tests, Total Threshold Limit Concentration (TTLC) tests, and Waste Extraction Tests (WET) were performed on granular activated carbon (GAC) samples from the reactors at the end of pilot testing
Metals	Minimal metals accumulation was observed on the GAC; all metals that were detected were below their hazardous waste threshold values.
Trace organics	No trace organics were detected on the GAC.
Uranium	Uranium was detected on the GAC, but at concentrations well below the threshold hazardous classification value.
MODELING	
General	A mathematical model was developed that took into account electron donor and electron acceptor gradients over the thickness of the biofilm and the distribution of normal heterotrophs, perchlorate reducing bacteria, and inert biomass. Kinetic and stoichiometric parameters were based on a previously calibrated model. The surface-to-volume ratio in the model was adjusted to match the observed complete perchlorate removal at an 8-minute EBCT.
Parameter sensitivity	Key parameters influencing model predictions that were evaluated were EBCT, electron donor addition ([D/A]), and influent nitrate and perchlorate concentrations. The model indicated that 1) complete perchlorate removal could be achieved with [D/A] ratios > 1.7, 2) complete perchlorate removal is associated with perchlorate reducing bacteria outcompeting other heterotrophic bacteria, and 3) complete perchlorate removal requires very low effluent oxygen concentrations (< 0.005 mg/L) and effluent nitrate concentrations below 2 mg/L.
Extrapolations	The model can be used to predict operating conditions with

	changed water characteristics. With influent perchlorate concentrations of up to 10,000 µg/L, effluent perchlorate concentrations of 25 µg/L were predicted with an EBCT of 25 min and a [D/A] of 1.8. An EBCT of 25 minutes was predicted to allow for perchlorate removal to below 5 µg/L with influent nitrate concentrations of up to 56 mg/L as long as [D/A] ratio of 1.8 was maintained. Extrapolations using the developed model should always be used with caution. Further testing at pilot or full-scale should be used to evaluate such extrapolations.

Post-Treatment Reoxygenation and Biofiltration Performance

STEADY STATE OPERATION	
Operating Parameter/Effluent Water Quality	**Performance and Comments**
Bed-depth and empty-bed contact time	A 5-ft bed depth and a 10-minute EBCT (3.7 gpm/ft^2) was used during steady state operation.
Hydrogen peroxide dose	A hydrogen peroxide dose of 4-8 mg/L was used during steady state operation.
Perchlorate	Since perchlorate removal was removed to below detection in the bioreactor, there was rarely any perchlorate in the feed to the biofilter. One exception was during the acetic acid feed failure robustness tests.
Dissolved oxygen	4-12 mg/L in the biofilter effluent.
Dissolved organic carbon	< 1 mg/L as carbon
Biodegradable organic carbon	Non-detect (<0.1 mg/L) to 0.5 mg/L as carbon
Hydrogen sulfide	Not detected analytically or by smell
Turbidity	0.35 NTU
pH	7.1
Total/fecal coliforms	Not detected
Heterotrophic plate counts	Too numerous to count (>5700 CFU/mL)
HAA5 disinfection by-product formation potential	6-15 μg/L
TTHM disinfection by-product formation potential	8-15 μg/L
Alum dose	Alum dosing upstream of the biofilter at up to 10 mg/L did not significantly lower effluent turbidity values beyond those observed without alum dosing.

Chlorine Disinfection Performance

STEADY STATE OPERATION	
Project Component/Chlorine Contact Tank Effluent Water Quality	**Performance and Comments**
Tracer test	A lithium (Li^+) tracer test was performed on the chlorine contact tank, which revealed a t_{10} of 10 minutes during steady state operation (i.e., 10-minute EBCT were used in the

	bioreactor and biofilter). When the bioreactor/biofilter EBCT were decreased to 5 minutes, the resultant t_{10} through the chlorine contact tank was 10 minutes.
Heterotrophic plate counts	Throughout the majority of pilot testing, free chlorine doses of 1-2 mg/L as Cl_2 were used for the final disinfection step, leaving residuals of approximately 0.5-1.2 mg/L as Cl_2. (i.e., CT = 8.5-20.4 mg-min/L). Typical resultant HPC in the effluent of the chlorine contact tank were 1-35 CFU/mL. A CT of 2 mg-min/L was also tested. This CT was achieved through two conditions: 1) a chlorine residual of 0.l2 mg/L as Cl_2 + a t_{10} of 17 minutes, and 2) a chlorine residual of 0.20 mg/L as Cl_2 + a t_{10} of approximately 10 minutes. Resultant HPC in the effluent of the chlorine contact tank were 44-430 CFU/mL.

Overall Treatment System - Bioreactor + Biofilter + Final Disinfection

STEADY STATE OPERATION	
Operating Parameter/Project Component	**Performance and Comments**
System recoveries	Overall system recoveries were 93-96%. Recovery = (total volume of water treated over a single run - the volume of water used for a single backwash of the bioreactor and the biofilter)/(total volume of water treated over a single run)*100.
Preliminary engineering	1,000- and 2,000-gpm conceptual FXB biological perchlorate treatment facility lay-outs were generated from design parameters developed during demonstration testing.
Cost model	A cost model was developed to estimate capital, O&M, and total water production (i.e., life cycle) costs for a 1,000- and 2,000-gpm FXB biological perchlorate treatment facility. The model can also be used to perform a wide variety of sensitivity analyses. Cost estimates indicated that total water production costs can be very low for full-scale FXB biological perchlorate treatment.

1. INTRODUCTION

1.1 Background

Perchlorate is a ground water contaminant that has recently received heightened attention. Its presence is often associated with facilities that once manufactured, handled, or stored ammonium perchlorate, a solid-rocket fuel oxidant. The severity and extent of perchlorate contamination was difficult to assess until 1997, when a new ion chromatographic method was developed to decrease the limit of detection (LOD) for perchlorate from 400 μg/L to 4 μg/L (CDHS, 1997). Since then, perchlorate has been detected in drinking water sources in 25 states (Brandehuber and Clark, 2004).

Both abiotic and biotic processes have been developed and evaluated for treating perchlorate-contaminated drinking water. Typical abiotic perchlorate treatment processes include ion exchange (Tripp et al., 2003; Gu et al., 2001), reverse osmosis/nanofiltration (Amy et al., 2003), electrodialysis reversal (Booth et al., 2000), and tailored granular activated carbon (Na et al., 2002). These processes separate perchlorate from the bulk solution by adsorption or diffusion-limited filtration.

The main drawback with abiotic approaches is that they each create a concentrated perchlorate waste stream that must be further treated or disposed. On the other hand, biological processes convert perchlorate to innocuous chloride and oxygen (Coates et al., 1999; Rikken et al., 1996), thereby eliminating perchlorate from the environment. Of the various available biological perchlorate treatment technologies, none has been tested more extensively on drinking water and has been demonstrated to be as simple, efficient, robust, and cost-effective as GAC-based heterotrophic (i.e., uses organic carbon sources) FXB bioreactors.

This demonstration project confirmed the advantages of biological perchlorate-reducing processes that have been identified through bench- and pilot-scale testing. These advantages include:

Perchlorate is not concentrated, but rather is converted to innocuous chloride and oxygen;
Multiple contaminants can be removed in a single reactor (e.g., perchlorate and nitrate);
Design and operation of FXB bioreactors are comparable to the design and operation of conventional granular media filters; and
Associated costs can be low.

1.2 Objectives of the Demonstration

The objective of this work was to evaluate the efficacy of using 1) FXB bioreactors to remove perchlorate from raw groundwater, and 2) a post-treatment reoxygenation, biofiltration, and final disinfection process to condition the water to potable standards. Using ten years of bench- and pilot-scale experience as a foundation, scale-up issues were identified by evaluating a

demonstration-scale FXB bioreactor system treating water from Rialto, California Well #2. Specific project emphases included the demonstration of sustained perchlorate removal capabilities, the identification and evaluation of process limitations and potential failure scenarios, and the development of realistic designs and cost estimates for full-scale, potable FXB biological perchlorate treatment.

1.3 Regulatory Drivers

There is no federal maximum contaminant level (MCL) for perchlorate. In February 2005, the USEPA adopted the National Academies of Science recommended perchlorate reference dose of 0.007 milligrams per kilogram per day, which correlates to a Drinking Water Equivalent Level (DWEL) of 24.5 μg/L. Individual states have established provisional perchlorate action levels ranging from 1 to 18 μg/L, while Massachusetts has set a primary drinking water MCL of 2 μg/L. California's 6 μg/L MCL went into effect on October 19, 2007.

1.4 Stakeholder/End-User Issues

There were a few overarching questions about FXB biological perchlorate treatment that were addressed by this demonstration project:

- Is the process robust, or is it susceptible to fluctuations in feed water quality or operating conditions?
- How well can the system handle relatively high concentrations of perchlorate in the raw water (e.g., ~1 mg/L)? This issue targets the question of whether the FXB bioreactor system can be applied at a remediation site (i.e., a non-potable application).
- What post-treatment is necessary to produce safe, aesthetically acceptable water?
- What are the associated treatment costs?
- What bacterial communities comprise the bioreactor beds?
- How well can the process be modeled?

2. TECHNOLOGY

2.1 Technology Development and Application

Technology Description. The technology relies on the premise that bacteria can gain substantial energy by mediating the transfer of electrons from an electron donor (such as acetic acid) to perchlorate. Thermodynamic data indicate that perchlorate is a strong oxidant (i.e., accepts electrons readily). Rikken et al. (1996) provided the free energies (at standard conditions and pH=7) for the stoichiometric reactions between acetate and dissolved oxygen (DO), acetate and nitrate, and acetate and perchlorate:

(1) $CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+$; $\Delta G^{o\prime} = -844$ KJ/mol acetate
(2) $CH_3COO^- + {}^3/_5NO_3^- + {}^{13}/_5H+ \rightarrow 2HCO_3^- + {}^4/_5H_20 + {}^4/_5\ N_2$; $\Delta G^{o\prime} = -792$ KJ/mol acetate
(3) ${}^1/_2CH_3COO^- + ClO_4^- \rightarrow HCO_3^- + {}^1/_2H^+ + ClO_2^-$; $\Delta G^{o\prime} = -801$ KJ/mol acetate[1]

Biological perchlorate treatment processes capitalize on this principle by maintaining an environment that fosters the growth of perchlorate-reducing bacteria (PRB). FXB biological processes utilize a stationary bed of media such as sand, plastic, or granular activated carbon (GAC) on which biofilms containing PRB develop. Water is drawn from a well, amended with an electron donor and then pumped across the media bed. Bacteria in the bed reduce DO, nitrate, and perchlorate. For convention during this project, electron donor (i.e., acetic acid) addition was dosed and adjusted in terms of a stoichiometric electron donor to electron acceptor ratio ([D/A]). [D/A] represents the stoichiometric acetic acid demand exerted by the raw water DO and nitrate concentration according to Equations (1) and (2) above. For simplicity only, the [D/A] calculation assumes that the fraction of electrons used for energy is 1 (i.e., $f_e = 1$). The cell synthesis half-reactions are ignored to simplify the calculation.

Post-Treatment. For remediation applications, it is unlikely that a perchlorate-reducing FXB bioreactor process would require substantial post-treatment (possibly reaeration and disinfection only). On the other hand, for drinking water treatment applications, treatment downstream of a FXB bioreactor process needs to have the ability to achieve the following treatment goals:

Reoxygenation: Since biological perchlorate reduction requires near anaerobic conditions, DO must be supplied during the post-treatment process;
Residual Organic Carbon Removal: The addition of an easily assimilable organic substrate can lead to the production of biologically unstable product water;
Sulfide and Turbidity Removal: Under anaerobic conditions, sulfate can be reduced to sulfide, which is odorous. Biomass that sloughs from the FXB reactor during production may produce turbidity; and

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[1] *Perchlorate-reducing bacteria reduce perchlorate to chlorite and then convert chlorite to chloride and oxygen during a dismutation reaction that yields no energy (Coates et al., 1999).*

Disinfection: As with any drinking water treatment process, a disinfection step must be included in the FXB biological perchlorate treatment train.

For this project, post-FXB bioreactor treatment included in-line reoxygenation (i.e, dosing of hydrogen peroxide), second stage biologically active filtration, and chlorination. A schematic of the demonstration treatment train is provided in Figure 2.1, and a 3-D model of the pilot system is provided in Figure 2.2.

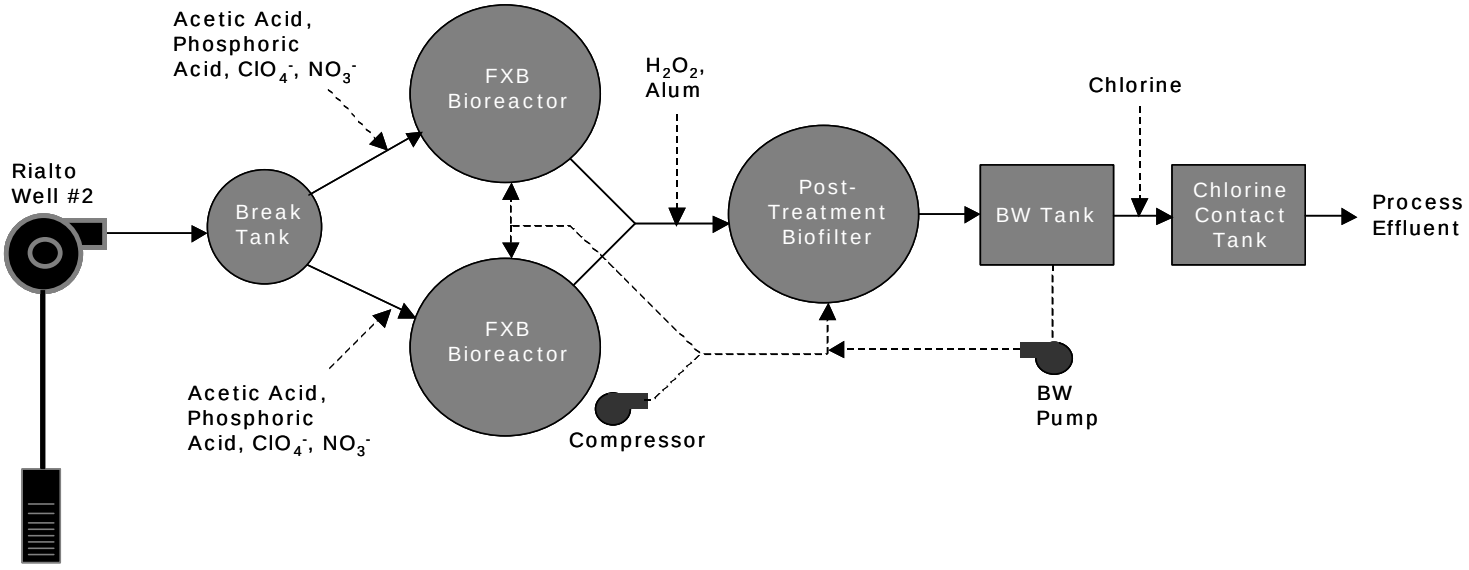


Figure 2.1 - Fixed-bed Bioreactor Treatment Train.

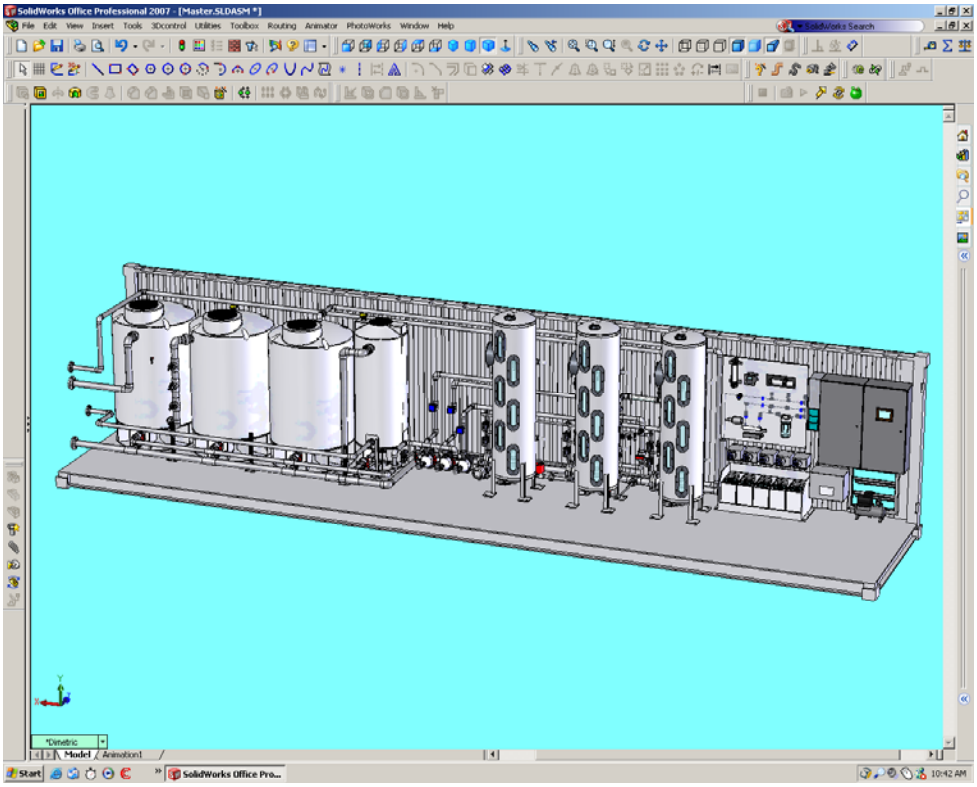


Figure 2.2 - Three-dimensional Model of the FXB Demonstration System.

Design Parameters. Design parameters were developed during demonstration testing that were used to construct preliminary process flow diagrams, facility layouts, and cost estimates. The critical design parameters for the FXB bioreactors included:

- Biogrowth support media selection;
- Empty-bed contact time (EBCT)/surface loading rate/bed depth;
- Efficiency/recovery;
- Acetic acid dosing requirements;
- Nutrient dosing requirements;
- Headloss trends/pumping requirements;
- Backwash protocol (frequency, air scour rate and duration, fluidization rate and duration); and

- Backwash wastewater quality, including volatile suspended solids (VSS), total suspended solids (TSS), biochemical oxygen demand (BOD), and total dissolved solids (TDS).

The critical post-treatment design parameters included:

- Hydrogen peroxide dosing requirements;
- Coagulant dosing requirements;
- EBCT;
- Headloss trends/pumping requirements;
- Backwash protocol (frequency, air scour rate and duration, fluidization rate and duration); and
- Chlorine dosing requirements.

2.2 Previous Testing of the Technology

2.2.1 Bench-Scale Testing

Since 1998, numerous bench-scale FXB bioreactors have been tested at the University of Illinois at Urbana-Champaign (Choi, 2005; Choi et al., 2003; Brown et al., 2003; Brown et al., 2002; Brown, 2002). This work has demonstrated that FXB bioreactors:

- Can achieve and sustain perchlorate removal to below detection (2 μg/L) using bacteria present in groundwater or dechlorinated tap water;
- Require the addition of only an electron donor (i.e., background nutrient concentrations are generally sufficient to sustain efficient perchlorate-reducing bioactivity;
- Require EBCT ranging from <1 to 25 minutes, depending on the concentration of DO and nitrate in the raw water. As raw water DO and nitrate concentrations increase, the required EBCT to remove perchlorate to below detection also increases. This is because biological perchlorate degradation is inhibited by the presence of DO and nitrate (i.e., bacteria typically utilize DO and nitrate as terminal electron acceptors before the utilize perchlorate as a terminal electron acceptor) This impact is especially pronounced in groundwater systems where perchlorate is typically an order-of-magnitude lower in concentration than DO or nitrate;
- Are robust with respect to fluctuations in raw water pH (6.5-9.0 tested), temperature (as low as 5^{o}C tested), perchlorate concentration (10-300 μg/L tested), and sulfate concentration (0-100 mg/L tested);
- Are robust with respect to electron donor feed system failures and filter bed cleaning events (comparable to backwashing events).

2.2.2 Pilot-Scale Testing

In January 2004, a six-month study in Southern California was completed that was designed to evaluate various technologies for removing perchlorate from groundwater. Pilot-scale FXB and fluidized-bed bioreactors were tested in parallel along with three single-pass, perchlorate-specific ion exchange (IX) resins (bench-scale). FXB bioreactor performance can be summarized as follows (Brown et al., 2005):

- Consistent perchlorate removal to below detection was achieved in the reactor using only organisms indigenous to the Saugus aquifer. With influent DO and nitrate concentrations of 7 and 15 mg/L (as NO_3^-), respectively, the lowest EBCT and acetic acid concentration that allowed consistent perchlorate removal to below detection were 15 minutes and 7.8 mg/L as carbon, respectively. 24-hour run times (i.e., length of production times between two backwashes) were used under these conditions, as headloss built up and had to be removed. For this pilot, the headloss value that triggered a backwash was 30 feet (13.0 psig), which was driven by feed pump capacity. A design EBCT of 25 minutes was chosen to allow for 48-hour run times;
- Effluent total organic carbon (TOC) and biodegradable organic carbon (BDOC) concentrations were generally below the detection limit of 0.1 mg/L;
- No fecal coliforms were detected in the feed or effluent of the reactor;
- Average feed and effluent turbidities were 0.5 and 0.6 NTU, respectively;
- Headloss across the reactor ranged from < 2 feet to 30 feet (0.9-13.0 psig), but was typically between 5 and 10 feet (2.2-4.3 psig);
- Backwashing with water containing 6-8 mg/L DO concentrations did not impact perchlorate removal performance;
- Fluctuations in feed perchlorate concentrations (5 μg/L to 300 μg/L) did not impact perchlorate removal performance;
- Gradual changes in feed DO and nitrate concentrations did not impact perchlorate removal performance;
- Periods of extended system shut-down (up to two weeks) did not impact perchlorate removal performance;
- A 24-hour acetic acid feed failure simulation did not impact perchlorate removal performance;
- 7-day Total Trihalomethane (TTHM) Formation Potentials of FXB bioreactor effluent using 3-5 mg/L free chlorine residual or 3-5 mg/L combined chlorine residual incubated for 7 days at 70-80 ^oF were 20 μg/L and <1 μg/L, respectively;
- 7-day Haloacetic $Acid_5$ (HAA_5) Formation Potentials of FXB bioreactor effluent using 3-5 mg/L free chlorine residual or 3-5 mg/L combined chlorine residual incubated for 7 days at 70-80 ^oF were 26 μg/L and 17 μg/L, respectively.

Based on the results of this pilot-scale work, Carollo Engineers submitted a comprehensive FXB biological perchlorate treatment engineering report to the California Department of Health Services [CA DHS]; now called the California Department of Public Health - CDPH) technology

acceptance application program (Brown et al., 2004). On November 15, 2004, CA DHS granted Carollo Engineers "Conditional Acceptance of Fixed-Bed Biological Treatment for the Production of Drinking Water from Perchlorate Contaminated Water" (Sakaji, 2004).

2.3 Advantages and Limitations of the Technology

Three processes that have received considerable attention for treating perchlorate include FXB bioreactors, fluidized-bed bioreactors, and single-pass IX. FXB bioreactors use a stationary bed of granular media for biogrowth support to which an organic electron donor is added. Contaminated water is passed through the bed and excessive biogrowth is removed during backwashing, which occurs approximately every 24 hours. Fluidized-bed bioreactors[2] are completely mixed systems that use recycle lines and high feed pumping rates to maintain a suspended bed of granular media for biogrowth support. An organic electron donor is added to the bioreactor, and biomass control is maintained using an off-line biomass/GAC separator (i.e., backwashing is not required). Single pass IX uses perchlorate selective resins to remove perchlorate from contaminated water. During this process, contaminants are adsorbed to the resin, and, once exhausted, the resin is removed and transported for incineration A general comparison of the strengths and weaknesses of each process is provided in Table 2.1.

[2]*The fluidized-bed reactor that has received CA DHS conditional approval for perchlorate treatment is a proprietary process developed by Shaw Environmental.*

Table 2.1 - Advantages and Limitations of Various Oxidant-Reducing Bioreactor Technologies Based on Available Data.

Configuration	Strengths	Weaknesses
Fixed-Bed	Can remove multiple contaminants in a single reactor (e.g., nitrate, perchlorate, volatile organic compounds) Short empty-bed contact times required (redox gradients allow efficient use of specific microbial metabolisms) Simple design and operation Robust with respect to operational and water quality upsets Low costs; costs are not highly sensitive to raw water quality or perchlorate treatment goals Received conditional CDPH certification for treating perchlorate-contaminated drinking water. Green technology (i.e., contaminants are degraded instead of concentrated). High recoveries	Backwash required Electron donor required and nutrient dose may be required. Post-treatment reoxygenation and filtration required. No full-scale potable installations in operation for perchlorate removal (20+ full-scale potable installations in operation for nitrate removal in Europe)

Configuration	Strengths	Weaknesses
Fluidized-Bed	Full-scale installations for remediation applications (i.e., non-potable) No off-line backwash required Low O&M costs Received conditional CDPH certification for treating perchlorate-contaminated drinking water. Green technology (i.e., contaminants are degraded instead of concentrated). High recoveries	High feed pumping rates Electron donor required and nutrient dose may be required. Recycle required Post-treatment reoxygenation and filtration required.
Single-Pass Ion Exchange	Full-scale installations in operation Simple design and operation High recoveries Low cost	Only targets perchlorate Not a green technology (i.e., contaminants are concentrated, then the exhausted IX resin is removed and transported for incineration).

3. PERFORMANCE OBJECTIVES

3.1 Summary

Performance objectives listed in Table 3.1 apply to the complete FXB bioreactor and post-treatment process train.

Table 3.1 - Performance Objectives.

Type of Performance Objective	Primary Performance Criteria	Success Criteria	Actual Performance Success Criteria Met?
Qualitative	Confidence in viability of the process	Utility/operator/DPH acceptance	Yes
	Ease of use	Operator acceptance	Yes
Quantitative	Sustained removal of raw water perchlorate to below detection under "steady state" optimized conditions (Phase 3 testing)	≥ 95% of effluent perchlorate concentrations below 2 μg/L over 6-week testing period	Yes
	Sustained removal of raw water perchlorate to below detection during periods of transient system upsets (Phase 4 testing)	≥ 95% of effluent perchlorate concentrations below 2 μg/L during each robustness test (includes high resolution sampling)	Yes
	High process efficiency	≥ 95% of raw water recovered for distribution	Yes
	Effluent DO levels	Effluent DO concentration = raw water DO concentration ± 1 mg/L	Yes

Type of Performance Objective	Primary Performance Criteria	Success Criteria	Actual Performance Success Criteria Met?
Quantitative	Biological stability of effluent	≥ 90% of effluent BDOC concentrations below detection (<0.1 mg/L) during 6-week Phase 3 testing period	No. Of the 6 BDOC samples taken during Phase 3 testing, 2 samples were <0.1 mg/L, and the other 4 samples ranged from 0.33-0.46 mg/L
	Aesthetic quality of effluent	No olfactory hydrogen sulfide detection in ≥ 95% of effluent samples during 6-week Phase 3 testing period	Yes
	Disinfection by-product formation potential (DBPFP) of effluent	<60 μg/L TTHMs and < 40 μg/L HAA_5 in all DBPFP tests during 6-week Phase 3 testing period	Yes
	Microbial quality of effluent	≥ 90% of effluent heterotrophic plate counts (HPC) ≤ 500 counts/mL during 6-week Phase 3 testing period	Yes

3.2 Performance Objectives

- **Confidence in the Viability of the Process.** It is important to demonstrate robust performance with any water treatment process so that utility managers, utility operators, regulators, and consumers can be confident that all water quality standards will be met regardless of raw water quality or operating conditions. For innovative processes with no full-scale track record, performance demonstration is particularly critical for establishing the viability of the process. Essentially, this objective reflects the accumulative demonstration of all other objectives listed in Table 3.1. If all other performance objectives are met, then utility, operator, and regulatory acceptance should follow.

- **Ease of Use.** Operators must be comfortable with the operation and maintenance of a treatment facility. The more complicated a process, the more opportunities for system failure, and the more time required to maintain the system. If a treatment process is simple, robust, and fully automated, operator attention requirements should be minimal. The FXB biological pilot is fully automated. Operator attention requirements were monitored through the demonstration to assess the Ease of Use performance criterion.

- **Sustained Perchlorate Removal.** Perchlorate concentrations in the effluent of the bioreactor were measured approximately every hour by an in-line ion chromatograph. Grab samples were also taken daily for duplicate analysis at a University of Michigan laboratory. Sustained removal was defined as detecting no perchlorate (<2 μg/L) in ≥ 95% of bioreactor effluent samples over the 6-week testing Optimal Operation testing phase (Phase 3 testing).

- **High Process Efficiency.** The availability of usable water supplies is diminishing, making it vital that water treatment facilities delivery as much of the water they treat as possible (i.e., minimize losses). Backwash frequencies and flow rates were logged daily. This information was combined with production rates to calculate process efficiencies throughout each phase of pilot testing.

- **Dissolved Oxygen.** Fluctuating or very low DO concentration in drinking water distribution systems can cause corrosion and taste and odor problems. To avoid these issues, a performance objective was established for the FXB bioreactor and post-treatment system to produce water with a DO concentration that was within 1 mg/L of the raw water DO concentration. Raw water and effluent DO concentrations were monitored continuously during the demonstration to evaluate this performance objective.

- **Biological Stability.** Biodegradable compounds in treated water promote biological growth in a distribution system, which could lead to corrosion and/or the generation of offensive tastes and odors. The related performance objective states that ≥ 90% of system effluent BDOC concentrations should be below the 0.1 mg/L detection limit. BDOC samples across the treatment system were collected once per week for analysis at a local laboratory.

- **Aesthetic Quality.** Consumers judge the health and safety of their drinking water based on aesthetics (taste, odor**,** clarity, etc.). To evaluate clarity, turbidity (i.e., a measure of cloudiness) was monitored and recorded daily. To evaluate odors, hydrogen sulfide, which confers a rotten-egg odor, was monitored daily as well. Occasionally, analytical hydrogen sulfide measurements were taken. However, since the human olfactory system has a lower limit of hydrogen sulfide detection (~0.5 μg/L) than field-based analytical techniques (~10-20 μg/L), an olfactory-based presence/absence data point was recorded each day for all sample locations throughout demonstration testing.

- **Disinfection By-Product Formation Potential.** Disinfection by-product (DBP) regulations limit the concentration of TTHMs and the HAA5s to <0.080 μg/L and <0.060 μg/L, respectively, measured as running annual averages of quarterly samples at four distribution system sites per treatment facility or entry point. Therefore, it is critical that water produced from a FXB bioreactor treatment plant have low potential to form DBPs. To quantify DBPFP, three, 7-day DBPFP tests were conducted using raw water, effluent from the FXB bioreactor, and effluent from the polishing biofilter.

- **Microbial Quality.** Per Environmental Protection Agency (EPA) regulations, utilities that have no detectable disinfectant in their distribution systems can meet the residual disinfectant requirement if they can show HPC below 500 counts/mL coming out of their treatment plant. Thus, to demonstrate the microbial quality of product water from the FXB bioreactor system, a performance objective was established that required ≥ 90% of effluent HPC samples to show ≤ 500 counts/mL during the Optimal Operation testing phase.

4. SITE DESCRIPTION

4.1 Test Site Description and History

Well #2 at the City of Rialto, California served as the demonstration site for this project. This well, which has been removed from production due to perchlorate contamination, has a capacity of 2,045 gpm and is not equipped with any form of treatment presently. Table 4.1 provides the available historical water quality data for Rialto Well #2 as well as perchlorate and nitrate data collected during demonstration testing.

Table 4.1 - Rialto Well #2 Water Quality.

Historical Raw Water Quality				
Raw Water Quality Parameter	Average	Minimum	Maximum	
Perchlorate (µg/L)	74	34	88	
Nitrate (mg/L as NO_3^-)	26	23	28	
Chloride (mg/L)	13	12	13	
Sulfate(mg/L)	12	11	12	
Carbonate/Bicarbonate (mg/L)	<3/210	<3/210	<3/210	
pH	7.8	7.7	7.9	
Total Dissolved Solids (mg/L)	260	260	260	
Volatile Organic Comounds (µg/L)	Not Detected	Not Detected	Not Detected	
Raw Water Quality Collected During Demonstration Testing				
Raw Water Quality Parameter	Average	Minimum	Maximum	95th Percentile
Perchlorate (µg/L)	53.5	37.0	61.0	57.8
Nitrate (mg/L)	27.8	24.9	38.6	30.2

Typical raw water DO concentrations were 8-10 mg/L. The high raw water DO and nitrate concentrations made Rialto Well #2 a challenging test site, as DO and nitrate can inhibit biological perchlorate degradation.

The area surrounding Well #2 has hosted several potential sources of perchlorate contamination over the last century. The Rialto Ammunition Storage Point, a ~2,800-acre area used during the 1940s for the storage of ordnance and explosives for WWII included the site of the present-day Well #2. The "160-acre parcel," located approximately 2 miles to the northwest of Well #2 has been used for many industrial purposes, including fireworks manufacturing and large-scale explosives disposal, both potential sources of perchlorate. Other areas near Well #2 have been used by a multitude of companies for ordnance and pyrotechnics manufacturing and for the treatment, storage, and disposal of explosive waste. The area was formerly used as a citrus grove, and those groves are believed to have used large qualities of Chilean sodium nitrate containing perchlorate. Table 4.2 details the Standard Industrial Classification (SIC) codes for the former manufacturing activities that have occurred near Well #2 (Geosyntec, 2006).

Table 4.2 - SIC Codes for Former Manufacturing Activities (OSHA, 2008)**.**

Activity	Description	SIC Code
Explosives Manufacturing	Establishments primarily engaged in manufacturing explosives.	2892
Fireworks Manufacturing	Chemicals and Chemical Preparations, Not Elsewhere Classified	2899
Ammunition Manufacturing	Ammunition Manufacturing, Except for Small Arms	3483

Well #2 is located in the Rialto-Colton Basin (Basin). Groundwater in the Basin occurs in alluvial sediments at depths usually below 450 feet, and groundwater flow is generally to the southeast. This groundwater flow is controlled by several barriers and faults in the vicinity. There are four hydrostratigraphic units in the Basin: river channel deposits and the upper, middle and lower water-bearing units. The middle water-bearing unit is the most relevant for Well #2 as it provides much of the water that is pumped by the well. It consists primarily of coarse to medium sand and interbedded silt and clay. The middle water-bearing unit ranges in thickness from about 240 to 600 feet. There are three laterally continuous aquifers in the middle water-bearing unit: the upper, the intermediate and the deep, regional aquifer. The deep, regional aquifer provides much of the groundwater that is pumped by municipal supply wells such as Well #2. The three aquifers are separated by aquitards that range from a thickness of a few feet to more than 30 feet. Some surficial soil borings in the area have revealed soil concentrations of perchlorate as high as 205 mg/kg near former pyrotechnics disposal ponds (Geosyntec, 2006).

4.2 Pre-Demonstration Testing and Analysis

Prior to the start of demonstration testing, the City of Rialto provided historical mean, maximum, and minimum Well #2 raw water quality, including perchlorate, nitrate, chloride, sulfate, carbonate, bicarbonate, pH, total dissolved solids, specific conductance, and volatile organics. Once Well #2 was started for demonstration testing, the DO, nitrate, and perchlorate concentrations were measured, which were used to establish initial operating conditions (EBCT and acetic acid dose) for the fixed-bed biological pilot. No additional pre-demonstration testing or analyses were performed.

5. TEST DESIGN

5.1 Conceptual Experimental Design

In February 2007, an 10-month demonstration study was initiated to treat perchlorate-contaminated groundwater from Rialto Well #2 using FXB bioreactor technology. Two first-stage, parallel FXB bioreactors (F120 and F130) treated groundwater to remove perchlorate. Acetic acid (electron donor) and phosphoric acid (nutrient) were fed to the process flow upstream of the bioreactors. See Figure 2.1 for a detailed process flow diagram. Effluent from these reactors was dosed with hydrogen peroxide to reoxygenate and oxidize residual organics and hydrogen sulfide. The reoxygenated water was then passed through a second stage FXB biofilter (F150) to oxidize any remaining organics and sulfide and to remove turbidity. The bioreactors and the biofilter had six, 12-inch windows that ran the length of each pressure vessel, which allowed for visual observation of bed depth, biogrowth, and mixing during backwash events. Effluent from the biofilter was discharged to a backwash tank and therefore served as the source water for backwashing the bioreactors and biofilter. Overflow from the backwash tanks was dosed with chlorine and flowed into the chlorine contact tank. A detailed description of the pilot testing phases is provided in Section 5.4.5

In parallel with the demonstration testing, a bench-scale FXB bioreactor was constructed to serve as a rapid screening process for identifying the effects of 1) nutrient addition, and 2) acetic acid dosing patterns on perchlorate removal performance. A mathematical model was developed and calibrated, which could be used to elucidate observed phenomena during pilot testing and to predict the perchlorate removal performance of a FXB bioreactor system at other sites. Additionally, molecular microbiological analyses were performed to quantify the relative abundance of specific bacteria within the mixed microbial community in the bioreactor bed.

5.2 Baseline Characterization

Baseline characterization for Well #2 consisted only of 1) gathering historical raw water quality data, and 2) measuring current raw water concentrations of DO, nitrate, and perchlorate. See Section 4.2 for additional details.

5.3 Treatability Results

During the 10-year period preceding this demonstration, numerous bench- and pilot-scale studies were completed that showed the treatability of perchlorate-contaminated groundwater using the FXB biological process. See Section 2.2 for additional details.

5.4 Field Testing

5.4.1 Demonstration Installation and Start-Up

The following site preparations were made at Rialto Well #2 (Shaw Environmental, Inc. coordinated these efforts):

- Well #2 pump motor was refurbished and the casing inspected;
- Power was expanded to handle multiple pilot systems;
- A National Pollutant Discharge Elimination System (NPDES) permit modification was acquired through the Santa Ana Regional Water Quality Control Board to allow for the discharge of raw and treated Well #2 water to an adjacent catch/percolation basin;
- A waste discharge line from the site to the adjacent catch/percolation basin was installed;
- Lighting and a new security gate were installed;
- Site was graded.

A new demonstration-scale FXB bioreactor skid was constructed for this project. A basic schematic of the process is provided in Figure 2.1. The skid was contained in a 40'x8'x8' trailer. The two-foot diameter parallel bioreactors and the two-foot diameter biofilter were filled with virgin Calgon F-816 GAC, with an effective size of approximately 1.4 mm. One bioreactor and the biofilter were filled to a depth of 4.7 feet, and the other bioreactor was filled to a depth of 3.9 feet. All three pressure vessels included depthwise sample ports, spaced six inches apart, which allowed for an evaluation depthwise DO, nitrate, and perchlorate profiles across the biological beds.

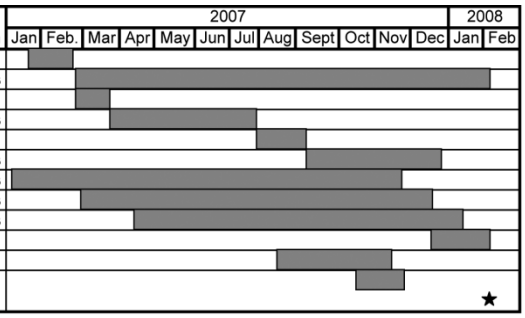
Once the skid was positioned on site, Schedule 80 Poly Vinyl Chloride (PVC) piping was installed to connect Well #2 with the raw water line on the outside of the trailer. Raw water was pumped from Rialto Well #2 into the bottom of a break tank at the head of the FXB bioreactor treatment train. However, the well water was supersaturated with gas, and gas bubbles formed in the bioreactor beds, causing rapid headloss build-up. To eliminate this problem, the well water was redirected to the top of the break tank and a spray nozzle was added to the pipe discharging into the break tank. This allowed supersaturated gas to come out of solution before reaching the bioreactor beds. Excess water overflowed from the break tank to a discharge line flowing to the adjacent catch/percolation basin. Treated effluent and backwash wastewater also discharged to this basin.

5.4.2 Period of Operation

Dates and durations for each component of the FXB biological perchlorate destruction demonstration project are listed in Table 5.1.

Table 5.1 - FXB Biological Perchlorate Destruction Demonstration Schedule.

TASKS	Start	Finish	Duration
1: Site Mobilization	1/22/2007	2/20/2007	29 days
2. Demonstration Testing	2/28/2007	2/5/2008	342 days
Phase 1: Bioacclimation	2/28/2007	3/20/2007	20 days
Phase 2: Optimization	3/21/2007	7/30/2007	133 days
Phase 3: Sustained Optimal Operation	7/31/2007	9/8/2007	39 days
Phase 4: Robustness Characterization	9/9/2007	12/20/2007	102 days
Bench-Scale Bioreactor Operation	1/3/2007	11/27/2007	328 days
Modeling	2/28/2007	12/20/2007	294 days
Microbiological Analyses	4/17/2007	1/19/2008	276 days
Media Characterization	12/20/2007	2/5/2008	16 days
3: a. Preliminary Designs & Conceptual Layouts	8/15/2007	11/14/2007	91 days
b. Cost Model Development	10/15/2007	11/27/2007	43 days
4. Demonstration Complete	2/5/2008	2/5/2008	0 days



5.4.3 Amount/Treatment Rate of Material to be Treated

Two FXB bioreactors were operated in parallel. EBCT, associated flow rates and hydraulic loading rates tested for the two bioreactors and the biofilter are listed in Table 5.2

Table 5.2 - Hydraulic Conditions Tested During the FXB Demonstration Study.

Parameter	Bioreactor F120				Bioreactor F130					Biofilter F150		
EBCT (min)	7	8	10	15	5	10	12	15	18	7	7.5	10
Flow (gpm)	13.1	11.5	9.2	6.1	22.1	11.0	9.2	7.4	6.1	15.8	14.7	11.0
Loading rate (gpm/ft^2)	4.2	3.7	2.9	1.9	7.0	3.5	2.9	2.3	1.9	5.0	4.7	3.5

The F130 Bioreactor operated at an average production rate of 11.0 gal/min (660 gal/hr, 15,840 gal/day) for 294 days, and the F120 Bioreactor operated at an average production rate of 9.2 gal/min (552 gal/hour, 13,248 gal/day) for 190 days. Therefore, the total volume of Rialto Well #2 raw water treated was approximately 7.2 million gallons.

5.4.4 Residuals Handling

Treated water and backwash wastewater were discharged to an adjacent catch/percolation basin per an NPDES permit modification.

5.4.5 Experimental Design

A 10-month FXB bioreactor demonstration program was conducted. The overall objective of this study was to refine design parameters for the full-scale implementation of FXB biological perchlorate removal from groundwater, to identify any process limitations or failure scenarios, and to develop operating and design parameters for a complete FXB biological treatment train.

Demonstration Testing Phase 1 (Biological Acclimation): The purpose of this phase was to develop efficient perchlorate-reducing biological activity in the filters using microorganisms indigenous to the local groundwater. One FXB bioreactor had a bed depth of 3.9 feet, and the other FXB bioreactor had a bed depth of 4.7 feet. An EBCT of 15 minutes was used and acetic acid (technical grade) was dosed at a concentration 50% above that required to stoichiometrically reduce all raw water DO and nitrate ([D/A = 1.5; $O_2 \Rightarrow H_2O$, $NO_3^- \Rightarrow N_2$]; for simplicity only, the [D/A] calculation assumes that the fraction of electrons used for energy is 1 [i.e., $f_e = 1$]. The cell synthesis half-reactions are ignored to simplify the calculation). This ensured that electron donor is not limiting. No phosphoric acid was added initially. However, only partial perchlorate removal was observed during the first few months of testing. Therefore, 96 days into demonstration testing, phosphoric acid dosing commenced at approximately 0.1 mg/L as PO_4-P.

Demonstration Testing Phase 2 (EBCT, Surface Loading Rate, Acetic Acid, and Backwash Optimization): The purpose of this phase was to determine the minimum EBCT ($EBCT_{critical}$) and minimum acetic acid dose ($AA_{critical}$) required to achieve perchlorate removal to below the 2 μg/L detection limit while maintaining process efficiencies of 95% or greater. Depth-wise sampling ports allowed for the simultaneous evaluation of multiple EBCT. Thus, it was possible to maintain a constant EBCT while varying the surface loading rate (i.e., effective bed depth changed by using different sample ports). This information was used to determine whether a design EBCT is independent of bed depth or surface loading rate.

Using the $EBCT_{critical}$, the acetic acid dose was incrementally decreased from [D/A] = 1.5 until perchlorate breakthrough was observed. An optimized backwashing protocol was also developed during this phase (e.g., frequency, air scour rate and duration, fluidization rate and duration).

Demonstration Testing Phase 3 (Optimal Operation): The purpose of this phase was to demonstrate sustained (6 weeks) perchlorate removal to below detection using the critical (or just above the critical) EBCT, acetic acid dose, and backwashing protocol determined during Phase 2. Six weeks provided sufficient time to evaluate the sustainability of the FXB biological perchlorate removal process under steady conditions.

Demonstration Testing Phase 4 (Robustness Characterization): The purpose of this phase was to determine how the FXB bioreactor responds to various process disturbances. The $EBCT_{critical}$ and $AA_{critical}$ remained fixed as operating parameters throughout most of this phase. Perchlorate removal performance during each disturbance was monitored, and required perchlorate removal performance recovery periods were measured. Five disturbances were tested:

1) Backwashing: Perchlorate concentrations were monitored in the backwash wastewater and were also measured in the effluent of the FXB bioreactors immediately following a backwash event;
2) Perchlorate feed fluctuation: The impact of step changes in feed perchlorate concentration were evaluated. Step feed perchlorate spikes to 100, 400, 600, 800, and 930 μg/L were tested.

Each dose was spiked for 1 to 4 days, and the EBCT and [D/A] were constant at 10 minutes and 1.7, respectively;
3) Nitrate feed fluctuation: The impact step changes in feed nitrate concentration were evaluated. Step feed nitrate spikes to 38 mg/L and 45 mg/L (as NO_3^-) were tested. Each spike was tested for 1-2 days, and the EBCT remained unchanged at 10 minutes. The acetic acid dose was increased to account for the additional nitrate, but remained at a 1.7 [D/A];
4) Electron donor feed failure simulation: The acetic acid feed system was turned off for up to a 24-hour period to simulate a full-scale chemical dosing system failure. Five different shut-down scenarios were tested, which varied backwash frequency, length of acetic acid shut-down, and acetic acid dose; and
5) Temporary system shutdown: The demonstration system was completely powered down for a 24-hour period and a 2-week period. These shut-down tests simulated an inadvertent full-scale system shut-down, but also helped elucidate an appropriate stand-by bioreactor rotation strategy.

Backwash Wastewater Characterization: Backwash wastewater composite samples were analyzed for TDS, VSS, TSS, and BOD. These analyses were performed three times throughout demonstration testing and were used to determine an appropriate handling/discharge strategy for backwash wastewater.

Post-Treatment: There were four main post-treatment goals: 1) reoxygenate, 2) remove residual BDOC, 3) remove sulfide, and 4) disinfect. Specific post-treatment performance targets associated with these goals are listed in Table 3.1. A short-term coagulant dosing test was also performed to see if alum could improve turbidity removal across the biofilter. Since the average biofilter effluent turbidity (0.35) was only 0.05 NTU higher than the average raw water turbidity (0.30 NTU), post-treatment testing did not include a turbidity removal optimization phase. The following post-treatment parameters were varied during demonstration testing to determine post-treatment requirements:

- Hydrogen peroxide dose;
- Alum dose;
- EBCT across Biofilter F150;
- Filter backwash protocol for Biofilter F150 (frequency, air scour rate and duration, fluidization rate and duration); and
- Chlorine dose and contact time (concentration multiplied by time or CT).

Modeling: As part of the demonstration, a mathematical was developed, which is capable of simulating all test phases proposed, including the effects of influent characteristics, EBCT, and backwashing. The purpose of the mathematical modeling was to make use of the experimental results from the demonstration scale reactors and to evaluate to what extent system performance can be extrapolated from the available results. The mathematical model had to be developed mainly based on bulk phase measurements of perchlorate, nitrate, oxygen, and acetate. These empirical observations were combined with well studied diffusion-reaction description of processes in the biofilm (Morgenroth, 2008). While the model structure for biofilm systems (i.e., the one dimensional diffusion-reaction modeling approach) is well established (Wanner et al.,

2006) the values of model parameters are not. In the current study, most of the model parameters are based on literature information and some are estimated based on observed reactor performance. To take into account the uncertainty of kinetic parameters, a range of reasonable parameter combinations are simulated to evaluate the sensitivity of model predictions to specific parameter values.

The mathematical modeling followed the following steps:

- Define model structure;
- Select standard model parameters from the literature and from calibrating against reactor performance;
- Use calibrated model to evaluate the influence of operating conditions on reactor performance;
- Influence of EBCT and electron donor addition;
- Influence of biofilm thickness and backwashing;
- Influence of influent perchlorate concentrations;
- Influence of influent nitrate concentrations

The modeling can be used to 1) elucidate phenomena observed during demonstration testing, and 2) predict perchlorate removal performance at other sites to facilitate a rapid preliminary design analysis (and economic analysis when combined with the cost model).

Bench-Scale System: A bench-scale FXB bioreactor was constructed to test how nutrient addition and intermittent electron donor addition patterns affect the microbial community and performance of a bioreactor. Tests were run using the bench-scale bioreactor that could not be easily conducted using the demonstration-scale system. The bench-scale system also provided "replicates" for the tests that were performed with both systems. The bench-scale FXB bioreactor system started in September 2006, and continued to operate under conditions closely matching the demonstration-scale operating conditions until September 2008 when the operating conditions were changed to suit a different research project.

The bench-scale FXB bioreactor was constructed with a GAC bed volume of 200 mm^3 (Calgon F-816 was used, which was also used for the demonstration-scale system). Synthetic groundwater was used as influent and pumped into the reactor in a down flow mode at the flow rate of 10 mL/min. The concentrations of DO, nitrate, and perchlorate in the influent were between 6 and 7 mg/L, 25 mg/L (as NO_3^-), and 75 μg/L, respectively. Based on stoichiometric calculation with an assumed net yield value of 0.4 g $COD_{biomass}$/g $COD_{acetate}$, 13 mg/L as C of acetic acid was needed to completely remove all three electron acceptors. With a safety factor of 1.5 applied, 20 mg/L as C of acetic acid was added to the reactor. These operating conditions were defined as the baseline for this system.

Intermittent addition of acetic acid to the BAC reactor was tested by dividing one backwash cycle (i.e., 48 hours) into four cycles. Each 12-hour cycle consisted of a 6-hour acetic acid

addition at a concentration twice the stoichiometric requirement (i.e., 26 mg/L as C) followed by addition at a concentration half of the stoichiometric requirement (i.e., 6.5 mg/L as C) for 6 hours.

Microbial Characterization: Biologically active carbon (BAC) samples were taken from the FXB Bioreactor F130 in May 2007 (~ one month before phosphorus addition was initiated) and again in September 2007 (a few months after phosphorus addition was initiated). A vertical core of the BAC bed was taken using a 1-inch PVC pipe. The core was placed in a 1-liter sample bottle and shipped to the University of Michigan for clone library analysis. Pre- and post-phosphorous BAC samples were also collected from the bench-scale BAC reactor.

By conducting clone library analyses on both biomass samples, the effects of phosphorus on the microbial community inside the bioreactor were elucidated, and the correlation between microbial composition and reactor performance was established. Similarly, biomass samples were also collected from the bench-scale BAC reactor, both before and after phosphorus addition. The microbial analyses for the bench-scale BAC reactor were compared with those for the bioreactor F130. Finally, along with the biomass samples from F130, a biomass sample from the bioreactor F120 was also collected in May 2007, and analyzed to determine the similarity between the microbial communities in the two demonstration-scale BAC reactors.

DNA samples were extracted from the BAC samples using FastDNA SPIN Kit by Qbiogene (Irvine, California). The DNA concentration of each sample was measured using a NanoDrop 1000 (NanoDrop Technology, Wilmington, DE) and DNA quality was evaluated by running a 1% agarose gel. DNA extracts were amplified in triplicates using the polymerase chain reaction (PCR) with the forward primer 8F (AGA-GTT-TGA-TCC-TGG-CTC-AG) and the reverse primer 1387R (GGG-CGG-(A/T)GT-GTA-CAA-GGC). The composition of the PCR reactions was adopted from the work by Briones and co-workers (1). The PCR reaction involved 30 cycles and started with 5 min of denaturation at 95˚C and ended with a final extension at 72˚C for 18 min. Each cycle consisted of denaturation at 95˚C for 30 s, annealing at 50˚C for 45 s, and extension at 72˚C for 2 min. Pooled PCR products were purified by running agarose gel electrophoresis and extracted using the MinElute Gel Extraction Kit (QIAGEN Inc, Valencia, California). Purified PCR products were cloned into TOPO vector (Invitrogen, Carlsbad, California), and transformed into chemically competent *Escherichia coli*. The transformed *E. coli* were plated on LB agar and incubated at 37˚C overnight. Colonies were picked and used to inoculate three 96-well microplates. Two of the three 96-well microplates were sent to the Genomic Center at Washington University (St. Louis, Missouri) for DNA sequencing.

Raw sequence readings obtained from the Genomic Center were entered into the Ribosomal Database Project (RDP) maintained by Michigan State University (East Lansing, Michigan). The raw DNA sequences were classified into various bacterial populations and the relative abundance of identified populations was quantified.

Media Characterization: Based on full-scale European biodenitrification experience, it is anticipated that the GAC would have to be replaced about every 10 years. To identify

appropriate disposal procedures for the spent GAC, total concentration leaching procedure (TCLP), total threshold limit concentration (TTLC), and waste extraction tests (WET) leaching procedure tests were performed on a mixed sample of GAC from both bioreactors at the end of demonstration testing. These tests, which simulate conditions that may be present in a landfill, are designed to extract constituents that are sorbed to the GAC media. Extraction fluids (e.g., citrate, sodium acetate) are added to a batch of BAC media and tumbled for up to 48 hours to extract any sorbed constituents that may ultimately leach during long-term storage in a landfill. Extracted metals, volatiles, semi-volatiles, pesticides, PCBs, herbicides, and perchlorate were quantified.

5.6 Sampling Methods

Figure 5.1 illustrates the various sampling locations and Table 5.3 lists the various water quality parameters that were measured, sampling location and frequency, and the associated laboratory responsible for the analysis. Increased perchlorate sampling frequencies during the robustness tests are described Section 5.4.5. Appendix F lists the analytical methods supporting the experimental design and Appendix G describes the elements of the quality assurance project plan.

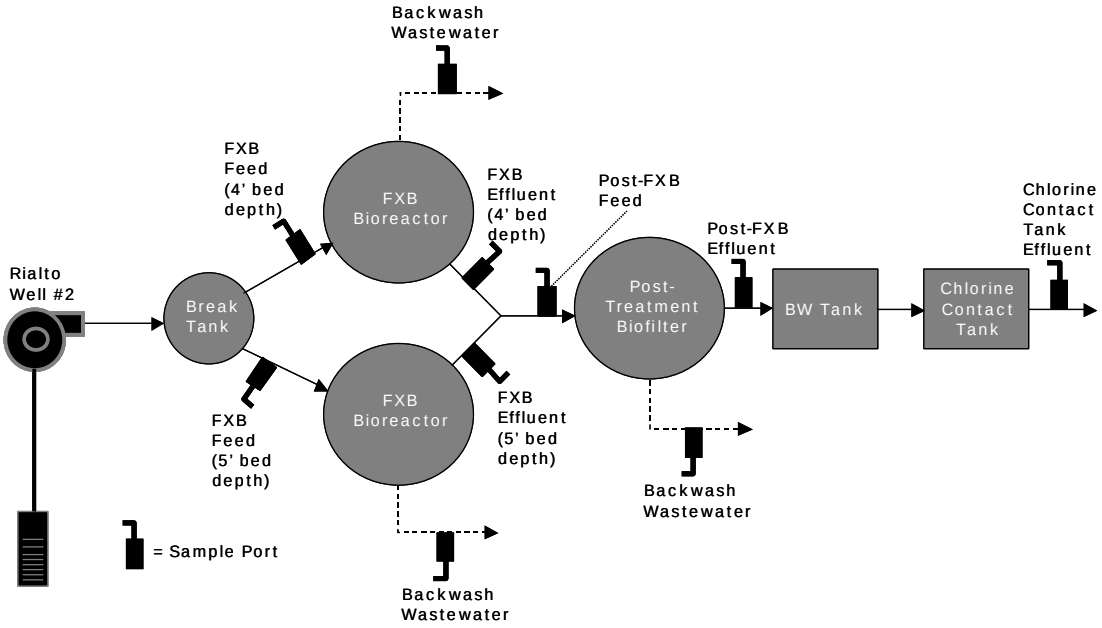


Figure 5.1 - Water Quality Sampling Points.

Table 5.3 - Testing Matrix for the FXB Bioreactor and Post-treatment Demonstration.

Parameter	Sampling Location	Sampling Frequency	Lab
Perchlorate	FXB[1] Feed 2 FXB Effluent FXB Feed 2 FXB Effluent Depth wise sample ports	3/week 3/week 1/two hours 1/two hours 1/week	University of Michigan and occasional checks with MWH[3] laboratories On-site: In-line Dionex ion chromatograph
Nitrate	FXB Feed 2 FXB Effluent FXB Feed 2 FXB Effluent Depth wise sample ports	3/week 3/week 1/two hours 1/two hours 1/week	University of Michigan and occasional checks with MWH On-site: In-line HACH NITRATAX nitrate probe Daily using a Hach DR 890 colorimeter
DO	FXB Feed 2 FXB Effluent Post-FXB[4] Feed Post-FXB Effluent Chlorine contact tank effluent Depth wise sample ports	1/two hours 1/two hours 1/two hours 1/two hours 1/two hours 1/week	On-site: In-line HACH sc100™ LDO™ probe
Chlorate	FXB Effluent	1/week	University of Michigan
Chlorite	FXB Effluent	1/week	University of Michigan

Table 5.3 Continued

Parameter	Sampling Location	Sampling Frequency	Lab
Nitrite	FXB Feed 2 FXB Effluent	1/week 1/week	University of Michigan and occasional checks with MWH
Sulfate	FXB Feed 2 FXB Effluent	1/week 1/week	University of Michigan
Phosphate	FXB Feed 2 FXB Effluent	1/ two weeks 1/two weeks	University of Michigan
Ammonia	FXB Feed 2 FXB Effluent	1/two weeks 1/two weeks	University of Michigan
Iron & Manganese	FXB Feed 2 FXB Effluent Post-FXB Effluent	1/month 1/month 1/month	University of Michigan
H_2S	Post-FXB Feed Post-FXB Effluent	1/week 1/week	On-site colorimetric method based on EPA 376.2
Dissolved organic carbon	FXB Feed 2 FXB Effluent Post-FXB Effluent Chlorine contact tank effluent	2/week 2/week 2/week 2/week	University of Michigan and occasional checks with MWH
Biodegradable organic carbon	FXB Feed 2 FXB Effluent Post-FXB Effluent	1/two weeks 1/week 1/week	MWH Laboratory
Free chlorine	Chlorine contact tank effluent	3/week	On-site: HACH DR 890 Colorimeter
TTHMs	$DBPFP^5$ tests	10 total DBPFP tests	MWH
HAA_5	DBPFP tests	10 total DBPFP tests	MWH

Table 5.3 Continued

Parameter	Sampling Location	Sampling Frequency	Lab
Heterotrophic plate counts	FXB Feed 2 FXB Effluent Post-FXB Effluent Chlorine contact tank effluent	1/week 1/week 1/week 1/week	MWH
Total & Fecal Coliforms	FXB Feed 2 FXB Effluent Post-FXB Effluent Chlorine contact tank effluent Backwash wastewater	1/week 1/week 1/week 1/week 4 total BW samples per each of 3 FXB reactors	MWH
Turbidity	FXB Feed 2 FXB Effluent Post-FXB Effluent	Daily Daily Daily	On-site: HACH DR 890 Colorimeter
pH	FXB Feed 2 FXB Effluent Post-FXB Effluent Chlorine contact tank effluent	Daily Daily Daily Daily	On-site : HACH pH probe
Temperature	FXB Feed	1/two hours	On-site: In line HACH sc100™ LDO™ probe
Head loss	Across all 3 FXB reactors	Continuous	On-site: In-line pressure transducer
Flowrate	Across all 3 FXB reactors	Continuous	On-site: In-line Magflow meter

Table 5.3 Continued

Parameter	Sampling Location	Sampling Frequency	Lab
Volatile suspended solids	Backwash wastewater	3 total BW samples per each of 3 FXB reactors	MWH
Total suspended solids	Backwash wastewater	3 total BW samples per each of 3 FXB reactors	MWH
Total dissolved solids	Backwash wastewater	3 total BW samples per each of 3 FXB reactors	MWH
Biochemical oxygen demand	Backwash wastewater	3 total BW samples per each of 3 FXB reactors	MWH

[1]*FXB: F120 and F130 Bioreactors*
[3]*MWH: Montgomery Watson Harza*
[4]*Post-FXB: F150 Biofilter reactor*
[5]*DBPFP: Disinfection By-Product Formation Potential (See Standard Method 5701B)*

5.6 Data Analysis, Interpretation and Evaluation

Water quality and operational data were compiled, tabulated, and plotted daily so that trends and instantaneous performance could be rapidly analyzed to determine appropriate system modifications. Optimal operating conditions established during Phase 2 Optimization testing were used during the Phase 3 Sustained Removal testing and Phase 4 Robustness testing. This ensured that design parameters were selected so that treatment objectives would be met and sustained during periods of constant (i.e., varying by less than 10%) and unsteady (i.e., varying by greater than 10%) water quality and operational conditions.

6. PERFORMANCE ASSESSMENT

6.1 Performance Criteria and Confirmation Methods

A detailed listing of the criteria and confirmation methods that were used to determine the effectiveness of FXB demonstration testing is provided in Table 6.1. Essentially, the effectiveness of the demonstration was defined by how efficiently the FXB biological treatment train (FXB bioreactor and post-treatment) produced perchlorate-free (i.e., perchlorate concentrations below 2 μg/L) potable water during steady and unsteady conditions. It was also important that the process train maintain an overall efficiency of ≥ 95% (i.e., raw water recovered for distribution). Water quality and operation performance parameters were selected to provide a comprehensive and, in many cases, continuous evaluation of treatment system performance. Details on sampling location, frequency, and associated analysis for these parameters are provided in Figure 3.2 and Table 3.2. Analytical and QA/QC methods are detailed in Appendix F and Appendix G, respectively.

Table 6.1 - Performance Criteria and Performance Confirmation Methods.

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Methods*	Actual (post demo)
PRIMARY CRITERIA (Performance Objectives) (Qualitative)			
Ease of Use Operator training requirements System maintenance requirements	1. Standard 2. Minor	Experience from demonstration operation	Monitored labor demand associated with system operation and maintenance
PRIMARY CRITERIA (Performance Objectives) (Quantitative)			
Contaminant Reduction Perchlorate Nitrate BDOC	1. ≥ 95% effluent perchlorate below 2 μg/L during 6-week optimal operation testing period (Phase 3) 2. ≥ 95% effluent nitrate below 1 mg/L (as NO_3^-) during same period 3. ≥ 95% effluent BDOC below 0.1 mg/L during same period	Analysis of water quality samples taken during demonstration testing. Duplicate and triplicate analyses of perchlorate and nitrate were occasionally performed.*	Analysis of water quality samples taken during demonstration testing.
Factors Affecting Technology Performance	1. ≤ 25 minutes 2. ≤ 50% above the	1. Continuos Magflow meter and occasional manual	1. Continuos Magflow meter and occasional

Table 6.1 Continued

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Methods*	Actual (post demo)
EBCT Acetic Acid Dose Nutrient Dose Raw water DO and nitrate conc. Backwash Effectiveness Process Upsets	stoichimetric raw water acetic acid demand based DO and nitrate concentrations 3. None required 4. No limit 5. 24-48-hour run time; ≤ 5 minutes air scour at ≤ 5 ACFM and ≤ 10 minutes fluidization at 10 gpm/ft^2 loading rate. 6. No measurable performance impact (see robustness)	calibration checks 2. Mass balance and regular DOC measurements 3. Experience from demonstration operation 4. Experience from demonstration operation 5. Experience from demonstration operation; air flow meter; Magflow meter 6. Experience from demonstration operation	manual calibration checks 2. Mass balance and regular DOC measurements 3. Experience from demonstration operation 4. Experience from demonstration operation 5. Experience from demonstration operation; air flow meter; Magflow meter 6. Experience from demonstration operation
Process Waste Process Efficiency Used Media	1. ≥ 95% of raw water recovered for distribution 2. Non-hazardous characterization of used GAC at end of demonstration testing	1. Calculation using throughput volumes and backwash waste volumes 2. TCLP test	1. Calculation using throughput volumes and backwash waste volumes 2. TCLP, TTLC, and WET tests
Robustness/Reliability Sustained Removal Performance during and after process upsets Backwashing Raw water quality fluctuation System shut-down periods Acetic acid feed failure	1. ≥ 95% effluent perchlorate below 4 µg/L during 6-week "steady state testing period" (Phase 3) 2. ≥ 95% effluent perchlorate below 4 µg/L during each robustness test (Phase 4); this includes high-resolution sampling	Analysis of water quality samples taken during demonstration testing. Duplicate and triplicate analyses of perchlorate and nitrate was occasionally performed.*	Analysis of water quality samples taken during demonstration testing.

Table 6.1 Continued

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Methods*	Actual (post demo)
Effluent Quality DO BDOC H_2S DBPs HPC	1. Effluent DO concentration = rater DO concentration ± 1 mg/L 2. ≥ 90% of effluent BDOC concentrations below detection (<0.1 mg/L) during six-week Phase 3 testing period 3. No olfactory hydrogen sulfide detection in ≥ 95% of effluent samples during six-week Phase 3 testing period 4. <60 µg/L TTHMs and < 40 µg/L HAA_5 in all DBPFP tests during six-week Phase 3 testing period 5. ≥ 90% of effluent HPC ≤ 500 counts/mL during six-week Phase 3 testing period	Analysis of water quality samples taken during demonstration testing. Duplicate and triplicate analyses of perchlorate and nitrate was occasionally performed.*	Analysis of water quality samples taken during demonstration testing.
SECONDARY PERFORMANCE CRITERIA (Qualitative)			
Safety Hazards	1. Acetic acid; chlorine	Experience from demonstration operation	Experience from demonstration operation and knowledge of standard chemical storage and handling protocols.
Scale-Up Constraints Heterogeneity of biological growth Backwash effectiveness	1. Uniform head loss build-up 2. Consistent "clean-bed" head loss	Continuous monitoring of head loss during demonstration operation	Head loss monitoring and visual inspection of biogrowth in the bioreactors

Table 6.1 Continued

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Methods*	Actual (post demo)
SECONDARY PERFORMANCE CRITERIA (Quantitative)			
Hazardous Materials Accumulated GAC adsorbates	1. "Non-hazardous" rating for used GAC	Toxicity Characteristic Leaching Procedure test	TCLP, TTLC, and WET tests

* Refer to Appendix F or Appendix G for further details

6.2 Demonstration Performance

Detailed demonstration data and figures are provided in Appendix B. A summary of the data as they relate to the performance criteria listed in Table 6.1 is provided above. To simplify this section, the text will focus on Bioreactor F130, which had a 4.7-foot bed depth. Bioreactor F120 (3.9-foot bed depth) performed well, but a full-scale system would be designed around the deeper bed depth to reduce the number of reactor vessels required.

6.2.1 Ease of Use

Ease of use relates to the complexity of system operation and addresses the issues of how much specialized training and operator attention are required. The demonstration pilot was automated with respect to production, backwashes, chemical dosing, and sampling (DO, nitrate, and perchlorate). The pilot operator was only required to maintain stock solutions of chemicals and sample for water quality parameters that were not measured in-line. Though some troubleshooting was also required during demonstration testing, it was minimal and was mostly associated with limitations of the piloting equipment. For example, one bioreactor underdrain lateral had to be repaired on several occasions. This would not be an issue with a full-scale system as the underdrain would be a nozzle-based system and would not be removable. Further, more automation would be included in a full-scale system (e.g., feed-forward control logic that would allow the acetic acid and phosphoric acid dosing to pace off of the raw water DO and nitrate concentrations automatically), so it is anticipated that full-scale operation would be even less complex than the pilot-scale demonstration proved to be. No specialized operator training requirements and minimal system maintenance requirements are anticipated for full-scale operation.

6.2.2 Contaminant Reduction

Perchlorate: Using an EBCT of 10-12 minutes, a phosphoric acid dose of 150 μg/L, and a [D/A] of 1.70, perchlorate was removed to below detection throughout the Optimal Operation testing phase (Figure B.10). EBCT as low a five minutes also resulted in steady removal of perchlorate to below detection. The detection limit for most perchlorate samples was 2 μg/L. However, numerous perchlorate samples were analyzed at a 0.5 μg/L reporting limit, and perchlorate was not detected.

Nitrate: To achieve biological perchlorate removal, nitrate must first be removed to low levels, so it was not surprising to see that effluent nitrate concentrations were low. Figure B.12 shows the effluent nitrate concentrations as measured during Optimal Operation testing by the in-line sensor, the University of Michigan (UM) laboratory, and the hand-held colorimeter. Effluent nitrate concentrations were typically 1 mg/L (as NO_3^-) or less during this phase except for two outliers in the handheld data. Concentrations measured by the UM laboratory were less than concentrations measured by the in-line sensor.

Biodegradable Organic Carbon: Figure B.3 shows the BDOC concentrations in the feed and effluent of Biofilter F150. Biofilter F150 feed is essentially the same as Bioreactor F130 effluent. Except for one outlier, BDOC concentrations coming out of Bioreactor F130 were very low, often non-detect (<0.1 mg/L). BDOC concentrations increased slightly across F150. During the Optimal Operation testing phase (Day 154-192), all BDOC measurements in the effluent of F150 were below 0.5 mg/L.

6.2.3 Factors Affecting Technology Performance

Empty-Bed Contact Time: With raw water DO and nitrate concentrations of 10 mg/L and 30 mg/L, respectively, it was anticipated that an EBCT of ~20 minutes would be required to achieve sustained perchlorate removal to below detection across F130. As indicated above, sustained perchlorate removal to below detection was achieved at EBCT as low as 5 minutes. A design EBCT of 10 minutes was selected for the cost estimates and facility lay-outs generated during this project.

Acetic Acid Dose: As shown in Figure B.8, sustained perchlorate removal was achievable when the [D/A] was 1.6 or greater (i.e., 60% above stoichiometric raw water acetic acid demand based on DO and nitrate concentrations). When [D/A] was 1.5 and 1.4, 20% and 40% perchlorate breakthrough was observed, respectively. A design [D/A] of 1.7 was selected for the cost estimates generated during this project.

Nutrient Dose: A phosphoric acid dose of ≥ 100 μg/L as PO_4-P was required to achieve sustained perchlorate removal to below detection. When no phosphoric acid was added, 40-60% perchlorate breakthrough was observed (Figure B.8). A design phosphoric acid dose of 150 μg/L as PO4-P was selected for the cost estimates generated during this project.

Raw Water Dissolved Oxygen and Nitrate Concentrations: Raw water nitrate concentrations matched the historical raw water quality data provided by the City of Rialto. No historical raw water DO concentrations were available, and start-up revealed that the raw water was supersaturated with gas. A spray nozzle was added to the raw water break tank to remove dissolved gas, and resulting feed DO concentrations were 8-10 mg/L.

Backwash Effectiveness: The ability of pilot-scale filters to effectively simulate a full-scale backwash system is severely limited for two reasons: 1) Uniformity of backwash and air scour flow is difficult to control at the pilot scale due to limitations in the underdrain system, and 2) It is difficult to control media loss during a pilot-scale backwash, which means that a simultaneous air scour/fluidization step must be very short. To get around these limitations, a 28-step backwash procedure was utilized that summed to fluidization (with surface wash) for 69 seconds at 4.8 gpm/ft^2, 12.7 gpm/ft^2 for 180 seconds, 3.2 gpm/ft^2 for 120 seconds, 6.7 gpm/ft^2 for 480 seconds, and 1.3 gpm/ft^2 for 30 seconds. 2-3.2 SCFM/ft^2 air scour was pulsed during the fluidization steps for a total of 24 seconds. Run times varied between 17 and 24 hours. It should be noted that a full-scale backwash procedure would likely include four steps: 1) drain, 2) air

scour (one loading rate), 3) combined air scour/fluidization (one loading rate for air scour and one loading rate for fluidization), and 4) fluidization (one loading rate).

A good metric for backwash effectiveness is low, consistent clean-bed headlosses. Figure B.11 shows typical head loss trends in Bioreactor F130. Clean-bed head losses were typically ~0.5 psig (1.2 feet), while the headloss at the end of a 17-24-hour run was typically just above 1 psig (2.3 feet).

Process Upsets: Several robustness tests were run using Bioreactor F130. These tests included backwash testing, system shutdowns, acetic acid shutdowns, perchlorate spiking, and nitrate spiking.

- ***Backwashing.*** Figure B.13 shows the results of the high-resolution backwash testing. For this test, a backwash was performed, and the pilot was then returned to production mode. Perchlorate samples were taken immediately after the backwash and at 15-minute intervals for 120 minutes. No perchlorate was detected.

- ***Perchlorate Spiking.*** Figure B.21 shows the results of the perchlorate spiking tests. Step changes in perchlorate simulate actual well field operations where pumps with differing water qualities come on and off line at different times and at varying intervals. Transient perchlorate loading episodes had very little impact on perchlorate removal performance in Bioreactor F130. Over an 11 day period, the feed perchlorate concentration was varied in step changes from 100 µg/L to 400 µg/L to 600 µg/L to 800 - 930 µg/L and back to the background concentration of 55 µg/L while the EBCT and the feed [D/A] ratio were maintained at 10 minutes and 1.70, respectively. For the majority of the test, the perchlorate concentration was at or below the limit of detection.

- ***Nitrate Spiking.*** Figure B.22 presents the results of the nitrate spiking tests performed on Bioreactor F130. Step changes in nitrate simulate actual well field operations where pumps with differing water qualities come on and off line at different times and at varying intervals. During the nitrate spiking tests, nitrate feed concentrations to the reactor were step-increased from 30 mg/L (background) to 38 mg/L and then to 45 mg/L (all as NO_3^-). During this test, the EBCT was constant at 10 minutes, and a [D/A] ratio of 1.70 was maintained. No perchlorate or nitrate breakthrough was observed.

- ***System-Shut Down Periods.*** Figures B.14 and B.15 show the results from the 24-hour and one-week shutdown tests, respectively. After each shutdown period, the pilot was put back into production and high-resolution samples were taken over the next 24-hour period. No perchlorate breakthrough was observed during either test.

- ***Acetic Acid Feed Failures.*** Simulated acetic acid feed failure experiments demonstrated that up to 10 hours are available after an acetic acid feed pump failure before perchlorate breakthrough occurs. The maximum perchlorate breakthrough after a 24-hour acetic acid

feed pump shut-off was 11 μg/L. After the pump was restarted at Hour 24, perchlorate removal to below detection was again achieved after approximately 4 hours. See Figures B.16-B.20.

6.2.4 Process Waste

Process Efficiency: System recovery is defined as the volume of treated raw water recovered for distribution OR [(total volume of water treated minus total losses)/total volume of water treated]*100. During the Optimal Operation testing phase, system recoveries were 93-96%. Higher recoveries are anticipated for a full-scale system, as air scour and backwash fluidization steps will likely be more efficient.

Used Media: Tables B.2 and B.3 present the results of the metals/uranium and trace organics media characterization tests, respectively. Hazardous waste threshold values are also provided, as defined in the California Code of Regulations, Title 22, Chapter 11, Article 3, Section 66261. No trace organics were detected in the leachate from the spent media sample. Additionally, minimal metals accumulation occurred on the GAC and all metals were detected below their hazardous waste threshold values. Fassell (2008) provided the uranium threshold values above which a waste is classified as low-level radioactive waste. Uranium detected on the media was well below the threshold value. No trace organics were detected on the media. Media disposal is expected to occur approximately every 10 years, at which point media characterization tests would need to be performed to identify appropriate disposal options.

6.2.5 Robustness/Reliability

Sustained Removal: See the Perchlorate subsection of Section 6.2.2.

Performance During and After Upsets: See the Process Upsets subsection of Section 6.2.3

6.2.6 Effluent Quality

Dissolved Oxygen: DO going into Biofilter F150 was typically < 1 mg/L and hydrogen peroxide was dosed to F150 at between 8 and 12 mg/L. The F150 effluent DO concentration averaged 5.3 mg/L and ranged from 1-12 mg/L.

Biodegradable Organic Carbon: See the Biodegradable Organic Carbon subsection of Section 6.2.2

Hydrogen Sulfide: Hydrogen sulfide was monitored daily. Occasionally, analytical hydrogen sulfide measurements were taken. However, since the human olfactory system has a lower hydrogen sulfide detection limit (~0.5 μg/L) than field-based analytical techniques (~10-20 μg/L), an olfactory-based presence/absence data point was recorded each day for all sample locations throughout demonstration testing. No hydrogen sulfide was detected analytically. A

slight hydrogen sulfide smell was detected in the effluent of F130 daily, but none was detected in the effluent of F150.

Disinfection By-Products: Figure B.23 shows the results of the three DBPFP tests. Each of the raw samples produced no appreciable HAA or TTHM. DBPFP was low coming out of Bioreactor F130 (21-42 μg/L HAA5 and 22-34 μg/L TTHM) and decreased across Biofilter F150 (6-15 μg/L HAA5 and 8-15 μg/L TTHM). Thus, all DBP measurements were well below Federal MCL.

Heterotrophic Plate Counts: HPC coming out of F130 and F150 were too-numerous-to-count (>5,700 counts/mL) Throughout the majority of pilot testing, free chlorine doses of 1-2 mg/L as Cl_2 were used for the final disinfection step, leaving residuals of approximately 0.5-1.2 mg/L as Cl_2. Based on a tracer test, the t_{10} through the chlorine contact tank was 17 minutes (i.e., CT = 8.5-20.4 mg-min/L) when an EBCT of 10 minutes was used through the post-treatment biofilter. Typical resultant HPC in the effluent of the chlorine contact tank were 1-35 CFU/mL.

A CT of 2 mg-min/L was also tested. This CT was achieved through two conditions: 1) a chlorine residual of 0.l2 mg/L as Cl_2 + a t_{10} of 17 minutes, and 2) a chlorine residual of 0.20 mg/L as Cl_2 + a t_{10} of approximately 10 minutes. Resultant HPC in the effluent of the chlorine contact tank were 44-430 CFU/mL.

6.2.7 Hazards

The primary hazards associated with FXB biological perchlorate treatment are associated with chemicals such as acetic acid, phosphoric acid, hydrogen peroxide, and chlorine. All these chemicals are NSF-60-Certified for drinking water applications, and standard protocols can be followed when they are stored and handled.

6.2.8 Scale-Up Constraints

Heterogeneity of Biological Growth: Meaningful depthwise pressure measurements were difficult to acquire as the depthwise sampling ports were typically blocked by biogrowth. Visual inspection showed that the heaviest biogrowth occurred in the top 12-24 inches of each bed, as expected. The biogrowth was a milky white and appeared evenly distributed at a given depth. Biogrowth was also observed in the deeper portions of the beds, but it appeared much less dense. Most importantly, the observed heterogeneity in biogrowth patterns did not appear to cause any short-circuiting through the bioreactors.

Backwash Effectiveness: See the Backwash Effectiveness subsection of Section 6.2.3.

6.2.9 Hazardous Material

See the Used Media subsection of Section 6.2.4.

7. COST ASSESSMENT

7.1 Cost Drivers

The main cost driver for FXB biological perchlorate removal systems is the concentration of DO and nitrate in the raw water. Because the presence of DO and nitrate inhibits biological perchlorate reduction, raw water DO and nitrate must be removed before perchlorate reduction to below detection is achieved. Therefore, the bioreactor system must be sized so that sufficient contact time (i.e., EBCT) is provided for the bacteria to reduce DO and nitrate. Perchlorate concentrations in groundwater are typically several orders of magnitude lower than DO and nitrate concentrations in groundwater. Therefore, no contact time beyond that provided for DO and nitrate reduction is necessary, regardless of raw water perchlorate concentration or target effluent perchlorate concentration. During this demonstration, feed water perchlorate concentrations were spiked all the way up to ~1 mg/L, and sustained perchlorate removal to below detection was achieved using the same EBCT and acetic acid dose used to remove background concentrations of perchlorate (~54 μg/L) to below detection.

It is interesting to note that the required EBCT (i.e., reactor sizing) is not nearly as sensitive to raw water DO and nitrate concentrations as originally thought. Performance data from this demonstration study and from a FXB biodenitrification pilot study recently completed in Riverside, California (Brown, 2008) support this assertion. During this demonstration study, with average raw water DO and nitrate (as NO_3^-) concentrations of 8 and 28 mg/L, respectively, perchlorate removal to below detection was achieved using an EBCT of 5 minutes (lowest EBCT tested), resulting in a design EBCT of 10 minutes. During the Riverside biodenitrification pilot testing, average raw water DO and nitrate (as NO_3^-) concentrations were 3 and 75 mg/L, respectively. Nitrate was removed to below 5 mg/L at the shortest EBCT tested, 5.8 minutes, suggesting that effective perchlorate removal could be achieved using very short EBCT, even when nitrate concentrations in the raw water are very high. The design EBCT for the biodenitrification system in Riverside was also set at 10 minutes. *This relative insensitivity of design EBCT to raw water quality is an important aspect of the FXB bioreactor process.*

Raw water DO and nitrate concentrations directly impact Operation and Maintenance (O&M) costs. Regardless of required EBCT, sufficient acetic acid must be dosed to the system to remove raw water DO and nitrate before achieving complete perchlorate removal. Since acetic acid dosing requirements are a function of stoichiometric oxidation, reduction, and cell synthesis reactions, the required acetic acid dose increases and decreases proportionally with increases and decreases in raw water DO and nitrate concentration. The cost model developed during this study showed that acetic acid costs account for over 80% of the total annual O&M costs of a

FXB biological perchlorate treatment system. Thus, fluctuations in unit acetic acid costs or raw water DO and nitrate concentrations will have a substantial impact on O&M costs.

7.2 Cost Assessment

This assessment was designed to provide a complete project cost estimate, including design, construction, and annual operating and maintenance costs for the 30-year lifecycle of the system.

7.3 Cost Model Basis

The cost model used to develop this assessment is based on data collected during the FXB biological perchlorate destruction demonstration conducted at the City of Rialto Well #2. Optimal operating criteria were developed during the pilot demonstration for key system parameters such as:

- GAC bed depth;
- Filter media depth;
- Empty bed contact time;
- Backwash frequency;
- Chemical dosages;

The design criteria from the demonstration have been combined with our extensive knowledge of project development and construction cost components to provide this detailed assessment.

7.3.1 Treatment Capacity Assessments

A 1,000-gpm system and a 2,000-gpm system were evaluated to demonstrate economies-of-scale. A process flow diagram and conceptual facility layouts for the 1000-gpm system and the 2,000-gpm system are provided in Figures 7.1, 7.2, and 7.3, respectively.

7.3.2 Basis of Design

The cost assessment includes redundant equipment for critical subsystems to provide reliability and to meet regulatory requirements. A standby bioreactor vessel and a standby biofilter vessel are included in the basis of design for use during backwash periods or media replacement maintenance to allow for uninterrupted operation per regulatory requirements. Similarly standby chemical metering pumps, back wash water pumps, and backwash air scour blowers are included to ensure system reliability. Chemical bulk storage is provided for 30-days of operation between product deliveries as required by Ten States Standards.

Figure 7.1 - Process Flow Diagram.

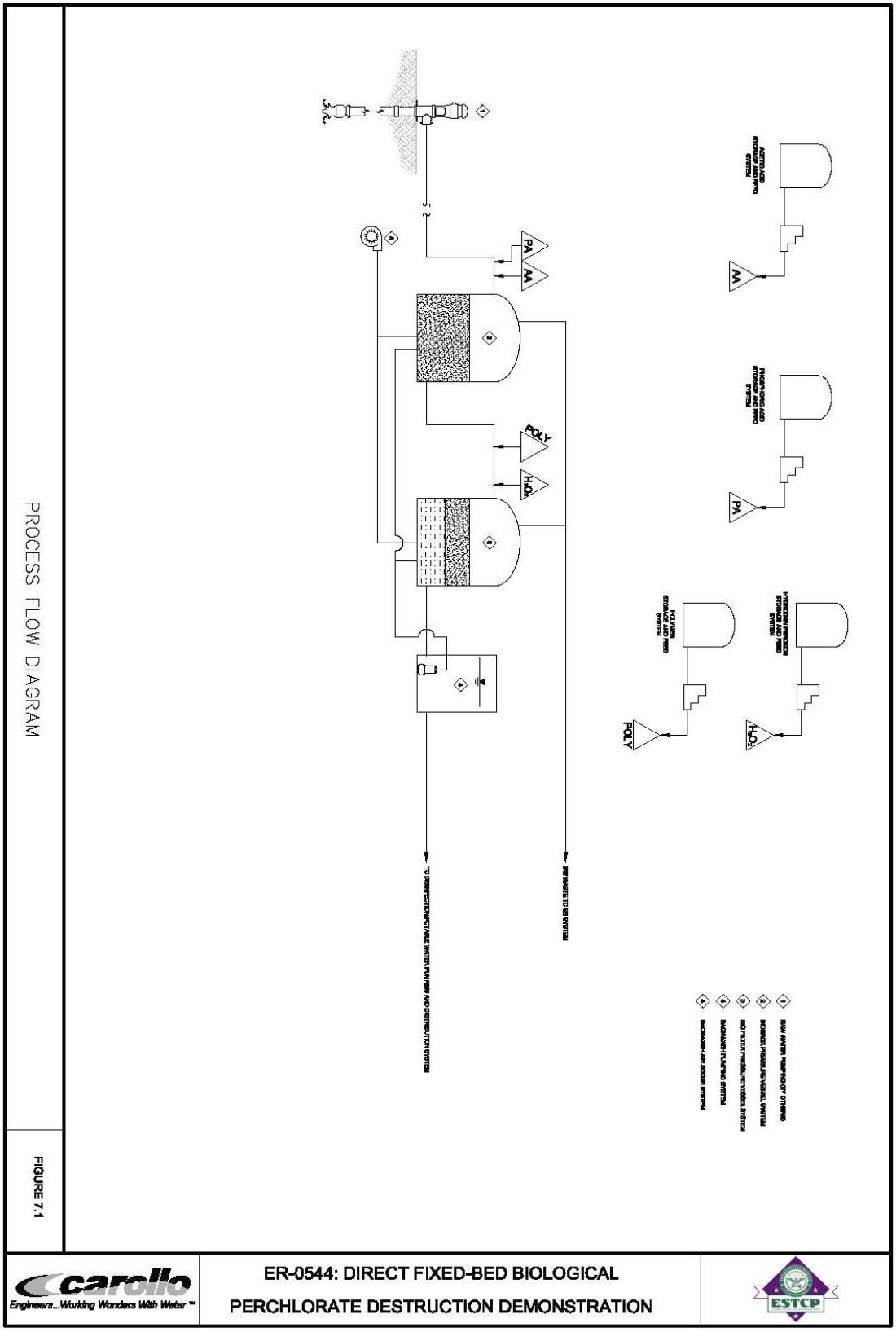


Figure 7.2 - 1000 gpm Conceptual Site Plan.

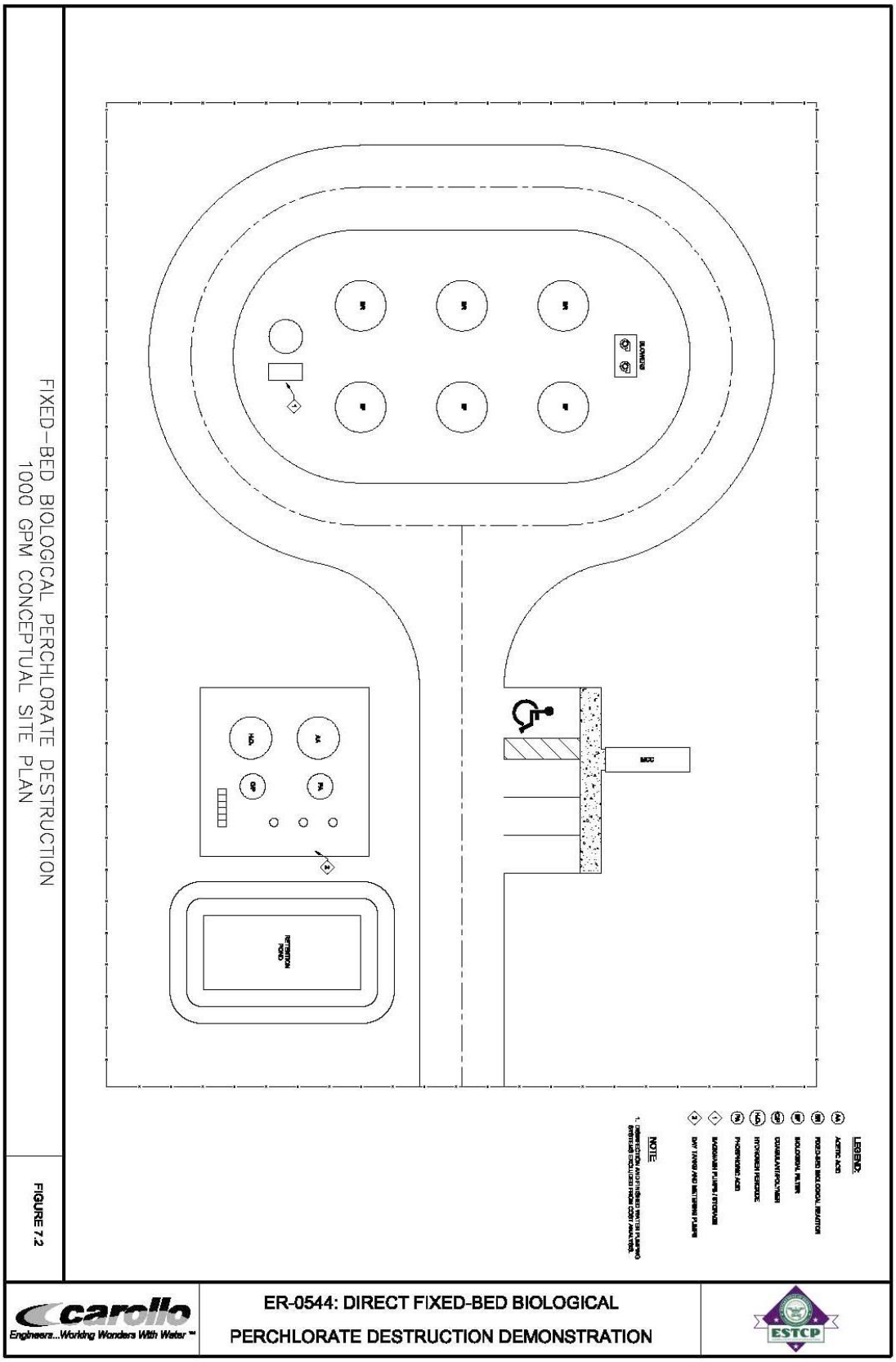
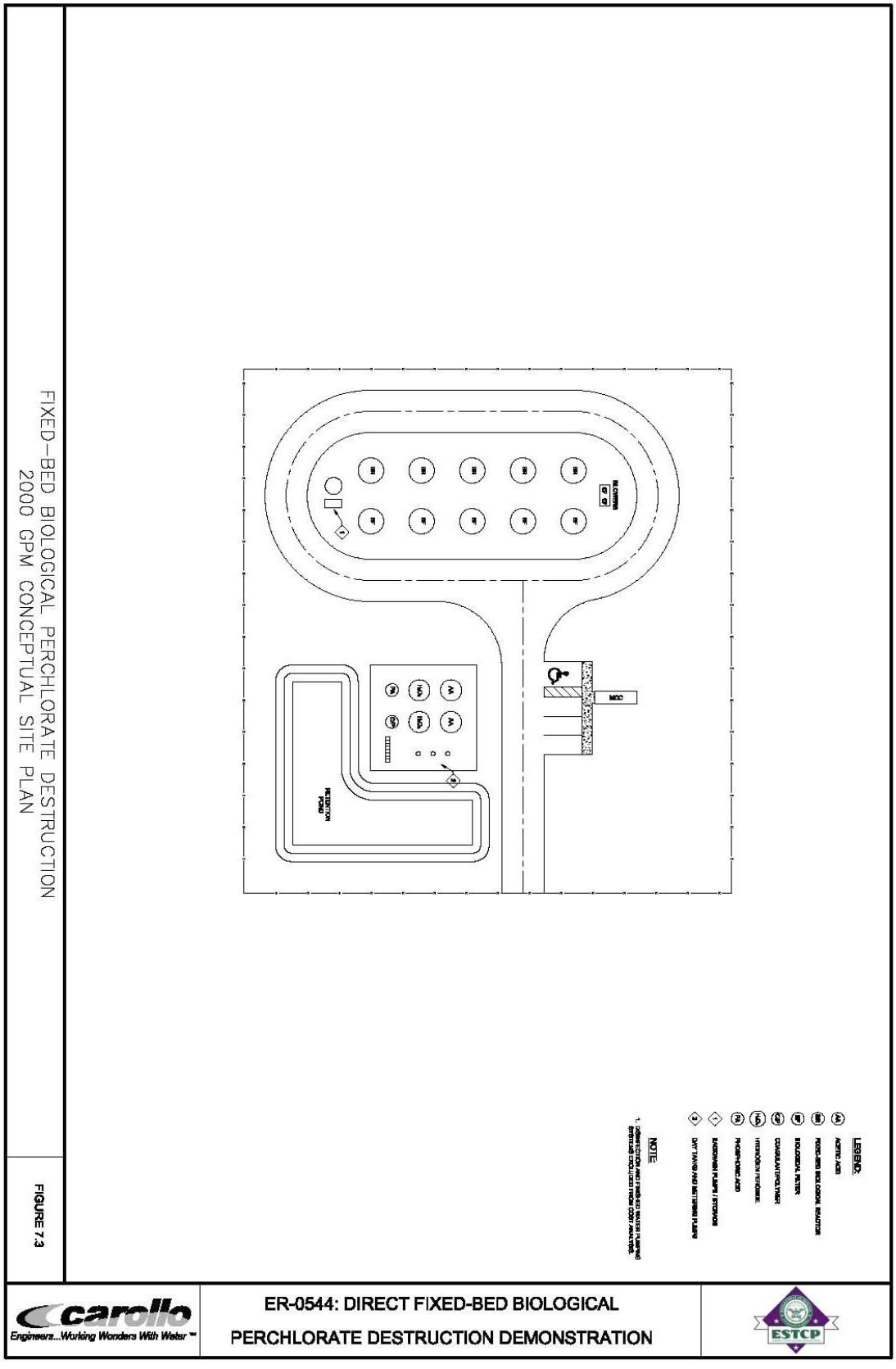


Figure 7.3 - 2000 gpm Conceptual Site Plan.



The demonstration study showed that perchlorate removal to below detection was achieved and sustained at EBCT as low as 5 minutes, which was the lowest EBCT tested. The resultant EBCT in the 1,000- and 2,000-gpm system cost estimates below are 8.5 minutes and 10.6 minutes, respectively, thereby providing considerable flexibility in capacity/contact time for each system. This assessment assumes that an in-line perchlorate ion chromatograph, which carries a sizable price-tag, will always be required during full-scale operation. Table 7.1 provides details of the basis of design system components.

Table 7.1 - Basis of Design Criteria.

Description	Finished Water Flowrate		
	Units	1,000 gpm	2,000 gpm
Biological Reactor System			
Number of Vessels (total)	No.	3	5
Redundant Vessels	No.	1	1
Flow per Vessel	gpm (mgd)	500 (0.72)	500 (0.72)
Perchlorate Levels Influent/Effluent	µg/L	5-1000/Nondetect.	
Biological Filtration System			
Number of Vessels (total)	No.	3	5
Redundant Vessels	No.	1	1
Flow per Vessel	gpm (mgd)	500 (0.72)	500 (0.72)
Turbidity[1] (effluent)	NTU	0.2	0.2
Acetic Acid System			
Metering Pumps (total)	No.	2	2
Redundant Metering Pumps	No.	1	1
Bulk Storage (30-day supply)	gal	4,100	8,100
Phosphoric Acid System			
Metering Pumps (total)	No.	2	2
Redundant Metering Pumps	No.	1	1
Bulk Storage (30-day supply)	gal	30	60
Coagulant System			
Metering Pumps (total)	No.	2	2
Redundant Metering Pumps	No.	1	1
Bulk Storage (30-day supply)	gal	130	260
Back Wash System			
Number of BW Water Pumps (total)	No.	2	2
Redundant BW Water Pumps	No.	1	1
Number of BW Air Scour Blowers	No.	2	2
Redundant BW Air Scour Blowers	No.	1	1
Number BW Water Holding Tank	No.	1	1
Volume BW Water Holding Tank	gal	1,000	2,000
Inline Perchlorate Analyzer			
Number of Perchlorate Analyzers	No.	1	1

	Manufacturer/Model/Type	DIONEX /DX900/Ion Chromatograph
Raw Water Quality	DO = 6 mg/L, NO_3 = 28 mg/L as NO_3^-, Sulfate = 12 mg/L, TDS = 260 mg/L	
[1]Less than or equal to 0.2 NTU in 95% of samples, never to exceed 1.0 NTU per CDPH regulations.		

7.3.3 Project Costs

Project costs include capital costs for system equipment and installation, along with standard project line item costs, including:

- Contractor mobilization/demobilization (1.5% of installed equipment costs),
- Site civil work,
- Yard piping,
- Electrical/I&C (30% of installed equipment costs),
- General Conditions (10% of installed equipment costs),
- Contractor Overhead and Profit (10% of installed equipment costs),
- Sales tax (7.75% of equipment material costs),
- Engineering design services (12% of project costs),
- Engineering construction phase services (4% of project costs),
- Owner's reserve for change orders (5% of project costs).

Excluded from the project cost assessment are:

- Land acquisition costs,
- Major site improvement work, such as fill material or substantial clearing,
- Raw water resource development and pumping/piping system,
- Disinfection system,
- Finished water storage,
- High service pumping system,
- Laboratory or staff office space,
- Bringing utilities to/from the site (water, wastewater, power, communications),
- Environmental assessment of site,
- Architectural accents to structures,
- Owner administration and legal fees.

Table 7.2 lists the detailed line items and estimated project costs for each of the two system capacities in the cost assessment.

Table 7.2 - Project Cost Estimate.

No.	Description	Finished Water Flowrate	
		1,000 gpm	2,000 gpm
1	Mobilization/Demobilization	$ 60,000	$ 107,000
2	Site Civil Installed Cost	$ 23,000	$ 46,000
3	Yard Piping Installed Cost	$ 60,000	$ 90,000
4	Biological Reactor System Installed Cost	$ 808,000	$ 1,511,000
5	Biological Filtration System Installed Cost	$588,000	$ 1,095,000
6	In-line Perchlorate Analyzer Installed Cost	$ 165,000	$ 154,000
7	Equipment Structures Installed Cost	$ 121,000	$ 238,000
8	Electrical/I&C Installed Cost	$ 548,000	$ 959,000
	TOTAL DIRECT INSTALLED COST	$2,373,000	$4,200,000
	CONTINGENCY	$ 366,000	$ 640,000
	GENERAL CONDITIONS	$ 281,000	$ 491,000
	CONTRACTOR OVERHEAD & PROFIT	$ 309,000	$ 540,000
	SALES TAX	$ 125,000	$ 229,000
	ENGINNEERING	$ 563,000	$ 986,000
	OWNER'S RESERVE FOR CHANGE ORDERS	$ 176,000	$ 309,000
	ESTIMATED TOTAL COST	$4,193,000	$7,395,000

7.3.4 Operation and Maintenance Costs

Annualized costs for operation and maintenance are estimated for the major equipment and system consumables based on a 30-year lifecycle. Costs for infrequent consumables, such as the filter sand media with an estimated 10-year life, are adjusted for inflation at 3 percent and distributed over the system lifecycle for inclusion with the annual operation and maintenance costs.

Consumables:

- Hydrogen peroxide 25% ($0.43/lb)
- Acetic acid 50% ($0.86/lb)
- Phosphoric acid 85% ($0.35/lb)
- Polymer 49% ($0.13/lb)
- Media Replacement:
- GAC 10-year life ($25/cf)
- Filter anthracite 10-year life ($10/cf)
- Filter sand 10-year life ($7/cf)
- Power ($0.12/kW-hr)

Excluded from the annual operation and maintenance cost estimate:

- Operations labor (no significant increase to a given utility's workload is anticipated),
- Raw water pumping power,
- Disinfection chemicals,
- Finished water pumping power,
- Minor equipment and lighting power

Table 7.3 provides estimated line item costs for the operations and maintenance.

Table 7.3 - Annual Operations & Maintenance Costs.

No.	Description	Finished Water Flowrate	
		1,000 gpm	2,000 gpm
1	Acetic Acid	$ 144,000	$ 289,000
2	Phosphoric Acid	$ 1,000	$ 2,000
3	Hydrogen Peroxide	$ 15,000	$ 30,000
4	Polymer	$ 1,000	$ 2,000
5	GAC	$6,000	$ 11,000
6	Filter Sand/Anthracite	$ 3,000	$ 6,000
7	Power	$ 5,000	$ 8,000
	ESTIMATED ANNUAL O&M COST	$ 175,000	$ 348,000

7.3.5 Treatment Costs

Per ESTCP requirements, the project costs were amortized utilizing the current Office of Management and Budget Real Discount Rate of 2.8% for the 30-year lifecycle assessment to obtain an annual budget estimate. Table 7.4 summarizes the amortized project costs, the O&M costs, and treatment costs.

Table 7.4 - Treatment Costs.

No.	Description	Finished Water Flowrate	
		1,000 gpm	2,000 gpm
1	Amortized[1] Project Costs	$ 209,000	$ 368,000
2	Annual O&M Costs	$ 175,000	$ 348,000
	ESTIMATED ANNUAL BUDGET	$ 384,000	$ 716,000
	TREATMENT COSTS $/1000-GAL	$ 0.73	$ 0.68
	TREATMENT COSTS $/AC-FT	$ 238	$ 222
[1]Amortized at the current Real Discount Rate of 2.8% and a 30-year lifecycle.			

7.3.6 Economy of Scale

The cost assessment indicates a 6.7% reduction in treatment costs due to economy of scale as the system finished water capacity is increased from 1,000-gpm to 2,000-gpm. Many of the process subsystems, such as air scour blowers, backwash pumps, and metering pumps require no additional equipment to process the increased treatment flowrate due to their flexible range of operation.

In each of the two flow rates assessed, a single standby biological reactor and a single standby biological filter pressure vessel is required to allow a duty vessel to enter a backwash cycle or for periodic maintenance of the media. Increased costs for the 2,000-gpm system include only those costs for duty vessels for the increased treatment capacity without the added cost of additional standby vessels, thus providing significant economy of scale.

7.4 Cost Comparison with Single-Pass Ion Exchange

The only process currently operating at full-scale for removing perchlorate from drinking water is ion exchange (IX). IX systems concentrate perchlorate onto a resin, which is removed and regenerated or incinerated (i.e., single-pass IX) once the resin is exhausted. IX systems are proprietary in nature and therefore cost and system data are not readily available.

Equipment and operational cost data for a 1000 gpm single-pass IX perchlorate selective system was obtained from Siemens Water Technologies Corporation for influent perchlorate concentrations of 50 μg/L and 270 μg/L. The supplier does not recommend the IX system for

perchlorate concentrations of 1000 μg/L. The IX cost data were provided in operating terms of 18 hrs/day, 300 days/year and were proportionally adjusted to operating terms of 24 hrs/day and 365 days/year for comparative purposes in this analysis.

7.4.1 IX Basis of Design

The IX system consists of a lead vessel followed by a polishing lag vessel that constitute a single treatment train. Each treatment train has an operating capacity of 1,000 gpm of finished water. As with the FXB biological system, the cost analysis provided for a redundant train to permit continuous operations during maintenance and resin change-out and to meet regulatory requirements for reliability. Table 7.5 lists the IX basis of design criteria.

Table 7.5 - IX Basis of Design Criteria.

Description		1,000 gpm Finished Water Facility Influent Perchlorate Level[1]		
	Units	50 μg/L	270 μg/L	1000 μg/L
Total Number of IX Lead-Lag Vessel Pairs[2]	No.	2	2	N/A
Redundant IX Lead-Lag Vessel Pairs[2]	No.	1	1	N/A
Effluent Perchlorate Level	μg/L	< 4μg/L		N/A
[1] Other raw water quality: DO = 6 mg/L, NO_3 = 28 mg/L as NO_3^-, Sulfate = 12 mg/L, TDS = 260 mg/L [2] One lead-lag vessel pair constitutes a single 1000 gpm finished water treatment system				

7.4.2 IX Project Costs

IX project costs include capital costs for system equipment and installation, along with standard project line item costs, including:

- Contractor mobilization/demobilization (1.5% of installed equipment costs),
- Site civil work,
- Yard piping,
- Electrical/I&C (30% of installed equipment costs),
- General Conditions (10% of installed equipment costs),
- Contractor Overhead and Profit (10% of installed equipment costs),
- Sales tax (7.75% of equipment material costs),
- Engineering design services (12% of project costs),
- Engineering construction phase services (4% of project costs),
- Owner's reserve for change orders (5% of project costs).

Excluded from the project cost assessment are:

- Land acquisition costs,
- Major site improvement work, such as fill material or substantial clearing,
- Raw water resource development and pumping/piping system,
- Disinfection system,
- Finished water storage,
- High service pumping system,
- Laboratory or staff office space,
- Bringing utilities to/from the site (water, wastewater, power, communications),
- Environmental assessment of site,
- Architectural accents to structures,
- Owner administration and legal fees.

Table 7.6 summarizes the project cost data for 1,000 gpm IX facilities at varying influent perchlorate levels.

Table 7.6 - IX Project Cost Estimate.

No.	Description	1,000 gpm Finished Water Facility Influent Perchlorate Level[1]		
		50 µg/L	270 µg/L	1000 µg/L
1	Mobilization/Demobilization	$ 53,000	$ 53,000	N/A
2	Site Civil Installed Cost	$ 23,000	$ 23,000	N/A
3	Yard Piping Installed Cost	$ 60,000	$ 60,000	N/A
4	IX System Installed Cost	$ 1,544,000	$ 1,544,000	N/A
5	Electrical/I&C Installed Cost	$ 489,000	$ 489,000	N/A
	TOTAL DIRECT INSTALLED COST	$ 2,169,000	$ 2,169,000	N/A[1]
	CONTINGENCY	$ 326,000	$ 326,000	N/A[1]
	GENERAL CONDITIONS	$ 250,000	$ 250,000	N/A[1]
	CONTRACTOR OVERHEAD & PROFIT	$ 275,000	$ 275,000	N/A[1]
	SALES TAX	$ 110,000	$ 110,000	N/A[1]
	ENGINNEERING	$ 501,000	$ 501,000	N/A[1]
	OWNER'S RESERVE FOR	$ 157,000	$ 157,000	N/A[1]

	CHANGE ORDERS			
	ESTIMATED TOTAL COST	$ 3,788,000	$ 3,788,000	N/A[1]
[1] Other raw water quality: DO = 6 mg/L, NO_3 = 28 mg/L as NO_3^-, Sulfate = 12 mg/L, TDS = 260 mg/L				

Annualized costs for operation and maintenance are estimated for the major equipment and system consumables based on a 30-year lifecycle.

Consumables:

- IX Resin
- Influent perchlorate = 50 μg/L ($219,000 per year)
- Influent perchlorate = 270 μg/L ($412,000 per year)
- Influent perchlorate = 1000 μg/L (NA)
- Power ($0.12/kW-hr)

Excluded from the annual operation and maintenance cost estimate:

- Operations labor (no significant increase to a given utility's workload is anticipated),
- Raw water pumping power,
- Disinfection chemicals,
- Finished water pumping power.

Table 7.7 - IX Annual Operations & Maintenance Costs.

No.	Description	1,000 gpm Finished Water Facility Influent Perchlorate Level[2]		
		50 μg/L	270 μg/L	1000 μg/L
1	Power (<100 kW/yr)	-	-	N/A[1]
2	IX Resin Replacement & Disposal	$ 219,000	$ 412,000	N/A[1]
	ESTIMATED ANNUAL O&M COST	$ 219,000	$ 412,000	N/A[1]
[1] Treatment of perchlorate at 1000 μg/L is not recommended by the IX supplier. [2] Other raw water quality: DO = 6 mg/L, NO_3 = 28 mg/L as NO_3^-, Sulfate = 12 mg/L, TDS = 260 mg/L				

7.4.3 IX Treatment Costs

The IX project costs are amortized utilizing the current Office of Management and Budget Real Discount Rate of 2.8% for the 30-year lifecycle assessment to obtain an annual budget estimate. Table 7.8 summarizes the amortized project costs, the O&M costs, and treatment costs.

Table 7.8 - Summarized IX Treatment Costs.

No.	Description	1,000 gpm Finished Water Facility Influent Perchlorate Level[1]		
		50 μg/L	270 μg/L	1000 μg/L
1	Amortized[2] Project Costs	$ 189,000	$ 189,000	N/A
2	Annual O&M Costs	$ 219,000	$ 412,000	N/A
	ESTIMATED ANNUAL BUDGET	$ 408,000	$ 601,000	N/A
	TREATMENT COSTS $/1000-GAL	$ 0.78	$ 1.14	N/A
	TREATMENT COSTS $/AC-FT	$ 254	$ 372	N/A

[1] Treatment of perchlorate at 1000 μg/L is not recommended by the IX supplier.
[2]Amortized at the current Real Discount Rate of 2.8% and a 30-year lifecycle.

7.4.4 Treatment Cost Comparison

When the raw water perchlorate concentration is approximately 50 μg/L, total treatment costs for the FXB biological system and the single-pass IX system are comparable (approximately $240-$250/AF for a 1,000-gpm system). As raw water perchlorate concentrations increase, the cost of the FXB biological system does not change, while the cost of the single-pass IX system increases (see Figure 7.4). The relative insensitivity of the FXB biological process to raw water perchlorate (and nitrate concentration - See Section 7.1) provides confidence that a FXB biological system installed today will be effective in the future without the need for additional treatment capacity even if raw water perchlorate (or nitrate) levels increase. Perhaps the most important difference between the two treatment approaches is that while the single-pass IX system only removes perchlorate, the FXB biological process removes nitrate and perchlorate in a single bioreactor. Other contaminants, such as halogenated organics, can be removed simultaneously in the FXB bioreactor as well (Brown, 2008), making the FXB biological system particularly well suited for multiple-contaminant treatment applications.

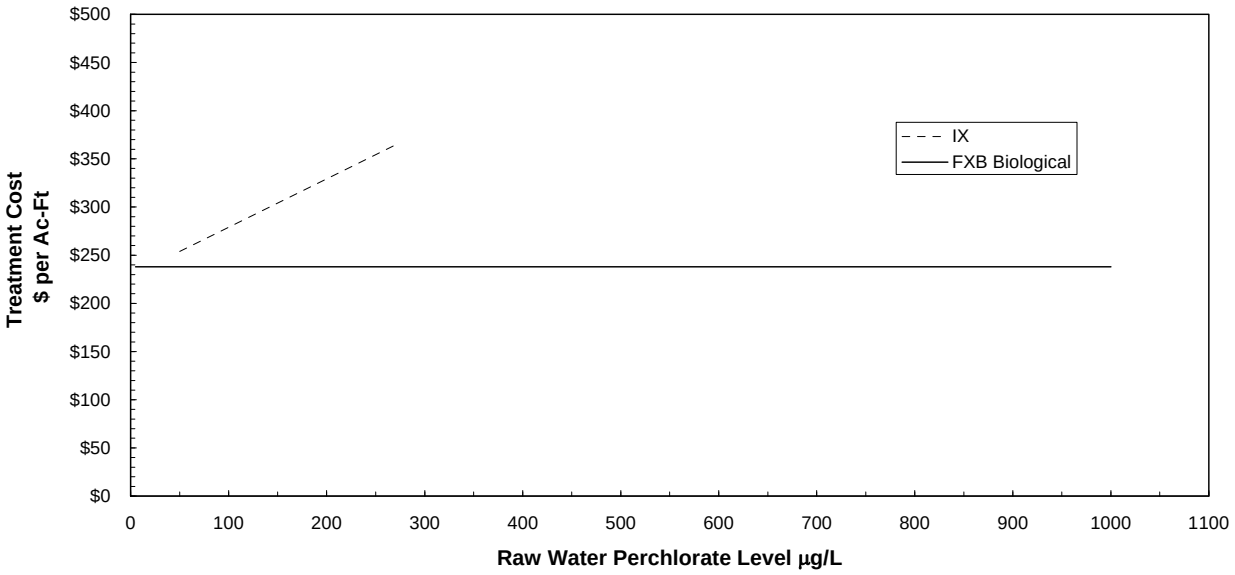


Figure 7.4 - FXB Biological and Single-Pass IX Treatment Costs as a Function of Raw Water Perchlorate Concentration.

8. IMPLEMENTATION ISSUES

8.1 Regulatory Considerations

Any full-scale, potable FXB biological perchlorate treatment process would be subject to all federal and state drinking water regulations. The majority of applicable drinking water regulations are established and well known. However, regulations associated specifically with potable biological perchlorate and nitrate treatment facilities have not been finalized. The CDPH is in the process of finalizing these regulations, which will deal with issues such as inactivation requirements, turbidity limits, and water quality monitoring requirements.

In addition to these established and emerging drinking regulations, which primarily apply to distributed water quality, utilities will also have to consider how to handle the backwash wastewater. This waste stream should be ≤ 3% of the total water treated and, as described in Section 5.0 of Appendix B, backwash wastewater is low strength. Therefore, it is expected that it can be discharged to the local sewer in many instances, though this would have to be confirmed on a site-specific basis. If no sewer discharge is allowable at a given site, a wastewater clarification and recycle process would need to be considered.

Lastly, a permit for full-scale installation and operation of a potable, FXB biological perchlorate treatment system must be applied for and received from the CDPH. Conditional CDPH approval for full-scale implementation of the FXB process was granted to Carollo Engineers in 2004 and discussions with CDPH in February 2008 indicated that, based on the performance data from various FXB biological perchlorate and nitrate treatment pilot studies, full-scale FXB biological treatment facility permitting should follow the standard schedule and protocol for any new water treatment facility in California.

8.2 End-User Issues

Previous bench- and pilot-scale testing showed that FXB biological perchlorate treatment is promising, and it led to the CDPH Conditional Approval for full-scale process implementation. The results of this Environmental Security Technology Certification Program (ESTCP) demonstration study showed that: 1) as FXB bioreactor treatment systems scale up, process efficiencies also go up (i.e., required contact times to achieve sustained, robust perchlorate removal decreased substantially relative to contact time requirements established during previous, smaller scale studies), 2) hydrogen peroxide reoxygenation, polishing filtration, and chlorination provide effective post-treatment, 3) system operation is straightforward, requiring no specialized training, 4) the bacterial communities in these systems are largely gram-negative Proteobacteria, 5) site-specific performance of these systems can be predicted using a mathematical model developed as part of this demonstration, and 6) total water production costs for a FXB system can be low.

In spite of the numerous strengths of FXB systems demonstrated during this project, one significant obstacle still hinders the widespread realization of these systems at full-scale: the lack

of the first full-scale, potable FXB biological perchlorate treatment facility. Though full-scale, anoxic/anaerobic FXB biological treatment processes have been used in Europe for over 20 years to remove nitrate from drinking water, no such facilities exist in the United States for perchlorate or nitrate treatment. Since it is more comfortable to select a process for full-scale treatment when there is a full-scale track record to affirm the selection, it is not easy for stakeholders to choose a novel process for their treatment system. In other words, the primary end user issue relates to the willingness to design, install, and operate a process with no full-scale track record. The most important outcome of this demonstration is that the results strongly suggest that this risk is small, while the potential benefits are considerable.

8.3 Procurement

While the expertise to design and operate a FXB biological perchlorate treatment system is not common in the drinking water industry, the process itself is not proprietary. FXB biotreatment is a modified form of standard granular media filtration and therefore would be procured through a typical bidding process. Specifications for the FXB bioreactor vessel have been developed based on 1) the performance observed during demonstration testing, 2) the Project Team's experience with two full-scale FXB bioreactor projects (different applications but similar characteristics), and 3) full-scale European biodenitrification experience.

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Appendix A - Points of Contact

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Appendix B - Demonstration Testing Results

1.0 Biological Acclimation Testing Phase

The purpose of the biological acclimation testing phase was to develop conditions in the bioreactors that would allow bacteria indigenous to water from Rialto Well #2 to remove perchlorate. In this phase, well water, and acetic acid were fed to the reactor to develop the perchlorate-reducing biological activity. Since only partial perchlorate removal was achieved during the first few months of operation, phosphoric acid dosing started on Day 96. Typical raw water Dissolved Oxygen (DO), nitrate, and perchlorate concentrations throughout pilot testing were 8-10 mg/L, 25-30 mg/L (as NO_3^-), and 45-50 μg/L, respectively.

1.1 Bioreactor F120

Figure B.1 shows the results of the biological acclimation testing phase for Bioreactor F120. Initially some perchlorate removal was observed due to adsorption of perchlorate onto the virgin Granular Activated Carbon (GAC). On Day 8, some perchlorate breakthrough was observed as the carbon's adsorption capacity for perchlorate diminished. These data are consistent with other observed perchlorate/GAC breakthrough curves observed in the literature (Na et al., 2002; Brown et. al, 2002). At the beginning of the pilot, little to no nitrate removal was observed. However, on Day 8, bacteria began to remove dissolved oxygen and nitrate, and consistent nitrate removal to below 5 mg/L as NO_3^-) was achieved by Day 20.

1.2 Bioreactor F130

Figure B.2 shows the results of the biological acclimation testing phase for Bioreactor F130. The general trend for the acclimation of reactor F130 was very similar to the trend observed in Bioreactor F120. Initially, some perchlorate removal was observed, followed by later breakthrough as the adsorption capacity for perchlorate diminished. On Day 9, removal of DO and nitrate was observed, followed by a brief breakthrough on Day 12, followed by a continuation of nitrate and dissolved oxygen removal.

1.3 Biofilter F150

Biodegradable Dissolved Organic Carbon (BDOC), Dissolved Organic Carbon (DOC), and turbidity in the effluent of Biofilter F150 through the entire operation of the pilot are shown in Figures B.3, B.4, and B.5, respectively. During the acclimation phase (through Day 20), no hydrogen peroxide was added to the biofilter and no turbidity, DOC nor BDOC data were taken. The Empty Bed Contact Time (EBCT) in the biofilter was 10 minutes for the duration of the biological acclimation testing.

An eductor was used for interstage reoxygenation during the first 97 days of pilot testing. However, two different eductor sizes produced no appreciable DO in the effluent of Biofilter F150. Thus, hydrogen peroxide dosing began on Day 98.

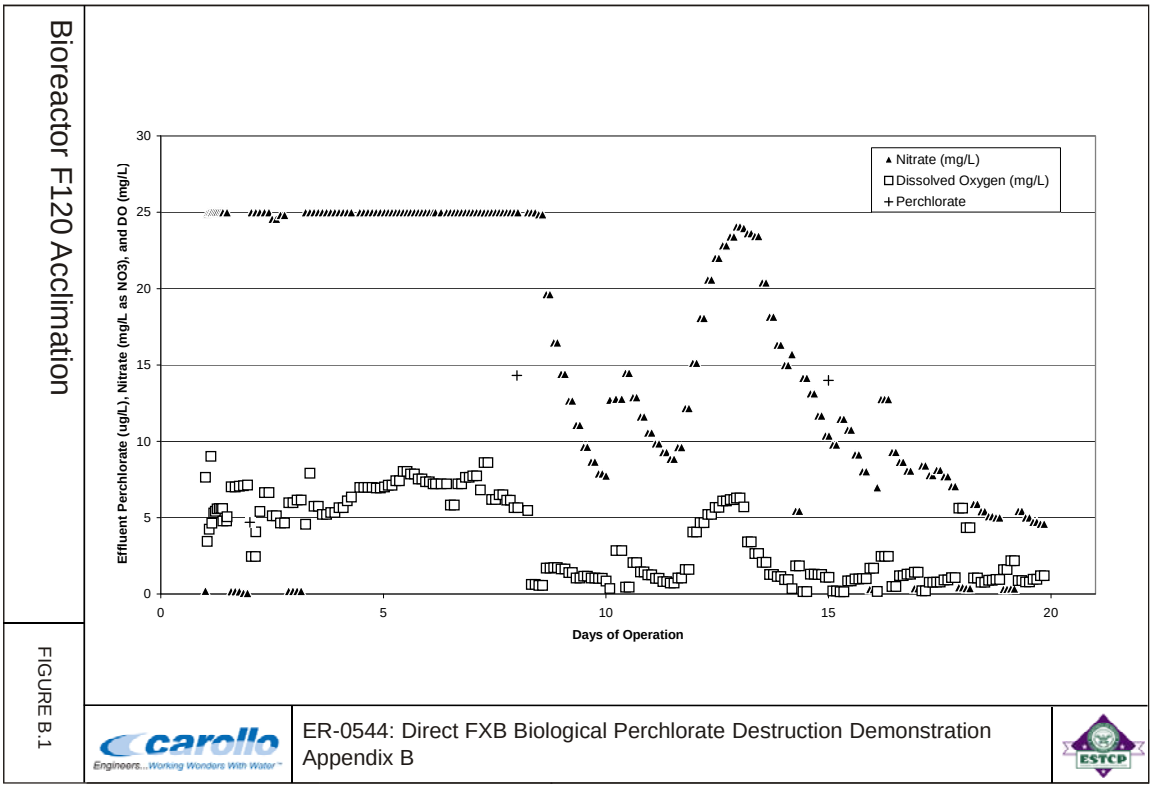

Figure B.1 - Bioreactor F120 Acclimation.

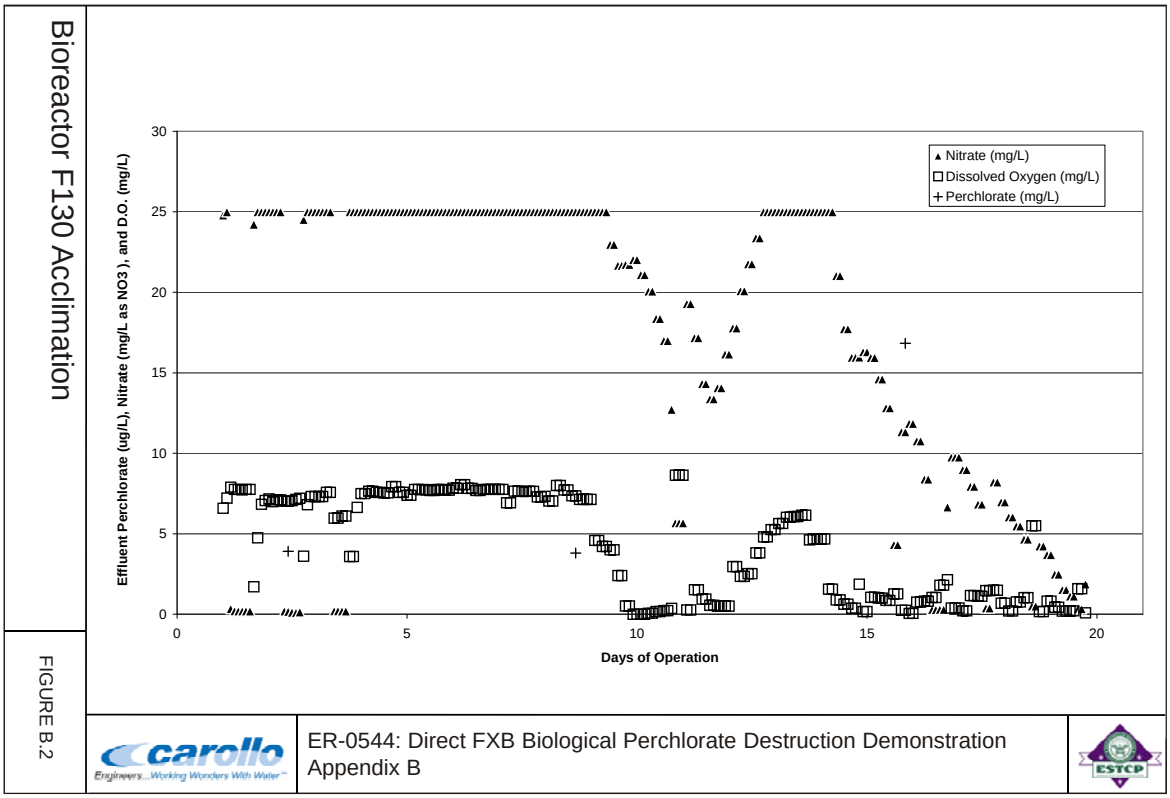

Figure B.2 - Bioreactor F130 Acclimation.

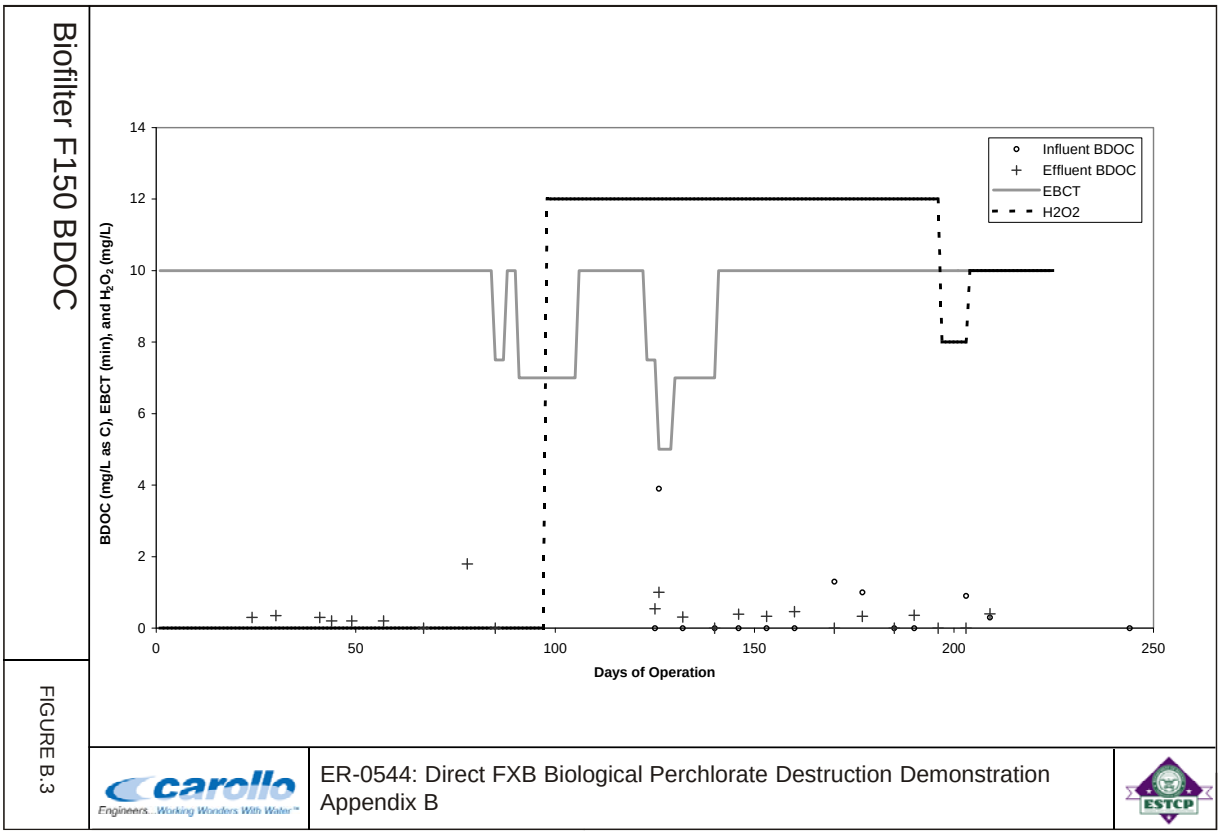

Figure B.3 - Biofilter F150 BDOC.

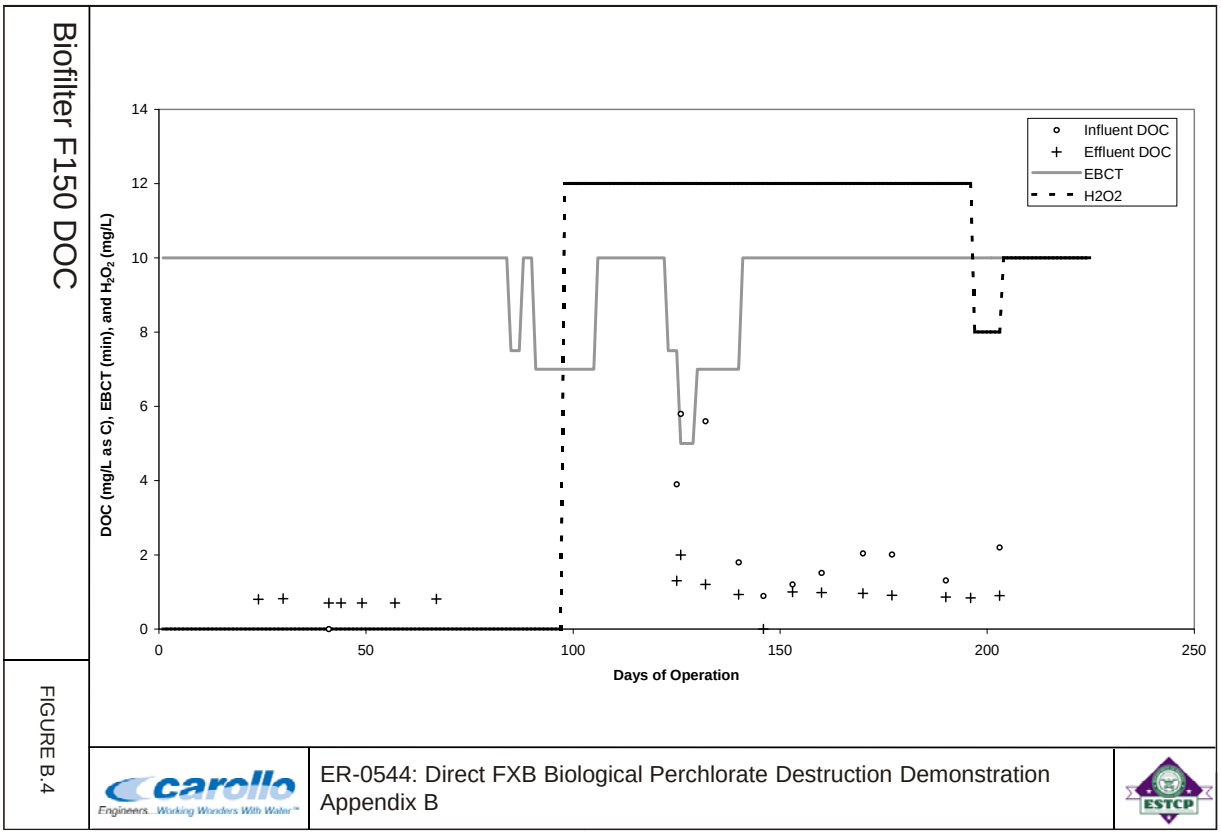

Figure B.4 - Biofilter F150 DOC.

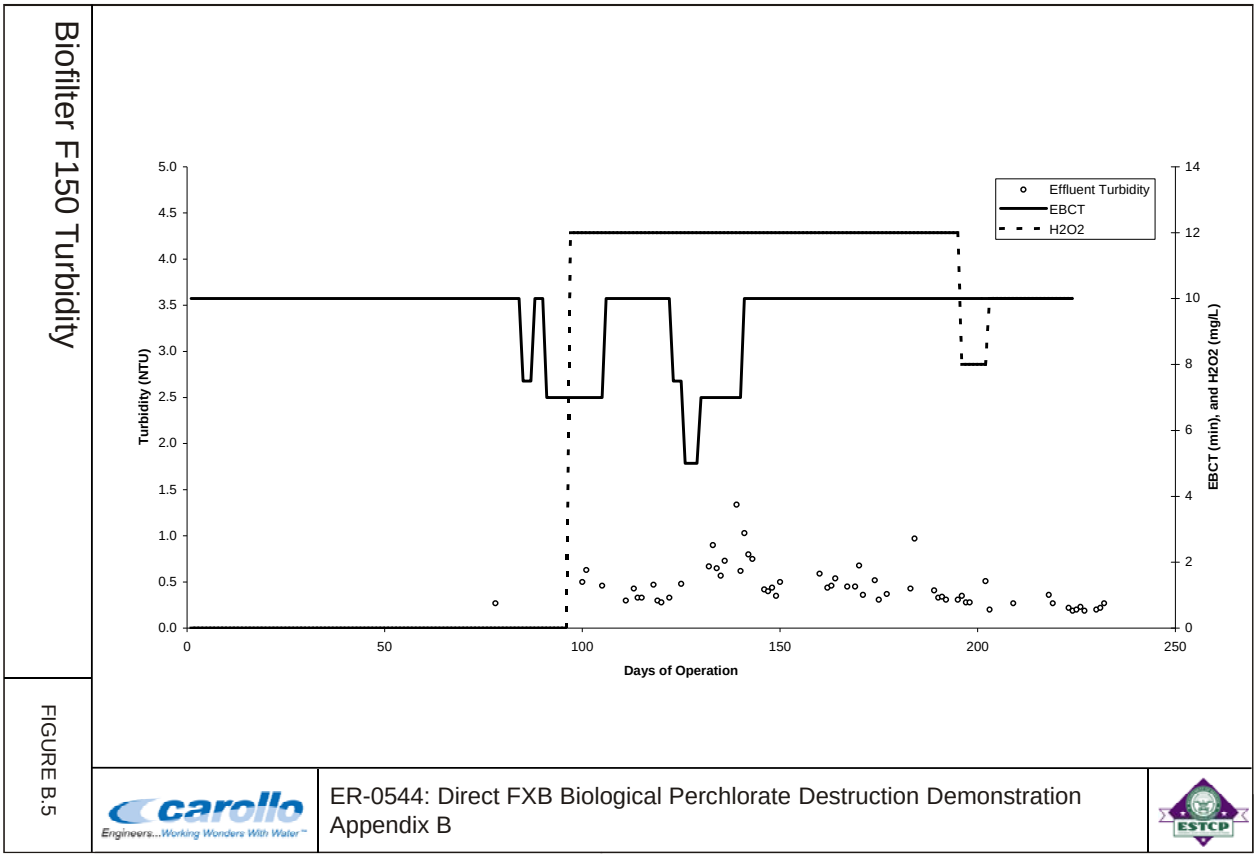

Figure B.5 - Biofilter F150 Turbidity.

1.4 Chlorine Contact Tank

Heterotrophic Plate Count (HPC), total coliforms, fecal coliforms, and free chlorine residual in the effluent of the chlorine contact tank during the acclimation phase (through Day 20) are plotted in Figure B.6. During the acclimation phase, only one data point for those parameters was collected. All parameters except HPC were not detectable. The value of HPC was 2.6 x 10^6 Colony Forming Units (CFU)/mL on Day 9, though no chlorine residual was detected.

2.0 Optimization Testing Phase

The purpose of the optimization testing phase was to determine the conditions under which the bioreactors removed perchlorate most efficiently. This was done by varying electron donor to acceptor ratio ([D/A]), phosphoric acid, and EBCT.

2.1 Bioreactor F120

Figure B.7 shows the results of optimization testing for Bioreactor F120, which utilized a 1.06 [D/A] and an EBCT of 15 minutes initially. Stoichiometric acetic acid percent excess is plotted instead of [D/A] for ease of comparison on a single graph. [D/A] is equal to the stoichiometric acetic acid percent excess divided by 100 plus 1. For example, a 50% acetic acid excess corresponds to a [D/A] of 1.5. Perchlorate removal was observed but not to below detection. Accordingly, [D/A] was increased to 1.50 on Day 37. Significant perchlorate breakthrough was still observed and [D/A] was progressively increased to 1.80 on Day 69. While perchlorate removal improved, removal to below detection was not achieved until phosphorus addition began on Day 97 (100 μg/L as PO_4-P). On Day 106, the EBCT was reduced to 10 minutes, with no significant effect on perchlorate removal. EBCT was reduced progressively to an EBCT of 7 minutes on Day 122, once again with no perchlorate breakthrough. [D/A] was then reduced to 1.40 on Day 142 and perchlorate breakthrough was observed. [D/A] was later increased to 1.70 and removal of perchlorate to non-detectable levels resumed. Optimization testing was completed on Day 153. Optimal operating conditions established by this phase of testing were 1) an EBCT = 10-12 minutes, 2) a [D/A] of 1.70, and 3) a phosphoric acid dose of 150 ug/L PO_4-P.

2.2 Bioreactor F130

Figure B.8 shows the results of optimization testing for Bioreactor F130. Bioreactor F130 started with an EBCT of 15 minutes and a [D/A] of 1.06, which was progressively increased to 1.75 on Day 44. Some perchlorate removal was observed, but approximately 30 μg/L perchlorate was still observed in the effluent. [D/A] was then increased to 2.0, which improved perchlorate removal. However, perchlorate removal to below detection was achieved only once phosphoric acid was dosed to the bioreactor (100 μg/L PO4-P initially). The EBCT was reduced to 12 minutes and [D/A] was decreased until breakthrough was observed at a [D/A] of 1.40 on Day 143. [D/A] was once again increased to 1.70 and removal of perchlorate to non-detectable levels was restored.

Optimization testing was completed on Day 154. Similar to what was observed for Bioreactor F120, the optimal operating conditions established during this phase of testing for Bioreactor F130 were 1) an EBCT = 10-12 minutes, 2) a [D/A] of 1.70, and 3) a phosphoric acid dose of 150 μg/L PO_4-P.

To better understand the interaction between DO, nitrate, and perchlorate across the depth of the bioreactor beds, water quality samples were taken on multiple occasions using the depthwise sampling ports. An example of an oxidant profile generated during these sampling events is shown in Figure B.9 (Day 114, Bioreactor F130). It is interesting to note that DO, nitrate, and perchlorate were all below detection limits approximately 3 feet into the bed. Perhaps more significant is the fact that substantial nitrate reduction occurred in the presence of > 5 mg/L DO, and substantial perchlorate reduction occurred even in the presence of > 5 mg/L DO and 4-15 mg/L nitrate (as NO_3^-). This highlights a unique aspect of the fixed-bed bioreactor technology: anaerobic microenvironments are established within the Biologically Active Carbon (BAC) bed, which allow efficient perchlorate degradation even when the bulk solution is not anaerobic.

2.3 Biofilter F150

BDOC, DOC, and turbidity data for Biofilter F150 during the optimization testing phase (Day 20 - 154) are presented in Figures B.3, B.4 and B.5, respectively. During that period, effluent DOC and BDOC values were less than 2 mg/L. Hydrogen peroxide was added on Day 98 at 12 mg/L and remained at 12 mg/L for the duration of optimization testing. The addition of hydrogen peroxide addition did not appear to significantly impact the removal of BDOC, DOC, or turbidity. The EBCT was lowered several times, which increased the effluent turbidity slightly, but did not significantly affect the removal of DOC or BDOC.

2.4 Chlorine Contact Tank

HPC, total coliforms, fecal coliforms, and free chlorine residual through the chlorine contact tank during the optimization phase (Day 20 -154) are plotted in Figure B.6. During optimization, the chlorine contact tank effluent had a measurable free chlorine residual of approximately 1 mg/L. Fecal coliforms were not detected (< 2 Most Probable Numer (MPN)/100 mL), and total coliforms were non-detect (<2 MPN/100 mL) for all but one sample during the optimization phase. HPC were generally very low but reached several million CFU/mL.

3.0 Optimal Operation Testing Phase

The purpose of this phase was to demonstrate that the Fixed-Bed (FXB) bioreactor process can achieve sustained (i.e., as many successive weeks as the testing schedule would allow) perchlorate removal to below detection. Operating conditions established during the Optimization Testing Phase were used during this phase of testing.

3.1 Bioreactor F120

Figure B.10 shows the results of the Optimal Operation testing for Bioreactor F120. The gaps in the data correspond to periods when the underdrain and/or the in-line perchlorate analyzer were being repaired. The bioreactor was operated at an EBCT of 10 minutes, phosphoric acid dosing of 150 μg/L PO4-P and a [D/A] of 1.70 during the Optimal Operation testing. Effluent perchlorate concentrations were typically below detection during this testing phase. Percent recovery for Bioreactor F120 during the Optimal Operation phase varied between 93 and 96 percent.

3.2 Bioreactor F130

Figure B.11 shows the results of Optimal Operation testing for Bioreactor F130. The gaps in the data correspond to periods when the underdrain and/or the in-line perchlorate analyzer were being repaired. The bioreactor was operated at an EBCT of 12 minutes, a phosphoric acid dosing of 150 ug/L PO4-P and a [D/A] of 1.70 during the Optimal Operation testing, with a decrease in EBCT to ten minutes on Day 181. Effluent perchlorate was not detectable throughout this testing phase. Percent recovery during the optimal operation phase for bioreactor F130 varied between 93 and 96 percent.

Figure B.12 presents typical trends in headloss for Bioreactor F130 during the Optimal Operation testing phase. After a backwash, headloss was typically 0.5 pounds per square inch (psi) or 1.2 feet and increased to just above 1 psi (2.3 feet) over the course of a 17-24 hour run.

Figure B.13 shows the effluent nitrate concentrations as measured during optimal operation testing by the in-line analyzer, the University of Michigan laboratory, and the hand-held colorimeter. Effluent nitrate concentrations were less than 5 mg/L during this phase except for two outliers in the handheld data. Concentrations measured by the University of Michigan laboratory were typically less than concentrations measured by the in-line sensor.

3.3 Biofilter F150

BDOC, DOC, and turbidity for Biofilter F150 during the Optimal Operation testing phase (Day 155 - 190) are shown in Figures B.3, B.4, and B.5, respectively. Biofilter F150 feed is the same as Bioreactor F130 effluent. The EBCT was 10 minutes and the hydrogen peroxide dose was 12 mg/L during this period. Except for one outlier, BDOC concentrations coming out of Bioreactor F130 were very low, often non-detect (<0.1 mg/L). BDOC concentrations increased slightly across F150, though the cause of this increase is unclear. During Optimal Operation testing phase (Day 154-192), all BDOC measurements in the effluent of F150 were below 0.5 mg/L. Typical Biofilter F150 feed and effluent DOC concentrations were 2 mg/L and 1 mg/L, respectively. Average effluent turbidity was 0.35 Nephelometric Turbidity Units (NTU), and the maximum

turbidity value was 0.97 NTU (Day 184). Interstage alum dosing tests were performed using alum doses of 1, 3, and 10 mg/L. The alum appeared to have no impact on Biofilter F150 effluent turbidity.

3.4 Chlorine Contact Tank

HPC, total coliforms, fecal coliforms, and free chlorine residual for the chlorine contact tank during the optimal operation phase (Day 154 -185) are plotted in Figure B.6. Free chlorine residuals ranged from 0.55 to 5.5 mg/L as Cl_2. Fecal and total coliforms were not detected during the Optimal Operation Testing Phase. HPC ranged from <2 to 34 CFU/mL.

Additional disinfection testing was performed outside the Optimal Operation Testing Phase. During these tests, a CT of 2 mg-min/L was tested. This CT was achieved through two conditions: 1) a chlorine residual of 0.l2 mg/L as Cl_2 and a t_{10} of 17 minutes, and 2) a chlorine residual of 0.20 mg/L as Cl_2 and a t_{10} of approximately 10 minutes. Resultant HPC in the effluent of the chlorine contact tank were 44-430 CFU/mL.

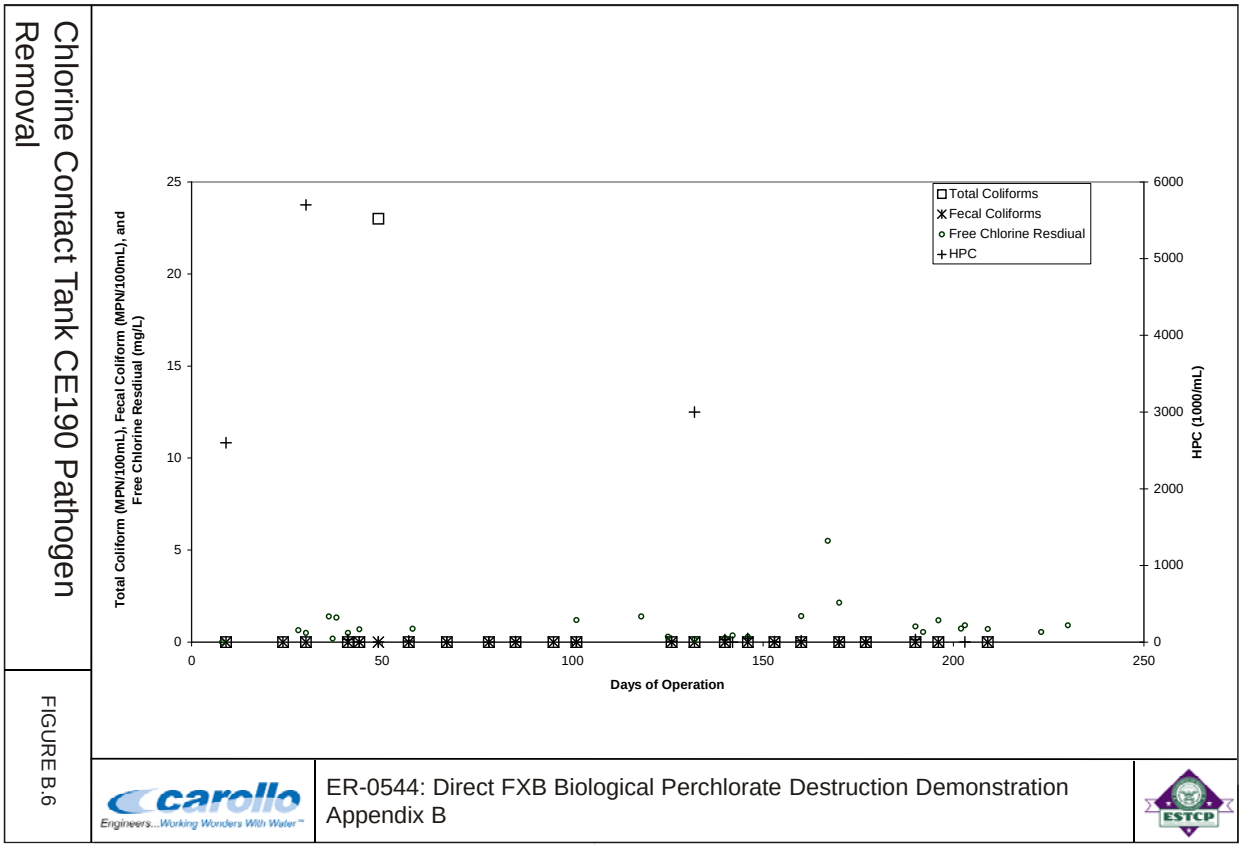

Figure B.6 - Chlorine Contact Tank CE190 Pathogen Removal.

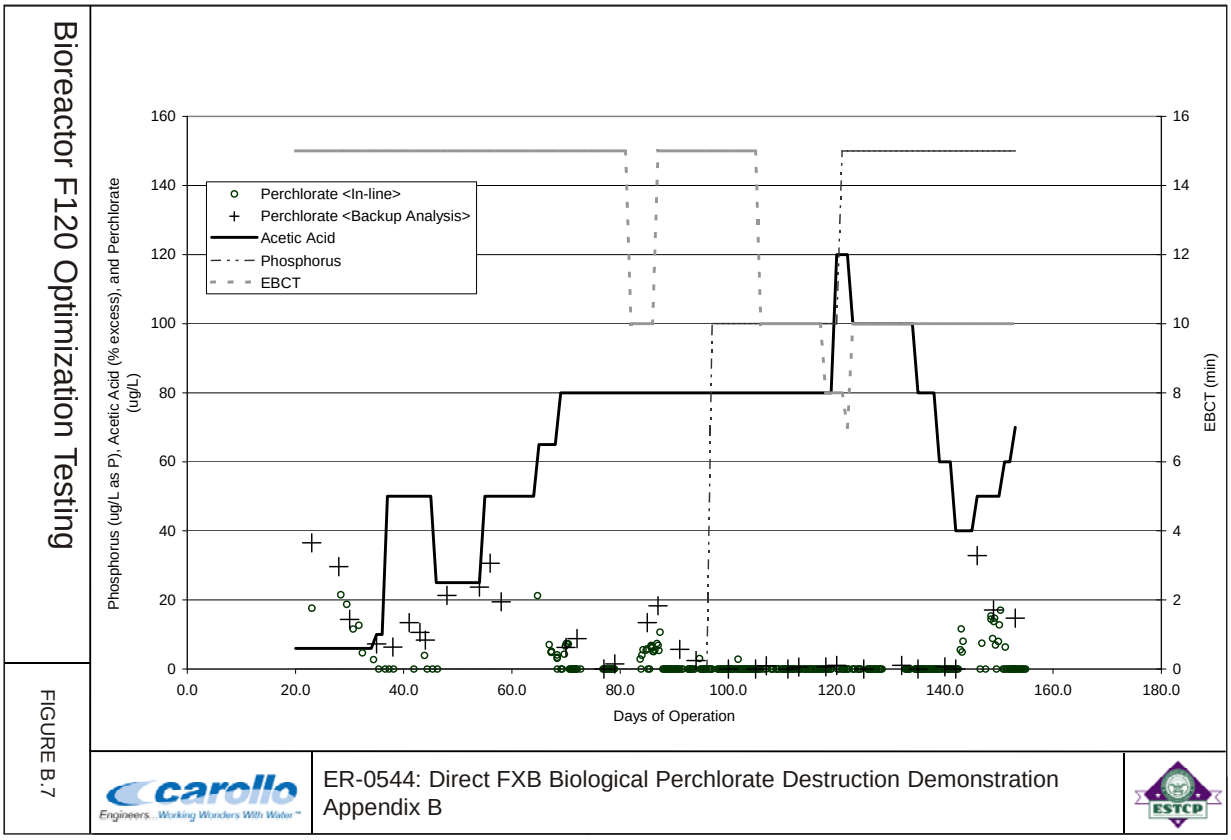

Figure B.7 - Bioreactor F120 Optimization Testing.

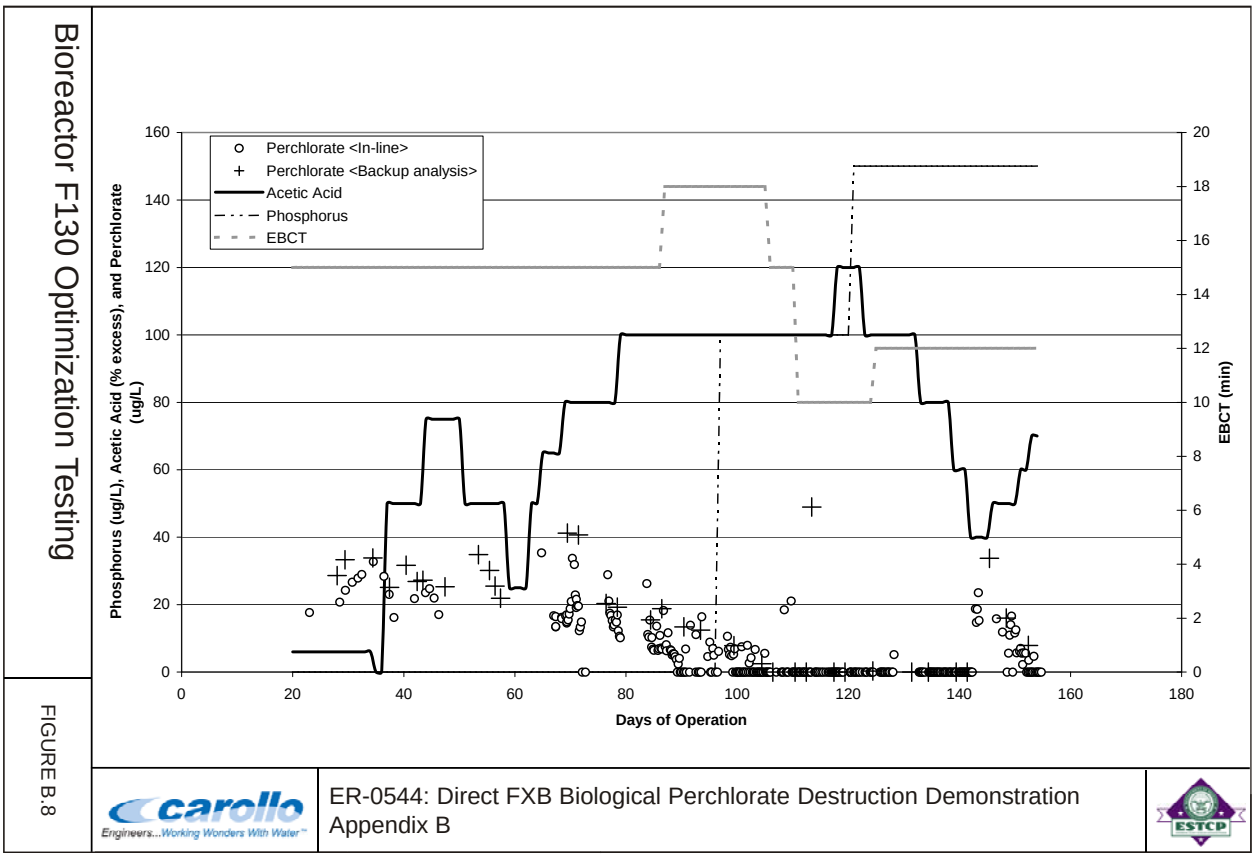

Figure B.8 - Bioreactor F130 Optimization Testing.

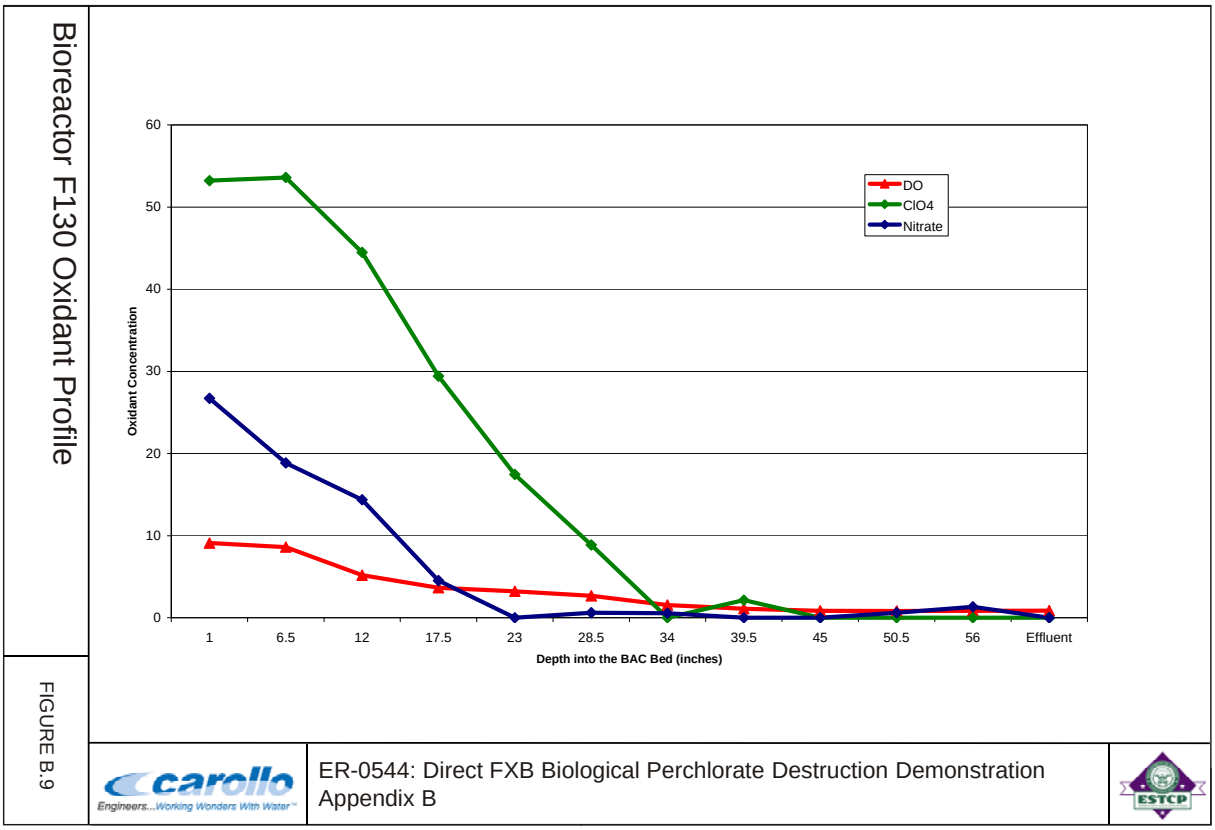

Figure B.9 - Bioreactor F130 Oxidant Profile.

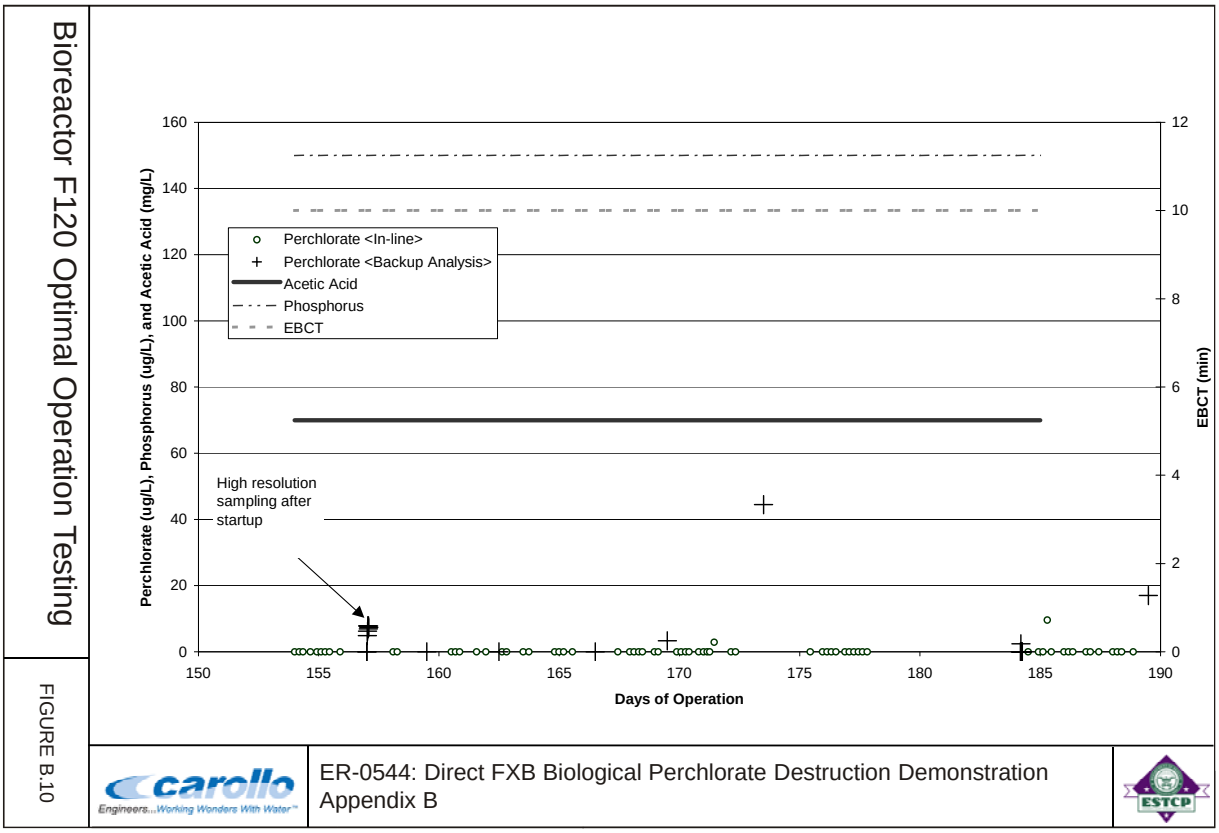

Figure B.10 - Bioreactor F120 Optimal Operation Testing.

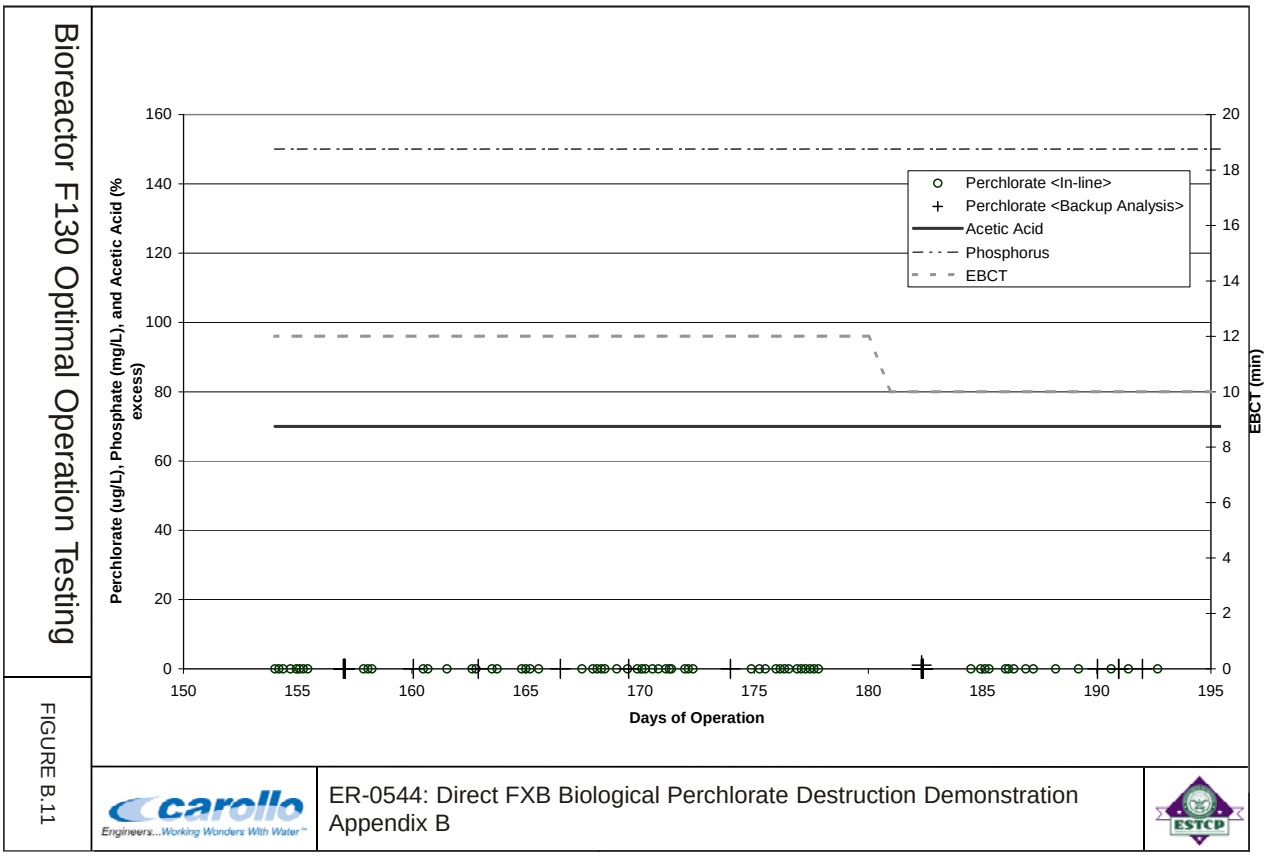

Figure B.11 - Bioreactor F130 Optimal Operation Testing.

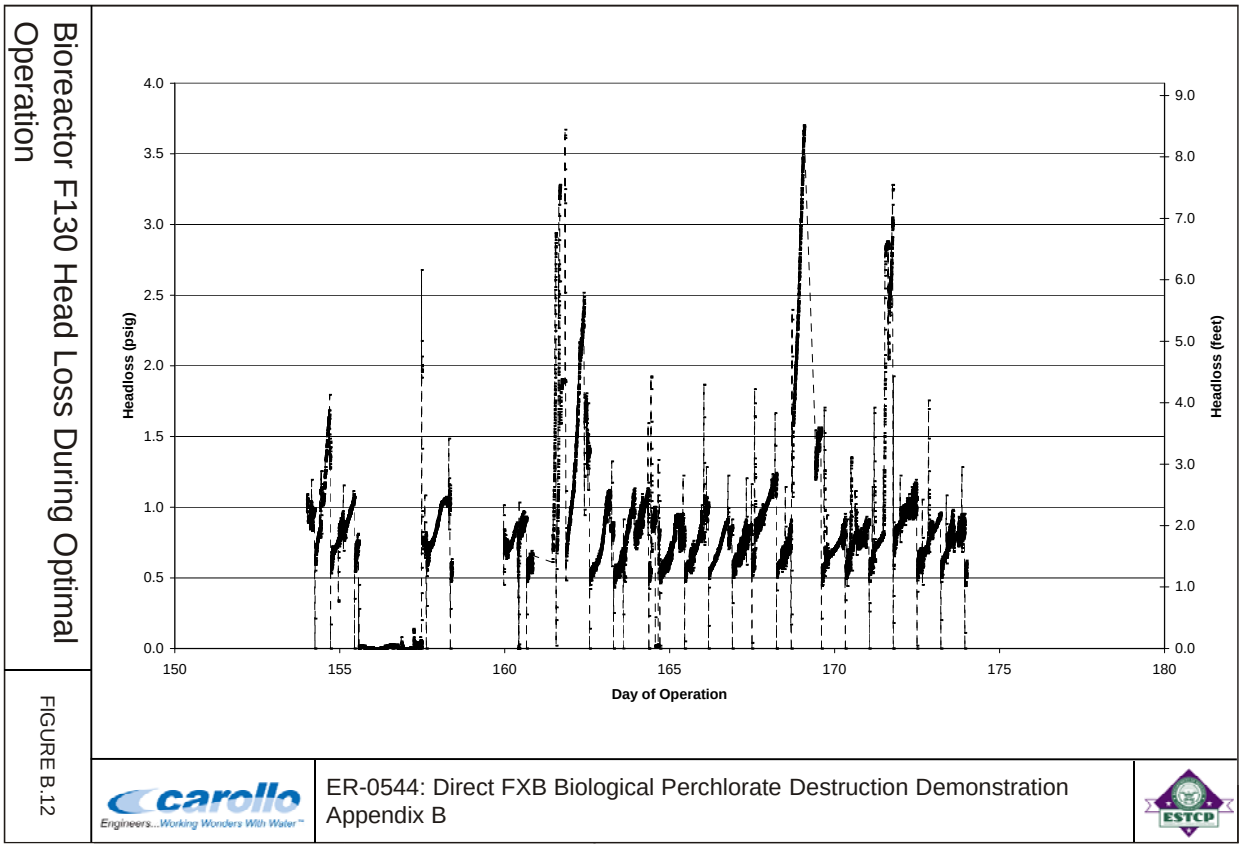

Figure B.12 - Bioreactor F130 Head Loss During Optimal Operation.

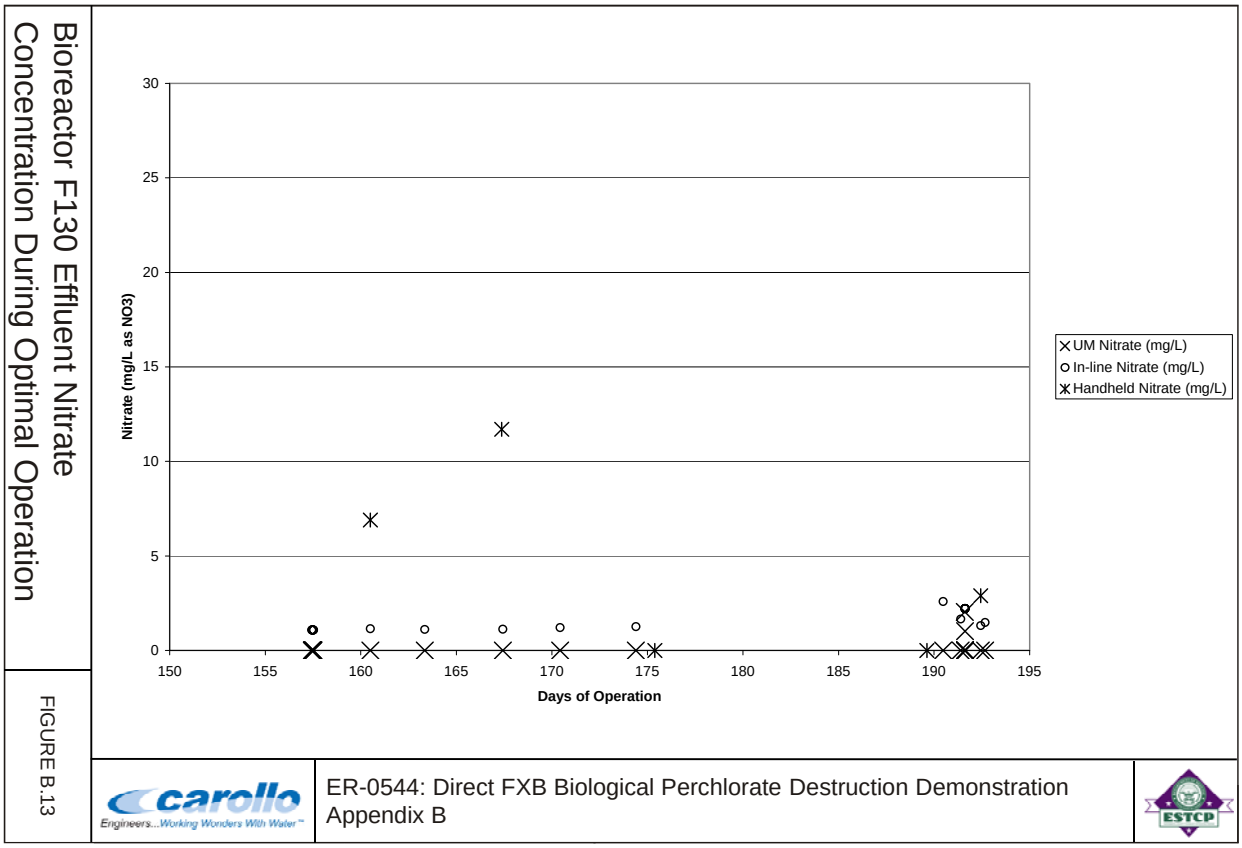

Figure B.13 - Bioreactor F130 Effluent Nitrate Concentration During Optimal Operation.

4.0 Robustness Testing Phase

Several robustness tests were run using Bioreactor F130. These tests included backwash testing, system shutdowns, acetic acid shutdowns, perchlorate spiking, and nitrate spiking. An EBCT of 10 minutes and a [D/A] of 1.7 was used during the robustness tests unless otherwise indicated.

4.1 Post-Backwash Testing

Figure B.14 shows the results of the high-resolution backwash testing. For this test, a backwash was performed, and the pilot was then returned to production mode. Perchlorate samples were taken immediately after the backwash and at 15-minute intervals for 120 minutes. No perchlorate was detected.

4.2 System Shutdowns

Figures B.15 and B.16 show the results from the 24-hour and one-week shutdown tests, respectively. After each shutdown period, the pilot was put back into production and high-resolution samples were taken over the next 24-hour period. No perchlorate breakthrough was observed during either test.

4.3 Acetic Acid Shutdowns

4.3.1 Test #1:

Figure B.17 presents the results for acetic acid shutdown test #1. This test was performed using a 10-hour run time, a [D/A] of 1.70 (before and after the shutdown), and a 24-hour acetic acid shutoff. Two backwashes were performed while the acetic acid was shut off. There was substantial perchlorate breakthrough during this test, with Bioreactor F130 effluent perchlorate concentrations peaking at 41.2 ug/L. Post-treatment Biofilter F150 attenuated some of the peak, as Biofilter F150 effluent perchlorate concentrations reached only 11.2 μg/L. Bioreactor F120 required almost three days to re-achieve sustained perchlorate removal to below detection. The relatively slow recovery may have been due to the removal of critical biomass by the two backwashes performed during the acetic acid shutdown period.

4.3.2 Test #2:

Figure B.18 presents the results for acetic acid shutdown test #2. This test was performed using a 24-hour run time, a [D/A] of 1.70 (before and after the shutdown), and a 24-hour acetic acid shut-off. Only one backwash was performed while the acetic acid was shut off. During this test, there was a 6-hour lag period before perchlorate breakthrough was observed and the maximum effluent perchlorate concentration measured was 8.3 μg/L (4.5 μg/L in Biofilter F150 effluent) . Sustained perchlorate removal to below detection was also re-achieved in only a few hours after the acetic acid pump was restarted. The

reduced number of backwashes would likely have allowed more biomass to remain in the reactor, thereby improving removal and expediting performance recovery.

4.3.3 Test #3:

Figure B.19 presents the results for acetic acid shutdown test #3. During this test, the acetic acid was shutoff for 24 hours, a [D/A] of 1.70 was used (before and after the shutdown), and no backwashes were performed. Under these conditions, perchlorate breakthrough occurred after only a few hours. Therefore, eliminating backwashes during an acetic acid shutdown does not appear to be beneficial. Despite the marginal perchlorate removal performance in Bioreactor F130, Biofilter F150 removed perchlorate to nondetectable levels for 19 hours after the acetic acid was shut off. Sustained perchlorate removal to below detection was re-achieved by the system within a few hours of turning the acetic acid pump back on.

4.3.4 Test #4

The acetic acid in test #4 was only off for 3.5 hours (Figure B.20), no backwashes were performed during the acetic acid shutdown, and a [D/A] of 1.70 was used (before and after the shutdown). Despite the short shut-off period, a maximum perchlorate breakthrough of 10.2 μg/L was observed (5.2 μg/L in the effluent of post-treatment Bioflter F150). Sustained perchlorate removal to below detection was re-achieved within a few hours after the acetic acid pump was restarted.

4.3.5 Test #5:

Figure B.21 presents the results for acetic acid shutdown test #5. This test was operated under identical conditions as test #2: 24-hour shutoff, 24-hour run time and one backwash. The only difference was that a [D/A] of 2.25 during the acetic acid restart instead of 1.70. In spite of the increased acetic acid dose, the reactor still required a few hours before it re-achieved sustained perchlorate removal to below detection. This confirms that acetic acid was not a limiting substrate during perchlorate shut-down test #2.

4.4 Perchlorate Spiking

Figure B.22 shows the results of the perchlorate spiking tests. Transient perchlorate loading episodes had very little impact on perchlorate removal performance in Bioreactor F130. Over an 11 day period, the feed perchlorate concentration was varied in step changes from 100 μg/L to 400 μg/L to 600 μg/L to 800 - 930 μg/L and back to the background concentration of 55 μg/L while the EBCT and the feed [D/A] were maintained at 10 minutes and 1.70, respectively. For the majority of the test, the perchlorate concentration was at or below the limit of detection.

4.5 Nitrate Spiking

Figure B.23 presents the results of the nitrate spiking tests performed on Bioreactor F130. During the nitrate spiking tests, nitrate feed concentrations to the reactor were step-increased from 30 mg/L (background) to 38 mg/L and then to 45 mg/L (all as NO_3^-). During this test, the EBCT was constant at 10 minutes, and a [D/A] ratio of 1.70 was maintained. No perchlorate or nitrate breakthrough was observed.

5.0 Backwash Wastewater Characterization

Composite backwash wastewater samples were taken from Bioreactor F130 and Biofilter F150 on 9/19/2007, 9/24/2007 and 10/2/2007. Results for BOD (Biochemical Oxygen Demand), TDS (Total Dissolved Solids), Total Suspended Solids (TSS), and Volatile Suspended Solids (VSS) are presented in Table B.1. Typical untreated domestic wastewater has a TDS concentration of 250-850 mg/L, a TSS concentration of 100-350 mg/L, a VSS concentration of 80-275 mg/L, and a BOD of 110-400 mg/L (Tchobanoglous and Burton, 1991). The backwash wastewater was substantially lower in strength relative to typical untreated domestic wastewater, and therefore discharge to a sanitary sewer could be an option.

Table B.1 - Backwash Wastewater Characterization.

Sample Date	Reactor	BOD (mg/L)	TDS (mg/L)	TSS (mg/L)	VSS (mg/L)
9/19/2007	F130	14	272	54	28
	F150	24	274	69	40
9/24/2007	F130	45	248	130	122
	F150	8.7	240	34	34
10/2/2007	F130	36	304	52	60
	F150	not sampled	not sampled	not sampled	not sampled

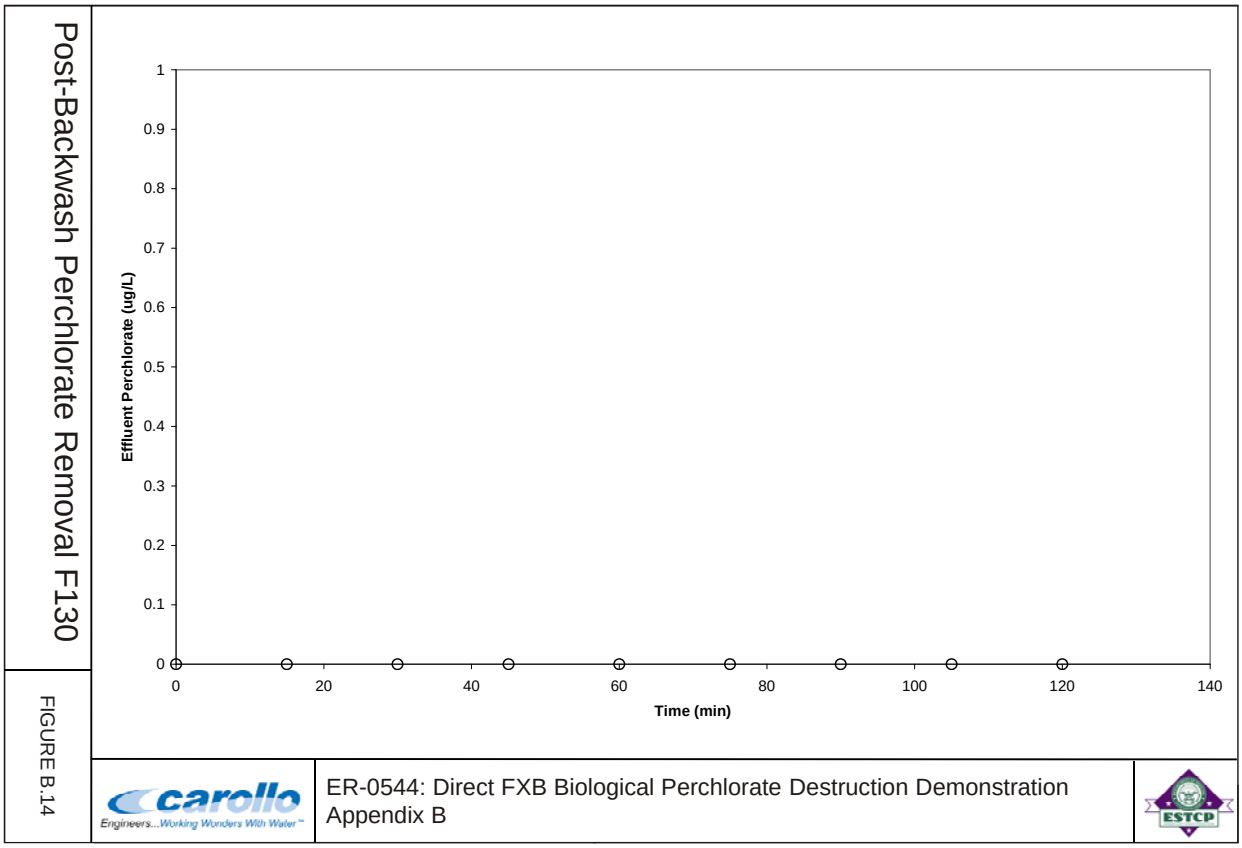

Figure B.14 - Post-Backwash Perchlorate Removal F130.

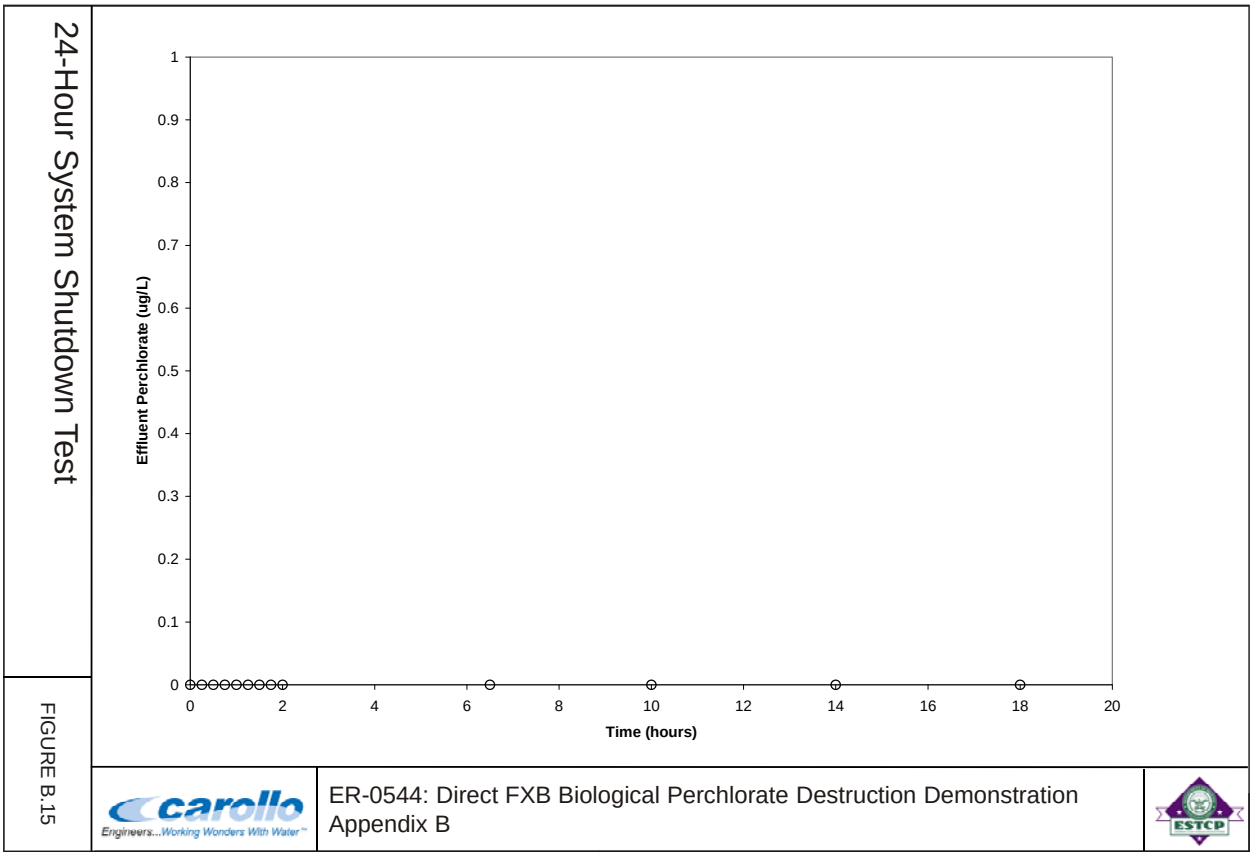

Figure B.15 - 24-Hour System Shut-Down Test.

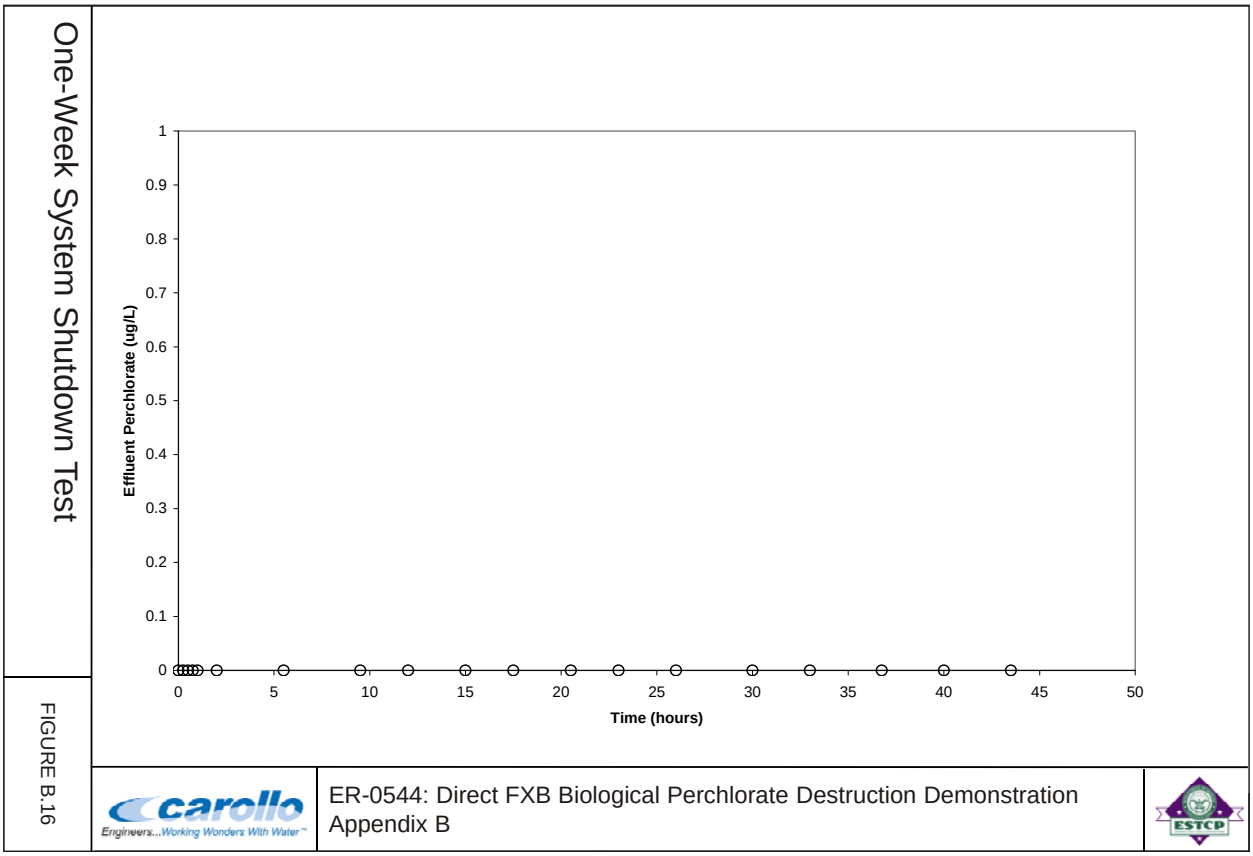

Figure B.16 - One-Week System Shut-Down Test.

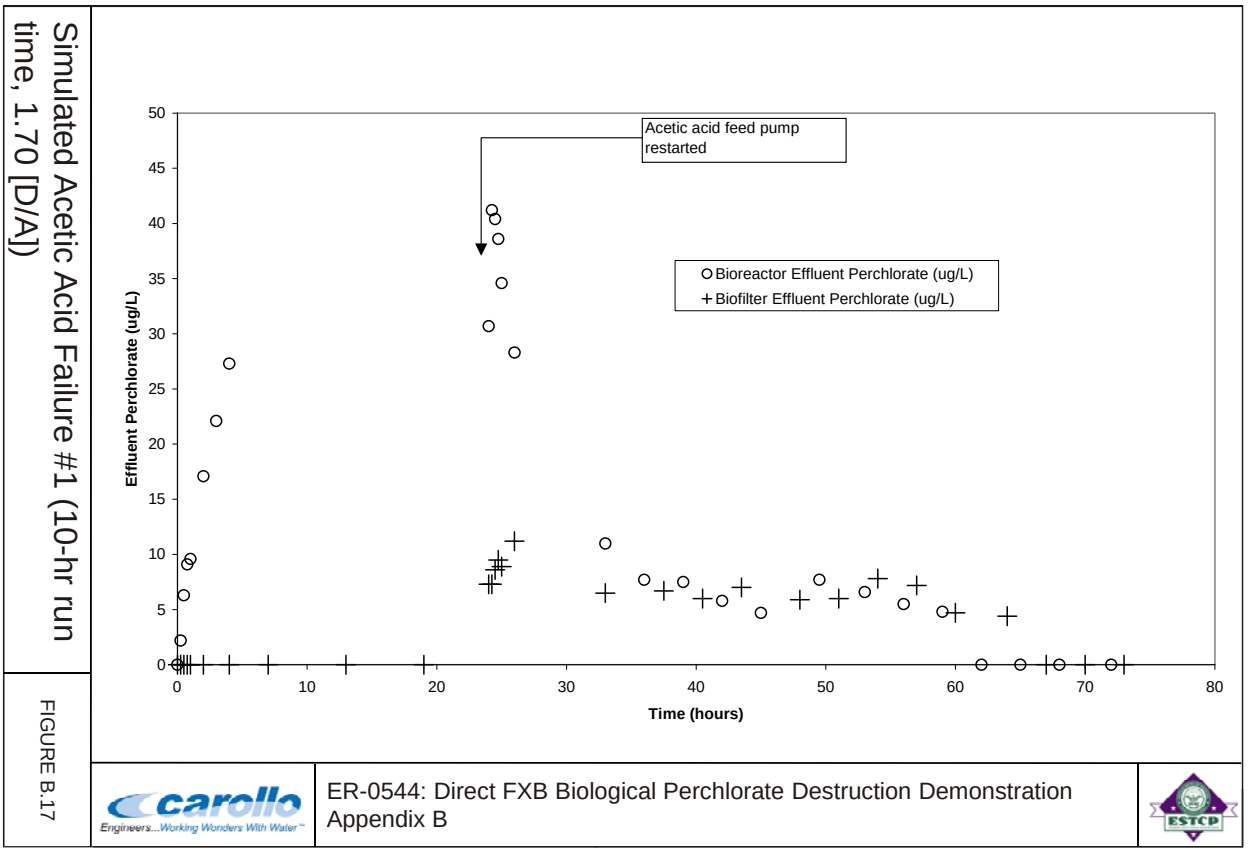

Figure B.17 - Simulated Acetic Acid Failure #1 (10-hr run time, 1.70 [D/A])**.**

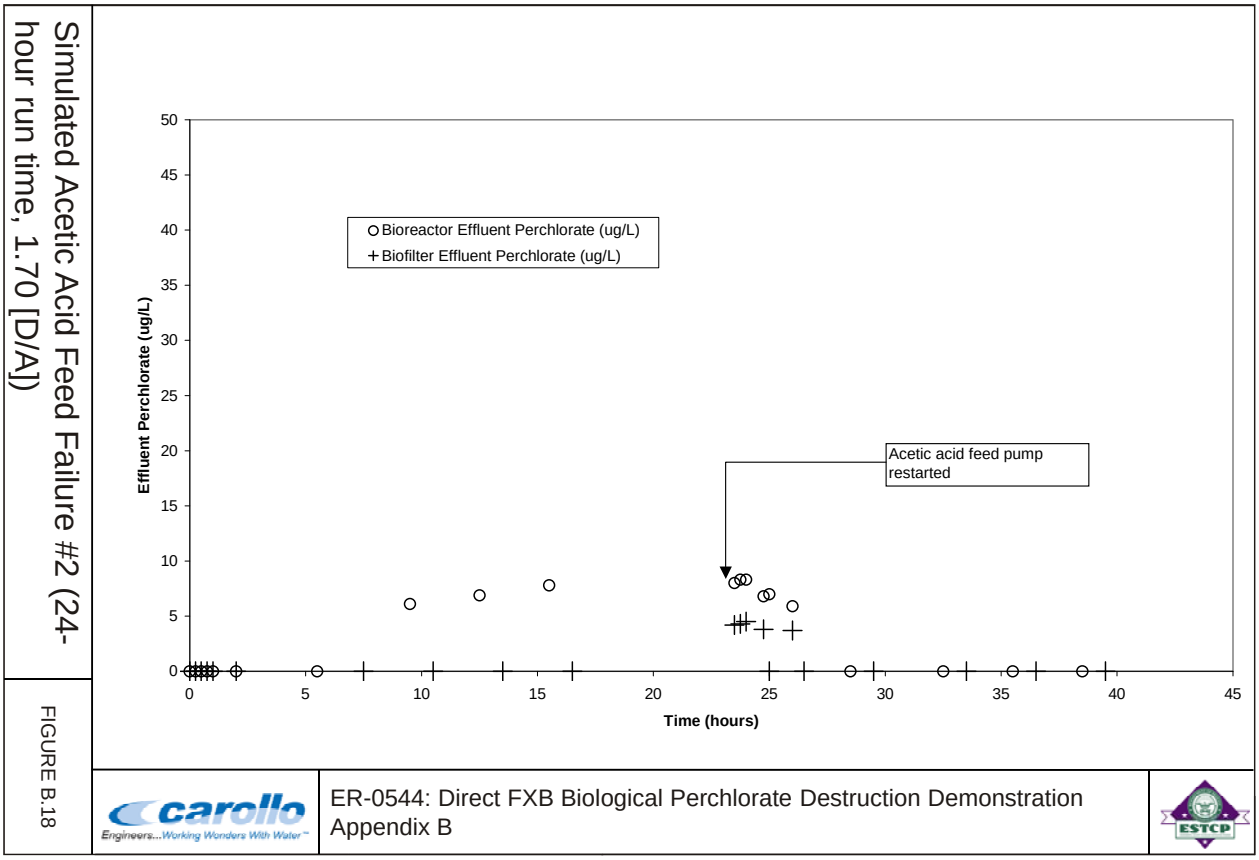

Figure B.18 - Simulated Acetic Acid Feed Failure #2 (24-hour run time, 1.70 [D/A])**.**

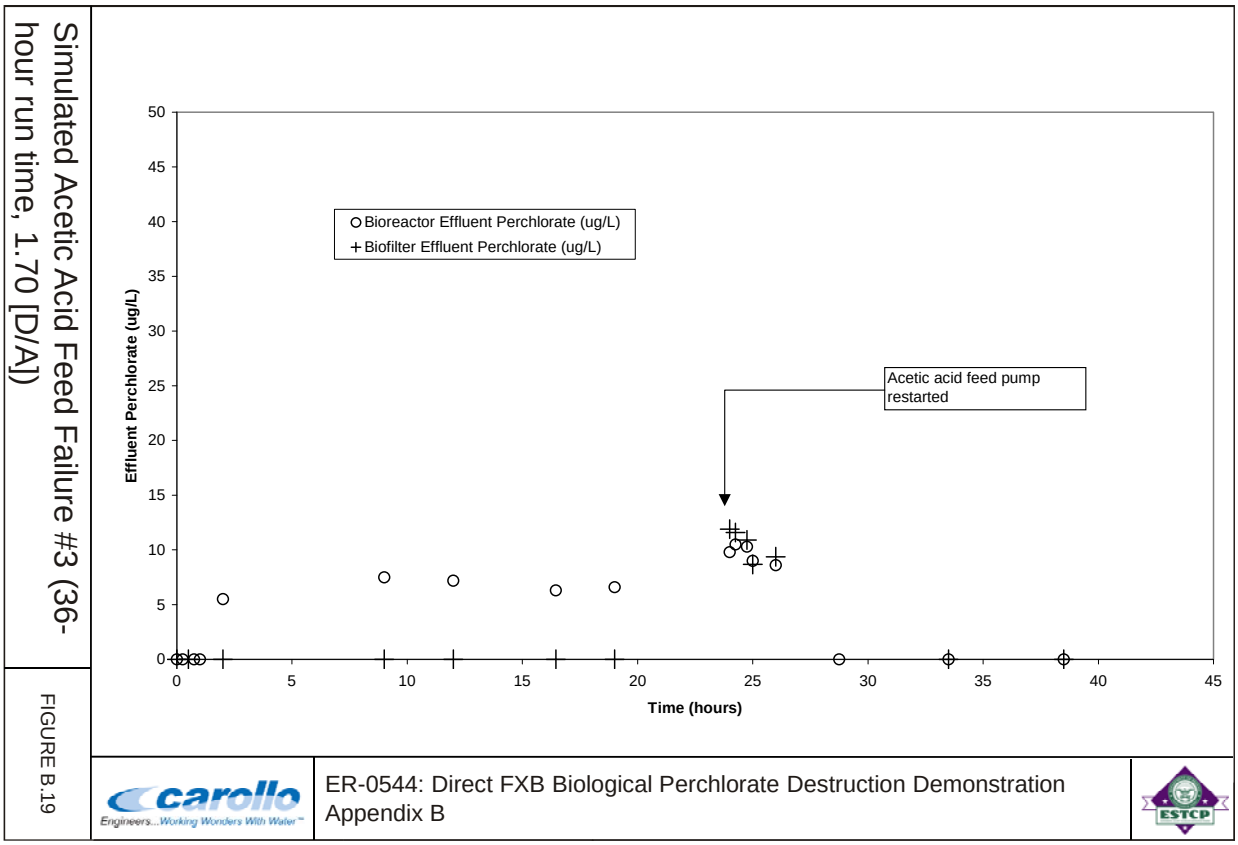

Figure B.19 - Simulated Acetic Acid Feed Failure #3 (36-hour run time, 1.70 [D/A])**.**

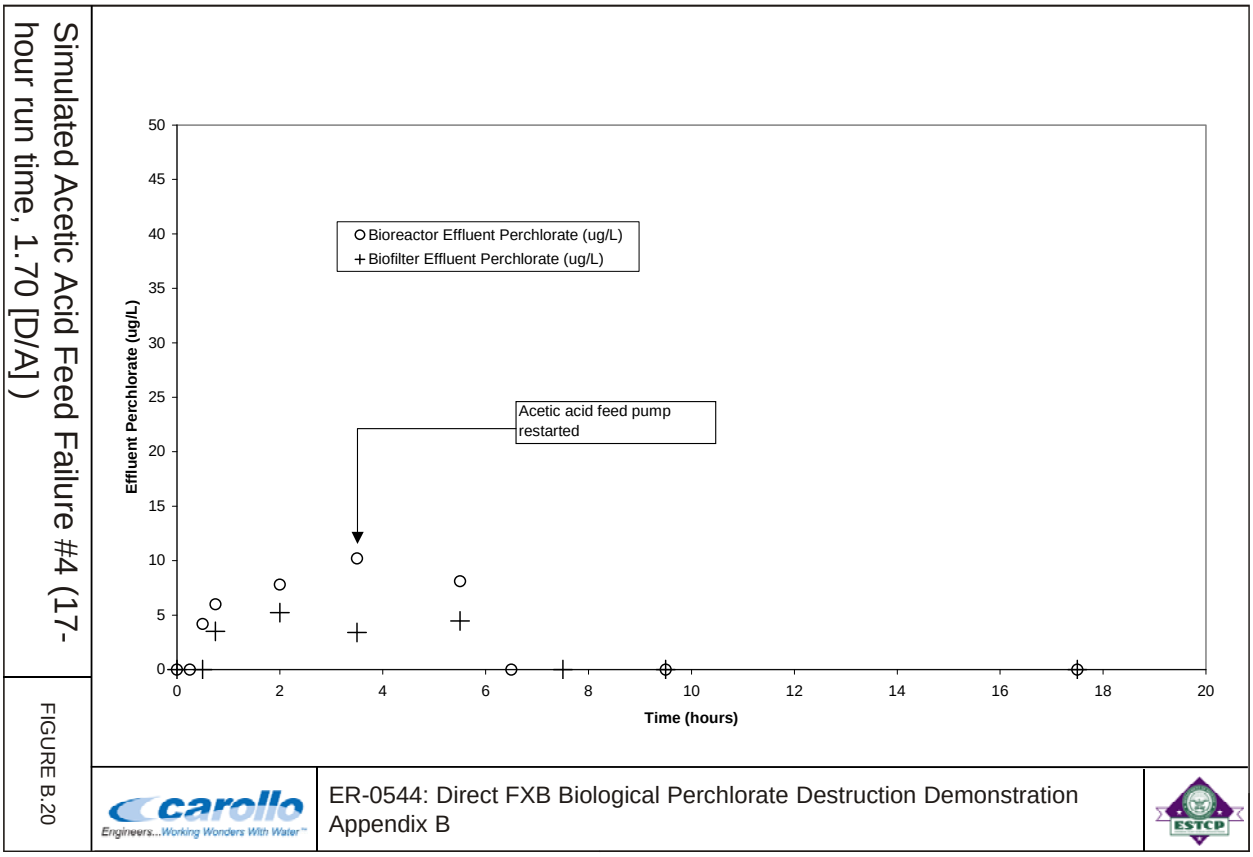

Figure B.20 - Simulated Acetic Acid Feed Failure #4 (17-hour run time, 1.70 [D/A]).

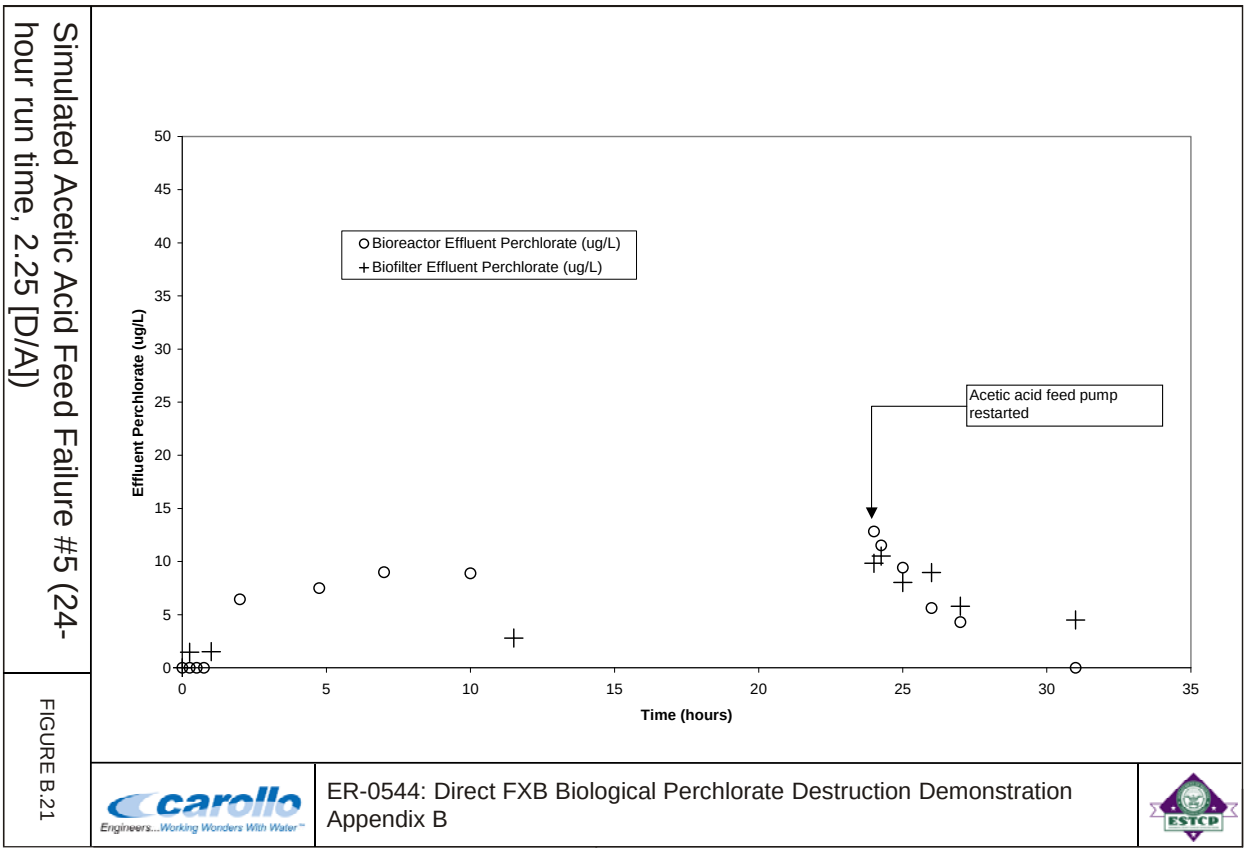

Figure B.21 - Simulated Acetic Acid Feed Failure #5 (24-hour run time, 2.25 [D/A])**.**

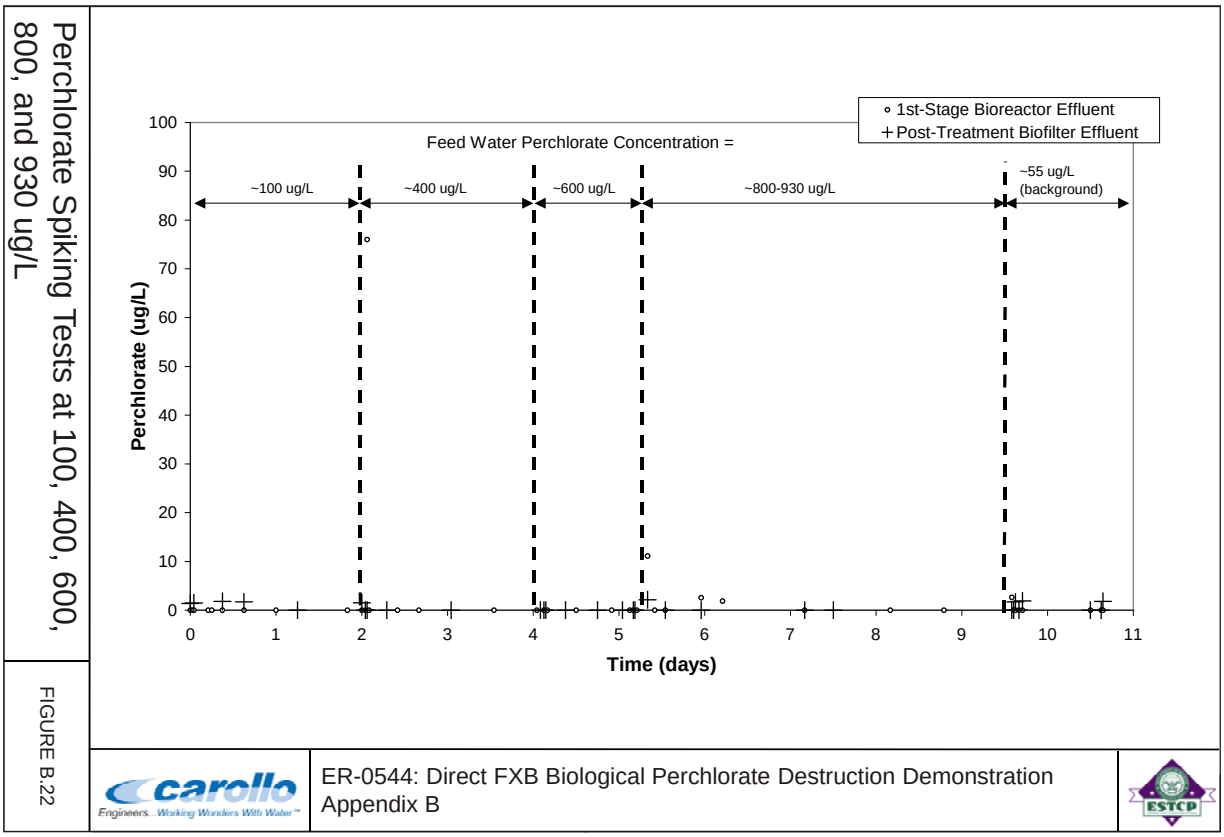

Figure B.22 - Perchlorate Spiking Tests at 100, 400, 600, 800, and 930 ug/L.

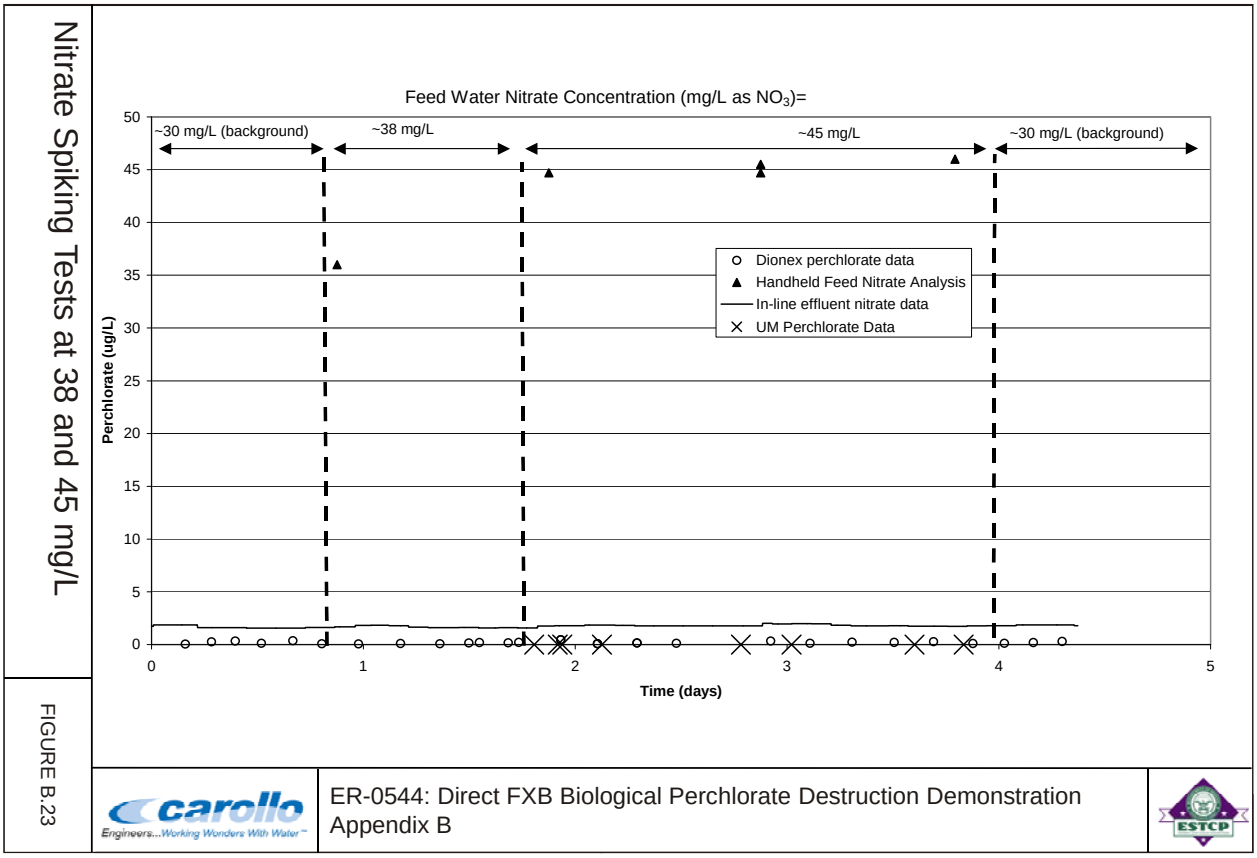

Figure B.23 - Nitrate Spiking Tests at 38 and 45 mg/L.

6.0 DBPFP Testing

On three separate occasions, samples were taken from the raw water, Bioreactor F130 effluent, and Biofilter F150 effluent for Disinfection By-Product Formation Potential (DBPFP) testing. All samples were dosed with free chlorine, and incubated for seven days inside the demonstration trailer. Table B.2 shows the DBPFP testing conditions and the results are provided in Figure B.24. Each of the raw samples produced no appreciable Haloacetic Acids (HAAs) or Total Trihalomethanes (TTHMs). DBPFP was low coming out of Bioreactor F130 (21-42 μg/L HAA5 and 22-34 μg/L TTHMs) and decreased across Biofilter F150 (6-15 μg/L HAA5 and 8-15 μg/L TTHMs). Thus, all DBP measurements were well below federal limits.

Sample Location & Test Number	Free Chlorine Dose (mg/L as Cl_2)	7-Day Free Chlorine Residual (mg/L as Cl_2)	pH	Temperature (^oF)
Raw 1	4.2	5.0	7.5	70-80
Raw 2	5.5	5.5	7.5	70-80
Raw 3	5.3	4.1	7.5	70-80
F130 Effluent 1	7.8	7.1	7.1	70-80
F130 Effluent 2	6.5	4.1	7.1	70-80
F130 Effluent 3	7.6	5.0	7.1	70-80
F150 Effluent 1	6.7	7.1	7.1	70-80
F150 Effluent 2	6.2	5.1	7.1	70-80
F150 Effluent 3	6.5	4.4	7.1	70-80

Table B.2 - DBPFP Testing Conditions.

7.0 Spent Media Characterization

Tables B.3 and B.4 present the results of the metals/uranium and trace organics media characterization tests, respectively. Hazardous waste threshold values are also provided, as defined in the California Code of Regulations, Title 22, Chapter 11, Article 3, Section 66261. Minimal metals accumulation occurred on the GAC and all metals were detected below their hazardous waste threshold values (Fassell, 2008). Uranium detected on the media was far below the threshold value. No trace organics were detected on the media. Media disposal is expected to occur approximately every 10 years, at which point media characterization tests would need to be performed to identify appropriate disposal options.

Table B.3 - Media Characterization Results.

	Parameter	Unit	RL	Rialto F130	Rialto F150	Limit
EML HASL Uranium	U-234	pCi/g		0.865	0.547	2000
	U-235	pCi/g	0.500	0.369	0.518	2000
	U-238	pCi/g	1.00	5.38	8.00	2000
TCLP Uranium	Total Uranium	pCi/L	1.40	ND	ND	
STLC Uranium	Total Uranium	pCi/L	7.0	290	210.00	
TTLC (WET) Metals	Antimony	mg/kg	18.4	ND	ND	500
	Arsenic	mg/kg	1.84	1.36	2.34	500
	Barium	mg/kg	1.84	53.1	65.2	10000
	Beryllium	mg/kg	1.84	0.643	0.821	75
	Cadmium	mg/kg	1.84	ND	ND	100
	Chromium	mg/kg	1.84	30	13.8	2500
	Cobalt	mg/kg	1.84	2.58	4.23	8000
	Copper	mg/kg	1.84	9.78	10.7	2500
	Iron	mg/kg	36.8	396	1620	N/A
	Lead	mg/kg	1.84	ND	ND	1000
	Mercury (7471A)	mg/kg	0.184	ND	ND	20
	Molybdenum	mg/kg	9.21	ND	10.6	3500
	Nickel	mg/kg	1.84	5.62	7.34	2000
	Selenium	mg/kg	1.84	2.82	0.92	100
	Silver	mg/kg	1.84	ND	ND	500
	Thallium	mg/kg	1.84	ND	ND	700
	Vanadium	mg/kg	1.84	53.7	78.9	2400
	Zinc	mg/kg	1.84	7.48	2.39	5000
STLC Metals	Antimony	mg/L	0.500	ND	ND	15
	Arsenic	mg/L	0.0500	0.0319	0.0744	5.0
	Barium	mg/L	0.0500	1.42	0.451	100
	Beryllium	mg/L	0.0500	ND	ND	0.75
	Cadmium	mg/L	0.0500	ND	ND	1.0
	Chromium	mg/L	0.0500	0.405	0.198	5
	Cobalt	mg/L	0.0500	ND	ND	80
	Copper	mg/L	0.0500	0.0187	0.0235	25
	Iron	mg/L	1.00	3.29	12.5	N/A
	Lead	mg/L	0.0500	ND	ND	5.0
	Molybdenum	mg/L	0.250	ND	0.322	350
	Nickel	mg/L	0.0500	ND	ND	20
	Selenium	mg/L	0.0500	ND	0.0668	1.0

	Parameter	Unit	RL	Rialto F130	Rialto F150	Limit
	Silver	mg/L	0.0500	ND	ND	5
	Thallium	mg/L	0.0500	ND	ND	7.0
	Vanadium	mg/L	0.0500	0.635	0.633	24
	Zinc	mg/L	0.0500	1.12	0.0313	250
TCLP Metals	Arsenic	mg/L	0.0500	ND	ND	5.0
	Cadmium	mg/L	0.0500	ND	ND	1.0
	Chromium	mg/L	0.0500	ND	ND	5.0
	Copper	mg/L	0.0500	0.0668	ND	N/A
	Lead	mg/L	0.0500	0.0343	ND	5.0
	Molybdenum	mg/L	0.250	ND	ND	N/A
	Nickel	mg/L	0.0500	ND	ND	N/A
	Selenium	mg/L	0.0500	ND	ND	1.0
	Zinc	mg/L	0.0500	0.737	0.515	N/A

Table B.4 - Trace Organics Measured.

Volatile Organics	Semi-Volatile Organics	Pesticides	Herbicides
Benzene	2,4,5-Trichlorophenol	Gamma-BHC (Lindane)	2,4-D
2-Butanone	2,4,6-Trichlorophenol	Heptachlor	2,4,5-TP (Silvex)
Carbon Tetrachloride	2,4-Dinitrotoluene	Heptachlor Epoxide	
Chlorobenzene	2-Methylphenol	Gamma-Chlordane	
Chloroform	4-Methylphenol	Alpha-Chlordane	
1,4-Dichlorobenzene	Hexachlorobenzene	Endrin	
1,2-Dichloroethane	Hexachlorobutadiene	Methoxychlor	
1,1-Dichlorooethane	Hexachloroethane	Toxaphene	
Tetrachloroethene	Nitrobenzene		
Trichloroethene	Pentachlorophenol		
Vinyl Chloride	Pyridine		
Note: None of the above compounds were detected			

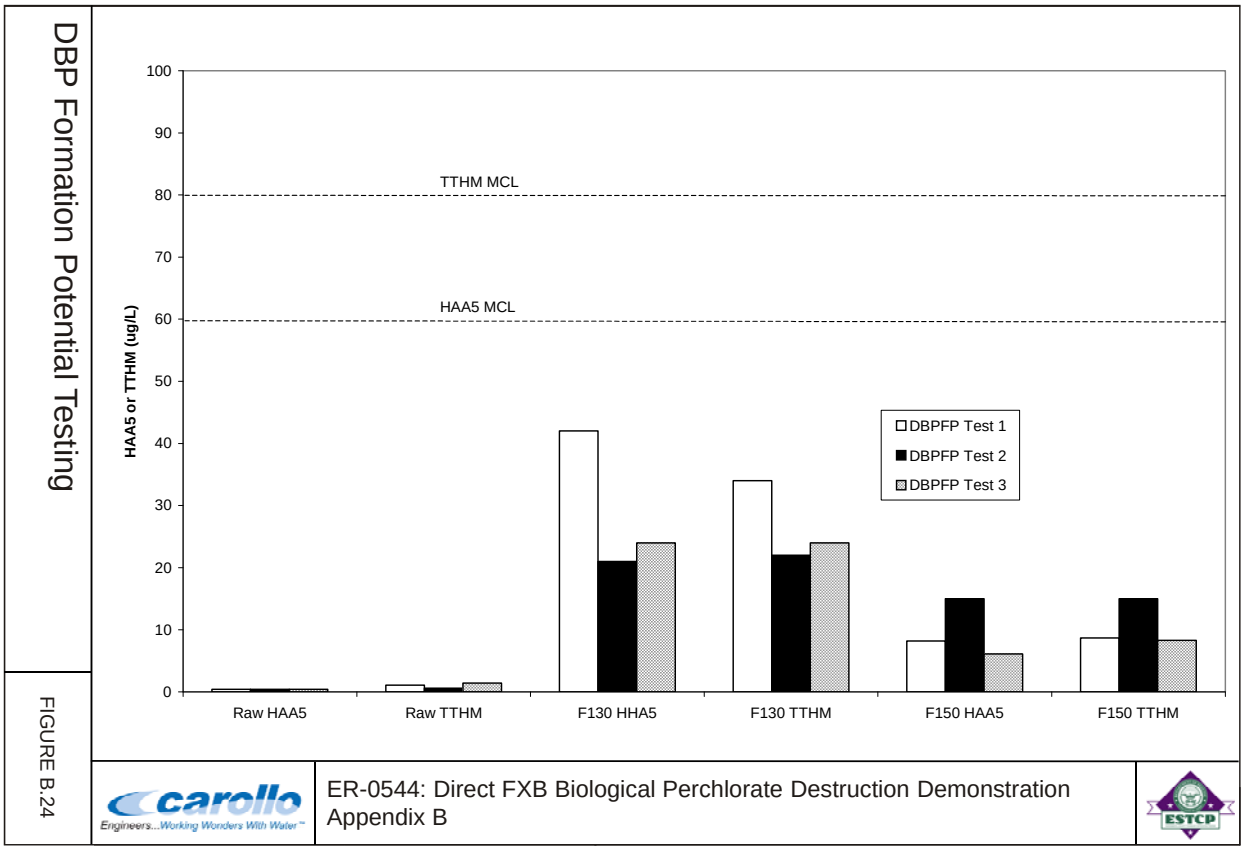

Figure B.24 - DBP Formation Potential Testing**.**

References

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Fassell, John (2008). California Department of Public Health, Radiologic Health Branch. Personal Communication.

Na, C., F.S. Cannon, and B. Hagerup, 2002. "Perchlorate Removal via Iron-Preloaded GAC and Borohydride Regeneration." Journal AWWA, 94(11): 90-102.

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Appendix C - Bench-Scale Results

1. Introduction

The bench-scale BAC system was constructed and operated to investigate the effects of various operating conditions on reactor performance (Appendix C) and microbial community (Appendix D). The operating conditions included the addition of phosphorus and the intermittent pattern of electron donor addition. Phosphorus is known as an essential element for microbial growth, however, its effect on microbial community structures is not well documented. Knowledge about the effects of phosphorus addition on microbial community structure will benefit the design and operation of biofilm reactors. Intermittent electron donor addition pattern was tested as an effort to minimize the residual electron donor in reactor effluent, which is critical to minimize microbial re-growth in distribution systems.

2. Experimental Procedure

The bench-scale fixed-bed BAC reactor consisted of a glass column with an inner diameter of 4.9 cm and a height of 26.0 cm. The height of the granular activated carbon (GAC) bed (bituminous F816, Galgon Carbon Corp., Pittsburgh, PA) was 10.6 cm resulting in an empty bed volume of 200 mm^3, and the rest of the height of the glass column was reserved for bed expansion during backwashing. A synthetic groundwater was pumped into the BAC reactor in a down-flow mode at a flow rate of 10 mL/min, resulting in an empty bed contact time (EBCT) of 20 min (Figure C.1). The synthetic groundwater composition was designed according to the composition determined for the Rialto groundwater, and reported in Table C1. Based on stoichiometric calculations with an assumed net yield of 0.4 g $COD_{biomass}$/g $COD_{acetate}$, 13 mg/L of acetic acid as C was needed to completely remove all three electron acceptors (i.e., DO, NO_3^-, and ClO_4^-). With a safety factor of 1.5 applied, concentrated acetic acid was added to the reactor using a syringe pump and resulted in a final concentration of 20 mg/L as C in the influent. The pH values of influent and effluent lied between 7.5 and 7.9. On day 115, phosphoric acid was added to the synthetic groundwater with a final concentration of 145 μg/L as P. In order to remove excess biomass, the bench-scale BAC reactor was backwashed every 48 hours. In each backwash, the BAC bed was fully fluidized by a mixture of deionized water (50 mL/min) and air for 4 min followed by rinsing flow of deionized water (500 mL/min) for 3 min. The BAC system was operated in a temperature control room set at 18°C. These operating conditions are designated as the baseline operating conditions. The bench-scale BAC system started on 9/26/2006 and continued to operate till 9/1/2008 when its operating condition was changed to suit another research project.

Intermittent addition of acetic acid to the BAC reactor was tested by dividing one backwash cycle (i.e., 48 hours) into four cycles. Each 12-hour cycle consisted of a 6-hour reactor run with an influent acetic acid concentration twice the stoichiometric requirement in influent (i.e., 26 mg/L as C) followed by a 6-hour reactor run with an

influent acetic acid concentration half of the stoichiometric requirement (i.e., 6.5 mg/L as C).

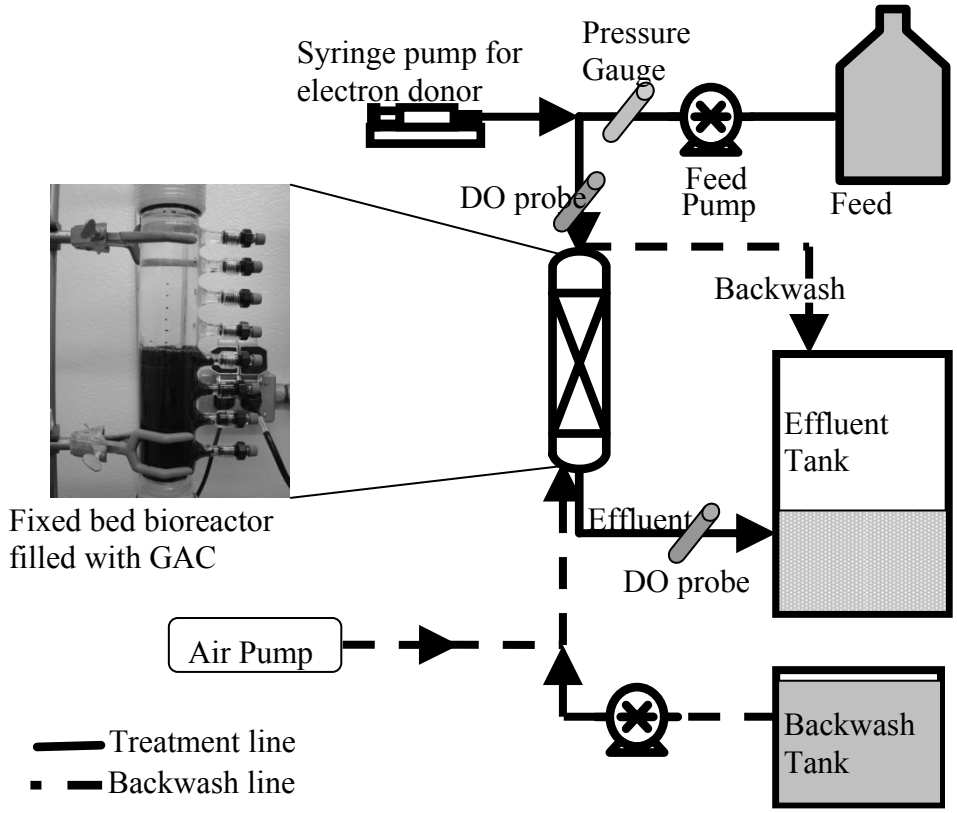

Figure C.1 - Schematic of the Bench-scale BAC Reactor at the University of Michigan.

Table C.1 - Influent Composition of the Bench-scale BAC Reactor at the University of Michigan.

Chemical added	Conc. (mg/L)
$NaClO_4$	0.075 as ClO_4^-
$NaNO_3$	25 as NO_3^-
NaCl	13 as (Cl^-) [1]
$CaCl_2$	
$MgCl_2$	
Na_2SO_4	12 as SO_4^{2-}
K_2CO_3	3 as CO_3^{2-}
$NaHCO_3$	210 as HCO_3^-
H_3PO_4	0.145 as P[2]
Acetic acid[3]	20 as C
DO	6-7 mg/L

[1] *Concentration of Cl^- from the three chemicals.*
[2] *Phosphorus was added since day 115.*
[3] *Acetic acid was added separately through the syringe pump*

3. Results and Conclusions

Because the bench-scale BAC reactor was operated for drinking water treatment, the addition of chemicals, in terms of both types and amounts, was intended to be minimized. Therefore, at the startup of the BAC reactor acetic acid was the only chemical added to the reactor influent (Figure C.1). Acetic acid was added at a relatively low concentration of 1.5 times the stoichiometric requirement (i.e., a safety factor of 1.5) in order to minimize the residual of acetic acid in the finished water, which can cause microbial contamination by supporting microbial growth in distribution systems. After a few months of operation at this condition, it was found that the reactor could not achieve complete removal of nitrate and perchlorate. After ruling out the possibility of electron donor being a limiting factor in the system, it was speculated that phosphorus deficiency in the reactor was hindering biological nitrate and perchlorate reduction. Thus, systematic studies were conducted to study the impacts of phosphorus on reactor performance and microbial community structure of the reactor. Succeedingly, electron donor addition was optimized by testing an intermittent electron donor addition pattern.

3.1 Phosphorus addition

Before the addition of phosphorus, the reactor was able to remove most of the influent DO, 68% of influent nitrate, and 13% removal of perchlorate (Figure C.2). After the addition of phosphorus on Day 115 (as phosphoric acid at a concentration of 145 μg/L as P), the effluent DO concentration quickly dropped below the detection limit of 0.1 mg/L, and both nitrate and perchlorate concentrations started to decrease. By Day 120, effluent nitrate concentrations dropped below the detection limit of 0.2 mg/L. By Day 130, effluent perchlorate concentrations were below the detection limit of 2 μg/L. The

simultaneous decreases in effluent concentrations of nitrate and perchlorate indicated that a single microbial population may be responsible for the reductions of these two electron acceptors.

Microbial analyses supported this speculation. The relative abundance of bacterial strains closely associated with the *Dechloromonas* genus increased from 15.7% to 46.2% after phosphorus addition (detailed microbial analysis are presented in Appendix D). Most known *Dechloromonas* strains can reduce both perchlorate and nitrate (Coates and Achenbach, 2004). Taken together, these findings suggest that phosphorus availability was important in ensuring biological perchlorate and nitrate reduction by maintaining a high relative abundance of perchlorate reducing bacteria (PRB) in the reactor.

3.2 Intermittent Acetic Acid Addition

Although the bench-scale BAC reactor was effective in removing nitrate and perchlorate after phosphorus addition, the influent acetic acid concentration of 1.5 times the stoichiometric requirement resulted in residual electron donor in the effluent. The presence of biodegradable material in finished drinking water can impair the safety of drinking water by supporting microbial growth in distribution systems. Therefore, the benefits of overdosing acetic acid to support biological activity need to be balanced with the need to maintain low effluent acetic acid concentrations to minimize microbial growth in distribution systems.

In order to optimize the electron donor addition, the adsorption capacity of GAC was investigated, as GAC may serve as a reservoir in controlling effluent acetic acid concentrations. It was hypothesized that when acetic acid is added in excess, the GAC could adsorb the acetic acid in excess and lower its concentration in the effluent; when acetic acid is added below the stoichiometric requirement for biological removal of contaminants, the acetic acid previously adsorbed on the GAC could be utilized by bacteria to support complete removal of nitrate and perchlorate, and the adsorption sites on GAC would be regenerated.

During the periods for which acetic acid was added twice the stoichiometric requirement (i.e., 26 mg/L as C), the GAC was able to adsorb the influent acetic acid present in excess and keep the effluent acetic acid concentration below the detection limit for one hour (Figure C.3 A). Then, the effluent acetic acid concentration gradually increased. The effluent nitrate and perchlorate concentrations remained below the detection limit during these periods (Figure C.3 B).

When the influent acetic acid concentration was switched from twice to half the stoichiometric requirement (i.e., 6.5 mg/L as C), the effluent acetic acid concentration quickly dropped to zero (Figure C.3 A). The effluent nitrate and perchlorate concentrations remained zero for one hour after the switch, and then started to increase (Figure C.3 B). The low concentrations of effluent nitrate and perchlorate likely were the result of biological activity supported by the acetic acid adsorbed onto GAC during the previous stage. It is likely that the acetic acid adsorption sites on the GAC were

regenerated during this stage, allowing adsorption during the next high influent acetic acid stage.

The results indicated that the adsorption capacity of GAC for acetic acid could serve as a reservoir for controlling the effluent acetic acid concentration. However, the breakthroughs of effluent acetic acid, as well as nitrate and perchlorate, one hour after the changes in influent acetic acid concentration indicated that the adsorption capacity was limited. That is, the adsorption capacity of GAC for acetic acid was not high enough to adsorb the excess acetic acid when the influent acetic acid concentration was twice the stoichiometric requirement for 6 hours. Consequently, the amount of acetic acid adsorbed onto the GAC was insufficient to support complete perchlorate and nitrate removal throughout the 6-hour stages when influent acetic acid concentration was half the stoichiometric requirement.

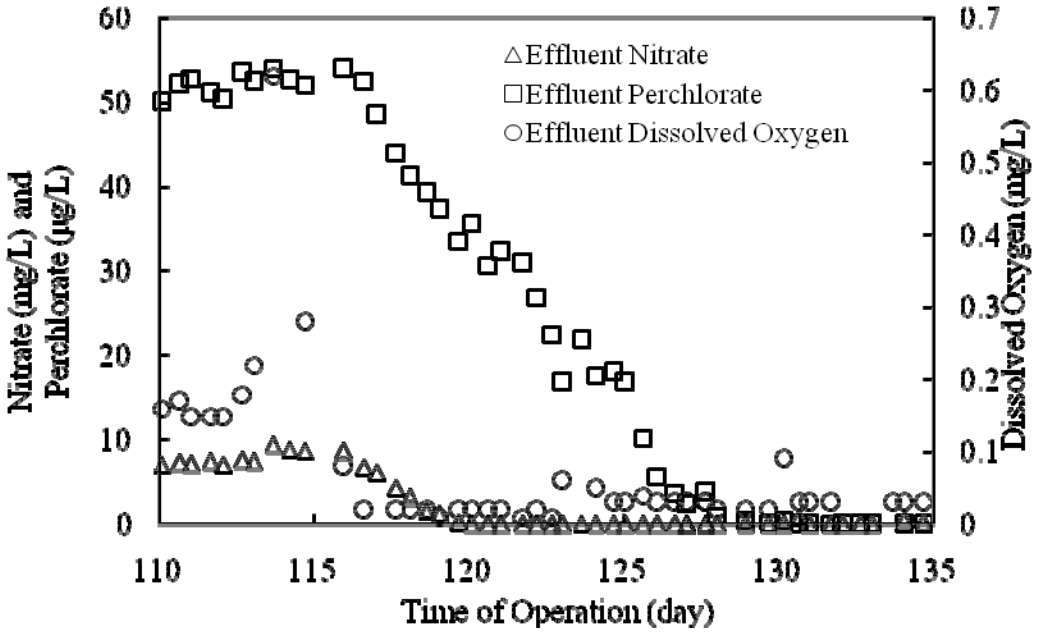

Figure C.2 - Concentrations of Oxygen, Nitrate (as NO_3^-), and Perchlorate in Effluent Before and After the Addition of Phosphorus on Day 115.

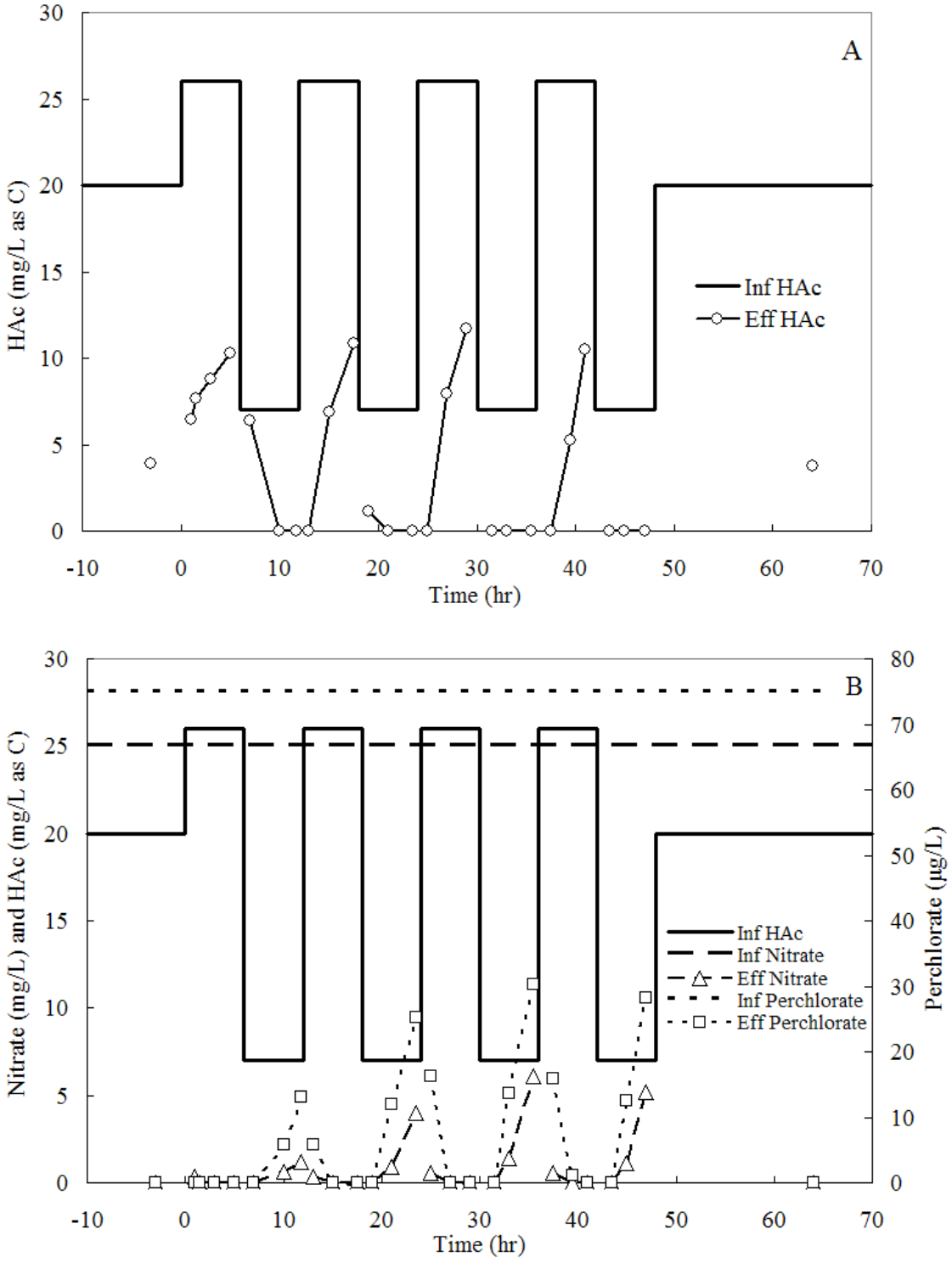

Figure C.3 - Performance of the Bench-scale BAC Reactor During the Intermittent Electron Donor Experiment: (A) **Influent and Effluent Concentrations of Acetic Acid** (HAc)**;** (B) **Influent and Effluent Concentrations of Nitrate** (as NO_3^-) **and Perchlorate** (influent acetic acid concentration was included for reference)**.**

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Appendix D - Microbial Characterization

1. Introduction

Reactor performance is determined to a large extent by the microbial community inside a bioreactor. Therefore, elucidating how microbial communities respond to reactor operation is crucial in optimizing reactor operating conditions to achieve satisfactory reactor performance. To this end, biomass samples from the bench- and demonstration-scale BAC reactors were collected both before and after phosphorus addition. These biomass samples were characterized using clone library technique. By combining clone library results and online DNA databases, the major bacterial populations in the reactors were identified, their relative abundances were estimated, and their change was correlated with the change in reactor performance. In addition, the microbial compositions of different BAC reactors were compared.

2. Experimental procedure

Five BAC samples were collected for clone library analyses: two from the bench-scale BAC reactor (Day 100 and 244, before and after phosphorus addition), two from the demonstration-scale BAC reactor F130 (Day 84 and Day 210, before and after phosphorus addition), and one from the demonstration-scale BAC reactor F120 (Day 84, before phosphorus addition). A vertical core of demonstration-scale BAC bed was taken using a 1" PVC pipe at each sampling time, and was shipped overnight to the University of Michigan. The BAC samples from the bench-scale BAC reactor were collected after vigorous shaking of the reactor. Each biomass sample contained a number of randomly selected BAC particles. All biomass samples were stored at -80°C until analyses.

DNA was extracted from the BAC samples using FastDNA SPIN Kit (Qbiogene Inc., Irvine, California) and quantified using a NanoDrop 1000 (NanoDrop Technology, Wilmington, Delaware). DNA quality was evaluated by electrophoresis on a 0.8% agarose gel. 16S rRNA genes were amplified in triplicate using the polymerase chain reaction (PCR) on a Mastercycler (Eppendorf International, Hamburg, Germany) with the forward primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') (Dojka et al., 1998) and the reverse primer 1387R (5'-GGG CGG [A/T]GT GTA CAA GGC-3') (Wobus et al., 2003). The composition of the PCR reaction mixture was adapted from the work by Wobus and co-workers (Wobus et al., 2003). The PCR reaction involved 30 cycles and started with 5 min of denaturation at 95˚C and ended with a final extension at 72˚C for 18 min. Each cycle consisted of denaturation at 95˚C for 30 s, annealing at 50˚C for 45 s, and extension at 72˚C for 2 min. Pooled PCR products were purified by electrophoresis on a 1% agarose gel and extracted using the MinElute Gel Extraction Kit (QIAGEN Inc., Valencia, California). Purified PCR products were cloned into TOPO vector (Invitrogen Inc., Carlsbad, California) and transformed into chemically competent TOPO10 *Escherichia coli*. The transformed *E. coli* cells were plated on Luria-Bertani agar that contained 50 μg/mL kanamycin and incubated at 37˚C overnight. Colonies were picked randomly and used to inoculate three 96-well microplates. Two of the three 96-well

microplates were sent to the Genomic Center at Washington University (St. Louis, Missouri) in glycerol stocks for sequencing.

Raw sequence information from the Genomic Center was entered into the Ribosomal Database Project (RDP) (Cole et al., 2007) or Greengenes (DeSantis et al., 2006). The sequences were classified into bacterial populations based on phylogenetic analyses, and the relative abundance of identified populations was determined. Microbial compositions among reactors were also compared using the "Compare Libraries" function provided by RDP.

3. Results and Conclusions

Bench-scale BAC reactor

The clone library results show that the relative abundance *Dechloromonas*-like bacterial strains increased from 15.7% to 45.2% after the addition of phosphorus (Figure D.1, Tables D.2, and D.3). In the meantime, the *Azospira*-like bacterial strains increased from 0.6 to 10% after the addition of phosphorus (Figure D.1, Table D.2, and D.3). It is worth mentioning that the type strain of *Azospira*, *A. oryzae*, has a subjective synonym *Dechlorosoma suillum*, the name used extensively in earlier literature. Most of the identified members of the two genera, *Dechloromonas* and *Azospira*, can use oxygen, nitrate, and perchlorate as electron acceptors (Coates and Achenbach, 2004). Therefore, it is speculated that phosphorus addition enhanced nitrate and perchlorate removal by promoting and maintaining high relative abundance of *Dechloromonas* and *Azospira* strains.

In addition to *Dechloromonas* and *Azospira*, *Zoogloea*-like bacterial strains were also abundant in the microbial community. Its relative abundance did not vary much after phosphorus addition (from 7.8% to 8.0%). Members of the *Zoogloea* genus are important in the formation of biofilm structure. Therefore, it is not surprising to detect *Zoogloea*-like bacterial strains in the biofilm reactor.

Demonstration-scale BAC reactors F120 and F130

No significant difference was detected between the microbial compositions of the two demonstration-scale BAC reactors, F120 and F130. Both *Dechloromonas*-like and *Zoogloea*-like bacterial strains were abundant in the two BAC reactors (12.4 and 12.4% in F120, and 8.0 and 17.8% in F130, respectively). The group Incertae sedis 5 represented a significant fraction of the community, 15.3% and 18.4% in F120 and F130, respectively. Two other genera, *Aquabacterium* and *Leptothrix*, were found at relatively high levels in these two reactors. *Aquabacterium* were first isolated from biofilms collected from a Berlin drinking water system. Members of this genus are microaerophilic and are able to use nitrate as an electron acceptor (Kalmbach et al., 1999). *Leptothrix* spp. are usually found in unpolluted natural environments with low concentrations of easily degradable organic nutrients. They can oxidize iron and manganese in water (Spring, 2006).

Demonstration-scale BAC reactor F130

The relative abundance of *Dechloromonas*-like bacterial strains, the only known perchlorate reducing bacteria detected in the reactor, decreased from 8.0 to 2.3% (Figure D.1, and Table D.4 and D.5). The other perchlorate reducing bacterial genus, *Azospira*, was not detected in either of the clone libraries. In the meantime, the relative abundance of *Zoogloea*-like bacterial strains, increased from 17.8 to 27.9% (Figure D.1, and Table D.4 and D.5). Another microbial population that increased significantly after phosphorus addition was *Acidovorax*-like bacterial strains: from non-detected to 19.8%.

The clone library result is counterintuitive: the relative abundance of *Dechloromonas*-like bacterial strains was expected to increase, because the reactor's ability of removing perchlorate improved significantly after phosphorus addition. One possibility is that the microbial community contained perchlorate reducing bacterial populations that have not been previously documented. For example, members of both *Acidovorax* and *Zoogloea* genera can use nitrate as an electron acceptor. It is possible that the increase in the relative abundance of these populations were responsible for the improvement in perchlorate reduction in the demonstration-scale bioreactor F130.

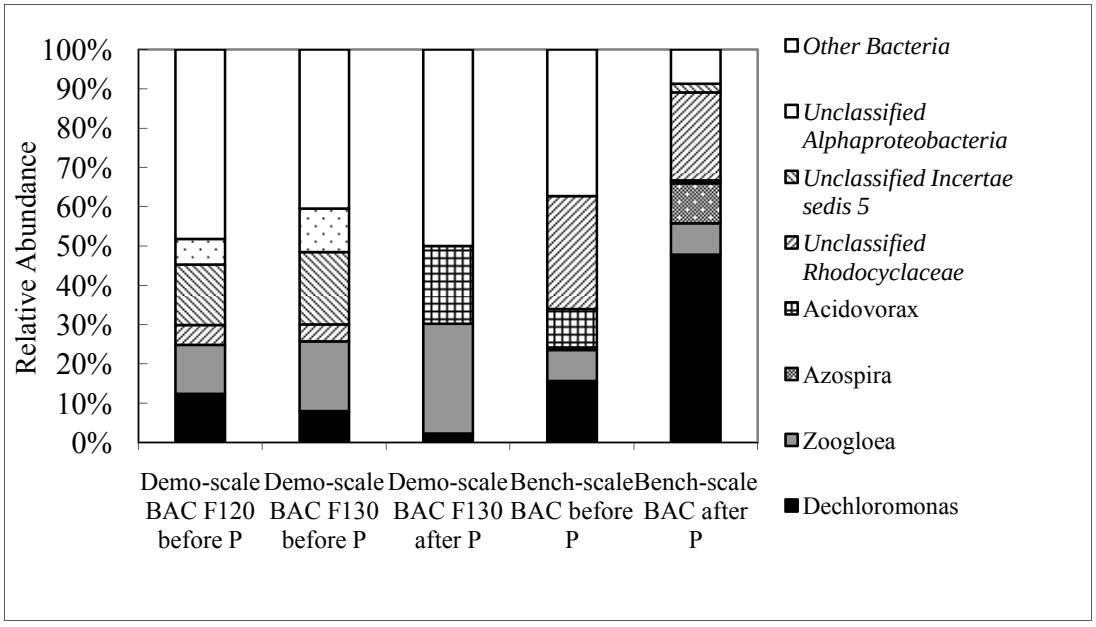

Figure D.1 - Relative Abundance of Microbial Populations in the Five Clone Libraries. For the Demonstration-scale Reactors, the "before P" and the "after P" Samples were Collected in May and September 2007, Respectively. For the Bench-Scale BAC Reactor, the "before P" and the "after P" Samples were Collected in December 2006 and May 2007, Respectively.

Table D.2 - Microbial Composition of the Biomass Sample Collected from the Bench-scale BAC Reactor at the University of Michigan in December 2006 (prior to phosphorus addition) [1].

Phylum	Class	Family	Genus	Number
Proteobacteria	Alpha-Proteobacteria	Hyphomicrobiaceae	*Pedomicrobium*	1
		Phyllobacteriaceae	*Mesorhizobium*	1
		Bradyrhizobiaceae	*Afipia*	2
			Unclassified Bradyrhizobiaceae	4
	Beta-Proteobacteria	Rhodocyclaceae	*Dechloromonas*	24
			Zoogloea	12
			Ferribacterium	1
			Azospira	1
			Unclassified Rhodocyclaceae	44
		Comamonadaceae	*Acidovorax*	15
			Hydrogenophaga	1
		Oxalobacteraceae	Unclassified Oxalobacteraceae	12
	Gamma-Proteobacteria	Vibrionaceae	*Vibrio*	3
			Unclassified Vibrionaceae	6
		Unclassified Gamma-Proteobacteria		6
	Delta-Proteobacteria	Polyangiaceae	*Byssovorax*	4
			Sorangium	1
			Unclassified Polyangiaceae	12
		Unclassified Myxococcales		1
	Unclassified Proteobacteria			1
Spirochaetes	Spirochaetes	Leptospiraceae	*Turneriella*	1
			Total:	153

[1.] *The numbers represent the numbers of the clones in the clone library.*

Table D.3 - Microbial Composition of the Biomass Sample Collected from the Bench-scale BAC Reactor at the University of Michigan in May 2007 (after phosphorus addition) [1].

Phylum	Class	Family	Genus	Number
Acidobacteria	Acidobacteria	Acidobacteriaceae	*Gp8*	1
Proteobacteria	Alpha-Proteobacteria	Bradyrhizobiaceae	*Bradyrhizobium*	1
			Afipia	1
	Beta-Proteobacteria	Rhodocyclaceae	*Dechloromonas*	66
			Zoogloea	11
			Azospira	14
			Azonexus	1
			Unclassified Rhodocyclaceae	31
		Incertae sedis 5	Unclassified Incertae sedis 5	3
		Comamonadaceae	*Curvibacter*	1
			Acidovorax	1
			Unclassified Comamonadaceae	1
		Oxalobacteraceae	Unclassified Oxalobacteraceae	1
		Unclassified Burkholderiales		1
	Gamma-Proteobacteria	Vibrionaceae	Unclassified Vibrionaceae	1
	Delta-Proteobacteria	Polyangiaceae	*Byssovorax*	1
			Sorangium	1
		Unclassified Delta-Proteobacteria		1
			Total:	138

[1] *The numbers represent the numbers of the clones in the clone library.*

Table D.4 - Microbial Composition of the Biomass Sample Collected from Carollo BAC Reactor F120 in May 2007[1].

Phylum	Class	Family	Genus	Number
Acidobacteria	Acidobacteria	Acidobacteriaceae	*Gp8*	1
Bacteroidetes	Unclassified Bacteroidetes			1
Proteobacteria	Alpha-Proteobacteria	Rhodospirillaceae	Unclassified Rhodospirillaceae	3
		Unclassified Rhodospirillales		2
		Unclassified Rhizobiales		2
		Unclassified Alpha-Proteobacteria		9
	Beta-Proteobacteria	Rhodocyclaceae	*Dechloromonas*	17
			Zoogloea	17
			Ferribacterium	7
			Propionivibrio	1
			Unclassified Rhodocyclaceae	7
		Incertae sedis 5	*Aquabacterium*	7
			Leptothrix	7
			Unclassified Incertae sedis 5	21
		Comamonadaceae	*Comamonas*	1
			Simplicispira	1
			Unclassified Comamonadaceae	13
		Unclassified Beta-Proteobacteria		1
	Gamma-Proteobacteria	Xanthomonadaceae	*Dyella*	2
			Unclassified Xanthomonadaceae	2
		Vibrionaceae	Unclassified Vibrionaceae	3
	Delta-Proteobacteria	Polyangiaceae	*Byssovorax*	1
		Unclassified Myxococcales		1
	Unclassified Proteobacteria			5
Chloroflexi	Anaerolineae	Caldilneacea	*Levilinea*	1
Firmicutes	Clostridia	Unclassified Clostridiales		1
Unclassified Bacteria				3
			Total:	137

[1.] *The numbers represent the numbers of the clones in the clone library.*

Table D.5 - Microbial Composition of the Biomass Sample Collected from Carollo BAC Reactor F130 in May 2007[1].

Phylum	Class	Family	Genus	Number
Acidobacteria	Acidobacteria	Acidobacteriaceae	*Gp8*	3
Bacteroidetes	Flavobacteria	Cryomorphaceae	*Crocinitomix*	1
			Unclassified Cryomorphaceae	1
		Unclassified Flavobacteriales		1
Proteobacteria	Alpha-Proteobacteria	Rhodospirillaceae	Unclassified Rhodospirillaceae	1
		Unclassified Rhodospirillales		3
		Unclassified Alpha-Proteobacteria		18
	Beta-Proteobacteria	Rhodocyclaceae	*Dechloromonas*	13
			Zoogloea	29
			Ferribacterium	8
			Unclassified Rhodocyclaceae	7
		Incertae sedis 5	*Aquabacterium*	7
			Leptothrix	8
			Pelomonas	1
			Unclassified Incertae sedis 5	30
		Comamonadaceae	*Comamonas*	1
			Simplicispira	1
			Unclassified Comamonadaceae	7
		Unclassified Burkholderiales		1
	Gamma-Proteobacteria	Xanthomonadaceae	*Aquimonas*	1
			Unclassified Xanthomonadaceae	2
		Vibrionaceae	*Vibrio*	2
	Delta-Proteobacteria	Cystobacteraceae	Unclassified Cystobacteraceae	2
		Polyangiaceae	*Byssovorax*	1
			Unclassified Polyangiaceae	2
	Unclassified Proteobacteria			7
Chloroflexi	Anaerolineae	Caldilneacea	*Leptolinea*	1
Firmicutes	Clostridia	Unclassified Clostridiales		1
Unclassified Bacteria				3
			Total:	163

[1.] *The numbers represent the numbers of the clones in the clone library.*

Table D.6 - Microbial Composition of the Biomass Sample Collected from Carollo BAC Reactor F130 in December 2007[1].

Phylum	Class	Order	Family	Genus	Number
Bacteroidetes	Bacteroidetes	Bacteroidales	Rikenellaceae	*Alistipes*	1
Proteobacteria	Beta-Proteobacteria	Rhodocyclales	Rhodocyclaceae	*Dechloromonas*	2
				Zoogloea	24
				Azoarcus	2
				Thauera	15
				Ferribacterium	1
		Burkholderiales	Incertae sedis 5	*Leptothrix*	2
				Ideonella	1
			Comamonadaceae	*Comamonas*	1
				Variovorax	1
				Hydrogenophaga	2
				Acidovorax	17
		Unclassified Beta-Proteobacteria			14
	Gamma-Proteobacteria	Xanthamonadales	Xanthamonadeceae	*Aquimonas*	1
Firmicutes		Bacillales	Bacillaceae	*Exiguobacterium*	1
				Propionispora	1
				total	86

[1] *The numbers represent the numbers of the clones in the clone library.*

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Appendix E - Mathematical Modeling Results

Modeling Approach

The purpose of the mathematical modeling was to make use of the experimental results from the demonstration scale reactors and to evaluate to what extent system performance can be extrapolated from the available results. The mathematical model had to be developed mainly based on bulk phase measurements of perchlorate, nitrate, oxygen, and acetate. These empirical observations were combined with well studied diffusion-reaction description of processes in the biofilm (Morgenroth, 2008). While the model structure for biofilm systems (i.e., the one dimensional diffusion-reaction modeling approach) is well established (Wanner *et al*., 2006) the values of model parameters are not. In the current study most of the model parameters are based on literature information and some are estimated based on observed reactor performance. And to take into account the uncertainty of kinetic parameters a range of reasonable parameter combinations are simulated to evaluate the sensitivity of model predictions on specific parameter values.

The mathematical modeling followed the following steps:

- Define model structure
- Select standard model parameters from the literature and from calibrating against reactor performance
- Use calibrated model to evaluate the influence of operating conditions on reactor performance
    - Influence of EBCT and electron donor addition
    - Influence of biofilm thickness and backwashing
    - Influence of influent perchlorate concentrations
    - EBCT requirements depend on influent perchlorate or nitrate concentrations

Model Description

1.1 Process Kinetics and Stoichiometry

A mathematical model was implemented in AQUASIM (Reichert, 1998) based on a model developed by Choi, 2005. The model Choi, 2005, was expanded to include nitrate as a state variable and bacterial growth using nitrate for both normal heterotrophic and perchlorate reducing bacteria. The stoichiometric and kinetic matrix is provided in the in

Table E.3. The details of the implementation of this model in AQUASIM are shown in Section 0.

1.2 Compartments in Series

All simulations were performed for three biofilm compartments in series. With a larger number biofilm compartments in series simulation time increases significantly. With n = 3 we can still get an idea about the different redox zones within the reactor with, for example, mainly aerobic removal in the first part of the reactor and the majority of perchlorate removal towards the end.

1.3 Backwashing

Regular backwashing was implemented as described in Morgenroth and Wilderer, 2000. The thickness of a biofilm results from the balance of growth (increasing the biofilm thickness) and decay and detachment (decreasing the biofilm thickness) (Morgenroth, 2008):

$$\underbrace{\frac{dL_F}{dt}}_{\text{Net change of biofilm thickness}} = \underbrace{\frac{Y \cdot J_{LF}}{X_F}}_{\text{Growth}} - \underbrace{b_{ina} L_F}_{\text{Decay}} - \underbrace{u_{d,S}}_{\text{Surface detachment velocity}}$$

For simplicity, most biofilm models assume a constant biofilm thickness by setting the rate of detachment ($u_{d,S}$) equal to the growth minus decay.

$$\underbrace{u_{d,S}}_{\text{Surface detachment velocity}} = \underbrace{\frac{Y \cdot J_{LF}}{X_F}}_{\text{Growth}} - \underbrace{b_{ina} L_F}_{\text{Decay}}$$

Dynamic reactor operation and stochastic biofilm dynamics results in variable biofilm thickness over time which can have significant implications of competition within a biofilm (Morgenroth and Wilderer, 2000). In this study we evaluated the influence of dynamic detachment. Biofilms were simulated assuming a constant thickness or with backwashing in daily or weekly intervals. During backwashing all biofilm above a user defined minimum base thickness is removed while between backwashing events the rate of detachment is set to zero resulting in a net increase of biofilm thickness until the next detachment event.

Parameter that define backwashing are

- R_Backwash_Int = Interval between backwashing events [d]
- R_Backwash_Duration = Duration of a backwashing event [d]
- R_L_Fdetach = Biofilm thickness after backwashing [m]
- R_DetachBetweenBackwash = Extent of biofilm erosion during backwashing events defined as fraction of biofilm eroded per biofilm growth[-]

1.4 How to Read the Plotted Output

The information automatically plotted by "CYC_read_AQUASIMouput.r" in R (R development core team, 2008) are the following:

- Above the plot
  - P: State variable that is plotted (Parameter)
  - T : Simulation time
  - C: Name of the compartment
  - S: Location inside of the biofilm where 1 is corresponds to the surface of the biofilm and 0 corresponds to the base of the biofilm
- Y-axis
  - Label: State variable that is plotted – same as P above the plot
  - Range: The range is automatically chosen by the data analysis software R (R development core team, 2008)
- X-axis

    - Label: One parameter that was varied between different simulation runs. Typically this is from one of the columns in CYC_vcmd.txt
    - Range: The range is automatically chosen by the data analysis software R (R development core team, 2008)
- Below the plot
    - Date/time when plot was prepared (helps to retrieve the appropriate PDF file containing plots)
    - Label: One parameter that is shown within the plot for each simulation. This could be a simulation number (e.g., Num_Sim) or another parameter in CYC_vcmd.txt.
- In the plot
    - Numbers next to data points: Value of the Label where the Label is defined below the plot.

Figure E1 is provided as an example of the standardized way that model output is presented. In Figure E1 perchlorate concentrations (C_PC) at the surface of the biofilm (S: 1) in the first biofilm compartment (C: Reactor_1) are plotted for different influent acetate concentrations (C_Sin) as the x-axis. Different scenarios are plotted where numbers in the plot refer to different simulations numbers (Num_Sim) where simulation numbers can be specified for different parameter combinations or operating conditions of the reactor. This standardized way of plotting is used for most plots in this report.

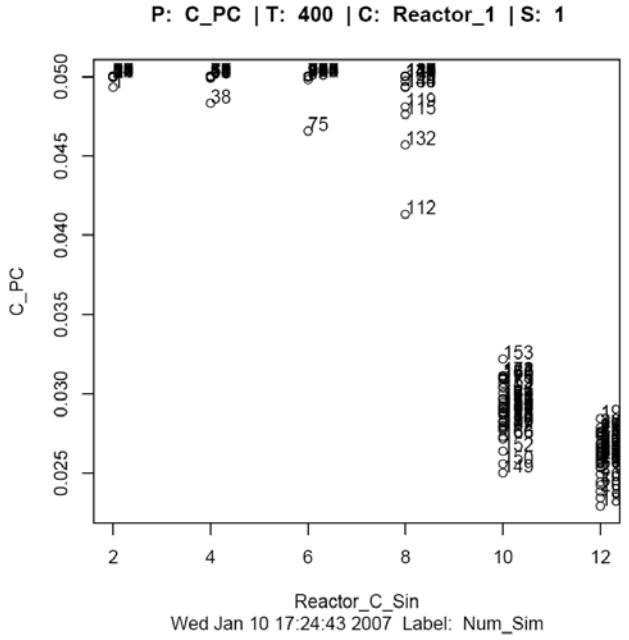

Figure E.1 - Description of How to Read the Model Output Plotted Using R (R development core team, 2008)**.**

Results from Pilot Scale and Bench Scale Testing

Pilot and bench-scale reactor operation evaluated the influence of EBCT and electron donor addition. Key results for the pilot plant (Optimization Summary.doc) and the bench scale (Summary of Bench-scale BAC.doc) demonstrated that complete perchlorate removal can be achieved with an EBCT larger than 8 min and a [D/A] larger than 1.7. These overall results were used to calibrate our mathematical model.

1.5 Standard Conditions

The following parameters were used to define standard conditions:

Table E.1 - Default Values of Key Process Parameters.

Symbol	Description	Unit	Value	Type[(a)]
EBCT	Empty bed contact time	min	e.g., 5 – 50	Ind
L_F, L_Fini	Biofilm thickness, initial biofilm thickness	m	600×10^{-6}	Ind
L_L	Boundary layer thickness	m	10×10^{-6}	Fix
a	Specific surface area of the biofilm = $A_{total}/V_{reactor}$	m^2/m^3	1,000	Fitted [(b)]
A_total	Total surface area of the biofilm support media	m^2	3.4	Fix [(c)]
V_reactor	Total reactor volume	m^3	$V_{reactor} = A_{total}/a$	Calc
Q	Influent flow rate	m^3/d	$Q = V_{reactor}/ EBCT = (A_{total}/a)/ EBCT$	Calc
n	Number of biofilm modules in series (see Figure E).	-	3	Fix
[D/A] or R_D_A_Ratio	Influent electron donor to electron acceptor ratio [(d)]	-	1 - 4	Ind
C_PC, C_PCin	Perchlorate concentration, influent perchlorate concentration	mg/L	0.050 - 5	Ind
C_O2, C_O2in	Oxygen concentration, influent oxygen concentration	mg/L	8	Ind
C_NO3, C_NO3in	Nitrate concentration, influent nitrate concentration	mg N/L	7 mg N/L = 31 mg NO_3^-/L	Ind
C_S, C_Sin	Organic substrate concentration, influent organic substrate concentration [(d)]	mg COD/L	$[D/A]*(C_{O2,in} + 2.85714* C_{NO3,in} + 0.643216*C_{PC,in})$	Calc

[(a)] Ind = independent parameter that was evaluated in this study, Fix = a fixed value was assumed for this parameter, Calc = this dependent parameter was calculated, Fitted = this parameter was fitted to experience from reactor operation as described in Section 1.6.
[(b)] The surface to volume ratio (*a*) was fitted based on observed removal in bench and pilot-scale reactors compared to model simulation in Figure E.2.

[c] Note that substrate removal is determined by the influent flow rate per available surface area and the influent flow rate is determined based on the EBCT and the specific surface area.
[d] [D/A] ratio is calculated as described in Appendix B Section 2.1, balancing electron donor and acceptor and not taking into account cell synthesis ($f_s = 0$)

1.6 Model Calibration

Most of the kinetic and stoichiometric parameters used to describe perchlorate reduction were derived from previous research studies (Choi, 2005). Results from pilot scale evaluation provided information on minimum EBCT and electron donor addition but did not allow for a detailed calibration of process kinetics and stoichiometry.

1.6.1 Surface to Volume Ratio

A key parameter for model that was fitted to experiences from pilot scale testing was the specific surface area of the biofilm (m^2 biofilm/m^3 reactor volume). Simulations were performed using surface to volume ratios ranging from 200 to 1,500 m^2/m^3 for different [D/A] with an EBCT of 8 min (Figure E.2). As discussed in the section above, with an EBCT of 8 min and a [D/A] of 1.7 good perchlorate reduction is expected. Based on these results a surface to volume ratio of 1,000 m^2/m^3 was fixed for all subsequent simulations (Table E.1).

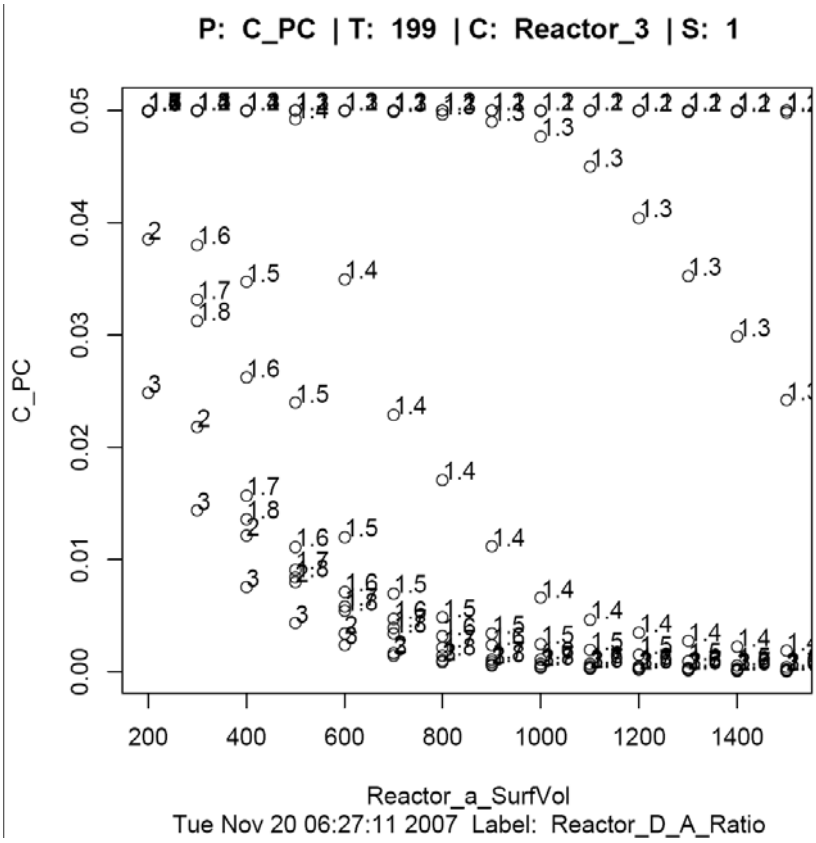

Figure E.2 - Evaluation of Reasonable Surface to Volume Ratio Based on Effluent Perchlorate Concentration as a Function of the Surface to Volume Ratio Evaluated for Different Electron Donor Additions at an EBCT of 8 minutes. Efficient Perchlorate Removal that was Observed in the Pilot Scale Reactor was Reproduced by the Model with a Surface to Volume Ratio of 1,000 m2/m3.

1.6.2 Biofilm Thickness

The biofilm thickness was chosen so that microbial competition was not limited by the total amount of biofilm but rather that competition was limited only by diffusive transport of substrate into the biofilm and competition of microbial species for locations close to the surface of the biofilm. In Figure E. the influence of the biofilm thickness on oxygen, nitrate, and perchlorate removal are shown for different sets of kinetic parameters for perchlorate reducers. In the first of the three reactors in series none of the electron acceptors (oxygen, nitrate, and perchlorate) are completely removed. However, increasing the biofilm thickness above 200 or 400 μm for the removal of oxygen or nitrate, respectively, does not improve removal of these electron acceptors. Significant removal of perchlorate in the first reactor can be achieved even with biofilm thicknesses of 200 μm but removal depends strongly on the kinetic parameters for the perchlorate reducing bacteria. Within the third reactor complete oxygen removal is achieved with biofilm thicknesses of 100 μm and effluent nitrate concentrations do not decrease below 3 mg/L for biofilm thicknesses of 300 μm. Stable perchlorate removal to less than 5 μg/L are achieved with biofilm thicknesses of 300 μm and, different from the first reactor, perchlorate removal is not strongly influenced by the choice of parameter combination. A standard biofilm thickness of 600 μm was, unless otherwise noted, assumed for all subsequent simulations (Table E.1). This standard biofilm thickness applied to all three biofilm compartments modeled in series as shown in Figure E.

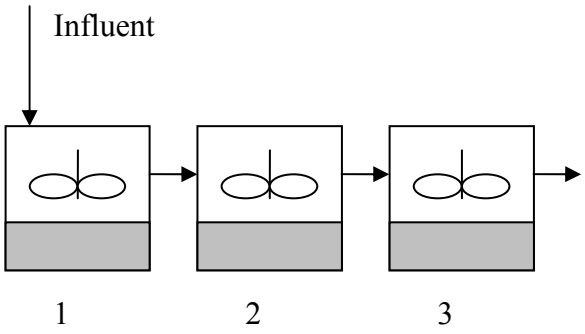

Figure E.3 - The Pilot Reactor was Modeled as three Biofilm Reactors in Series Where the Bulk Phase in Each Reactor was Completely Mixed and Mass Transfer into the Biofilm was Modeled Separately for the Three Biofilm Compartments (Morgenroth, 2008).

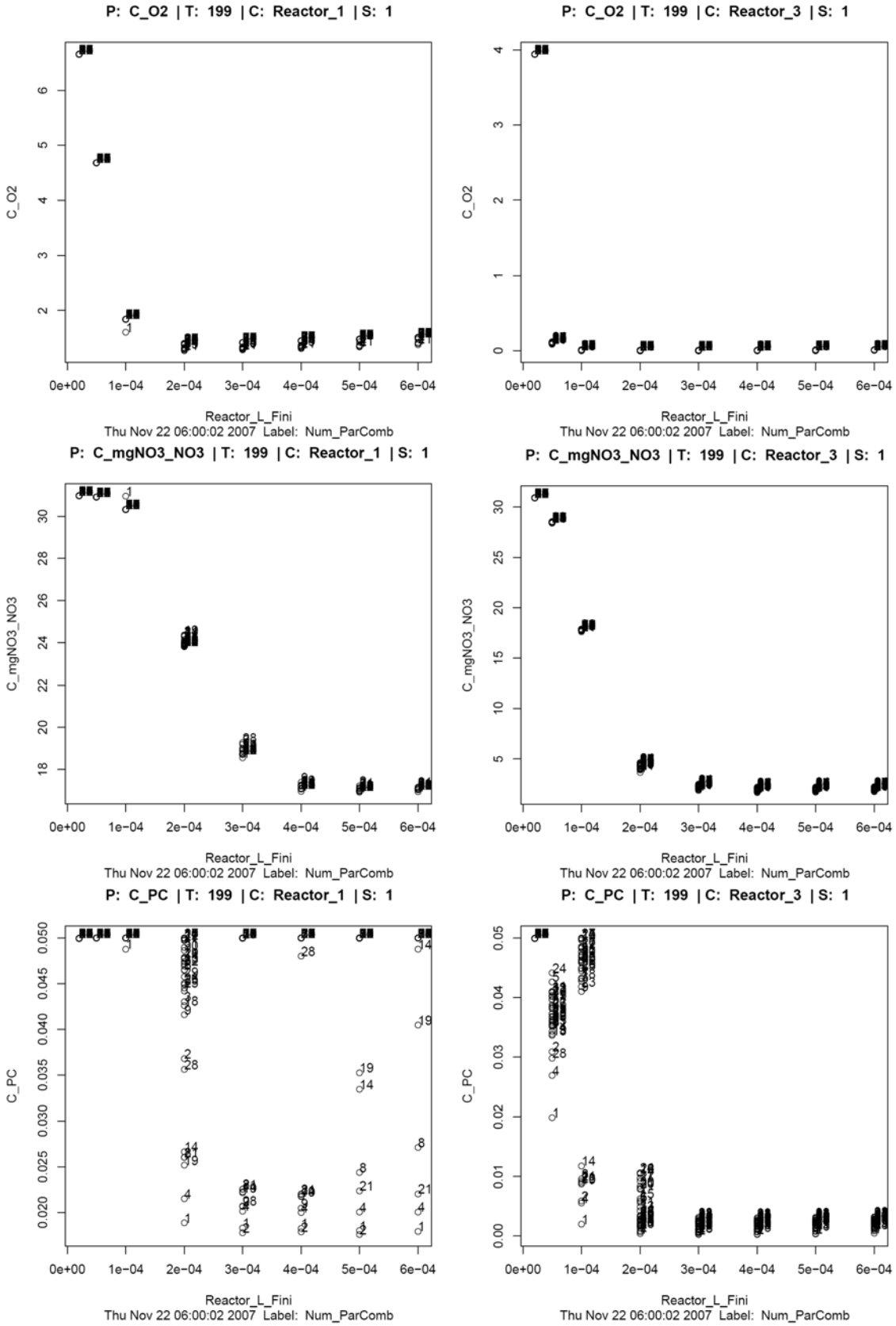

Figure E.4 - Influence of Biofilm Thickness (x-axis) and Kinetic Parameter Sets (numbers in plots) for Perchlorate Reducers on Effluent Concentrations of Oxygen (top), Nitrate (middle), and Perchlorate (bottom) in the First (left) and the Third (right) Reactor. Operating Conditions are 8 min EBCT and [D/A] = 2.

Results

1.7 Influence of EBCT and Electron Donor Addition

Simulations were performed to evaluated the combined effect of EBCT and the [D/A] ratio on the removal of all electron acceptors (oxygen, nitrate, and perchlorate) for two different influent perchlorate concentrations. Results are presented in Figure E.5. Almost complete removal of oxygen (effluent oxygen concentrations below 0.04 mg/L) was observed for all [D/A] ratios and EBCT evaluated where effluent oxygen concentrations decreased with increasing [D/A] ratios and EBCT. Nitrate and perchlorate removal was mainly influenced by [D/A] ratios where both nitrate and perchlorate removal required [D/A] ratios larger than 1.7. This limiting [D/A] ratio applied independent of the influent perchlorate concentration.

The extent of perchlorate removal in the three biofilm compartments is shown in Figure E. Only partial perchlorate removal was achieved in the first biofilm compartment [D/A] ratios larger than 1.7 where perchlorate removal increased significantly with increased EBCT. For [D/A] ratios larger than 1.7 the overall reactor effluent contained significant amounts of organic substrate where effluent perchlorate and substrate concentrations are correlated as shown in Figure E.8 and Figure E.9Figure E. In the first compartment perchlorate removal was biomass rather than substrate limited and both effluent perchlorate and substrate from the first compartment decreased with increasing EBCT.

In Figure E.7 the volume fractions of heterotrophic and perchlorate reducing bacteria are shown. It can be seen that with [D/A]s larger than 1.7 the mathematical model predicts an outcompetition of heterotrophic by perchlorate reducing bacteria.

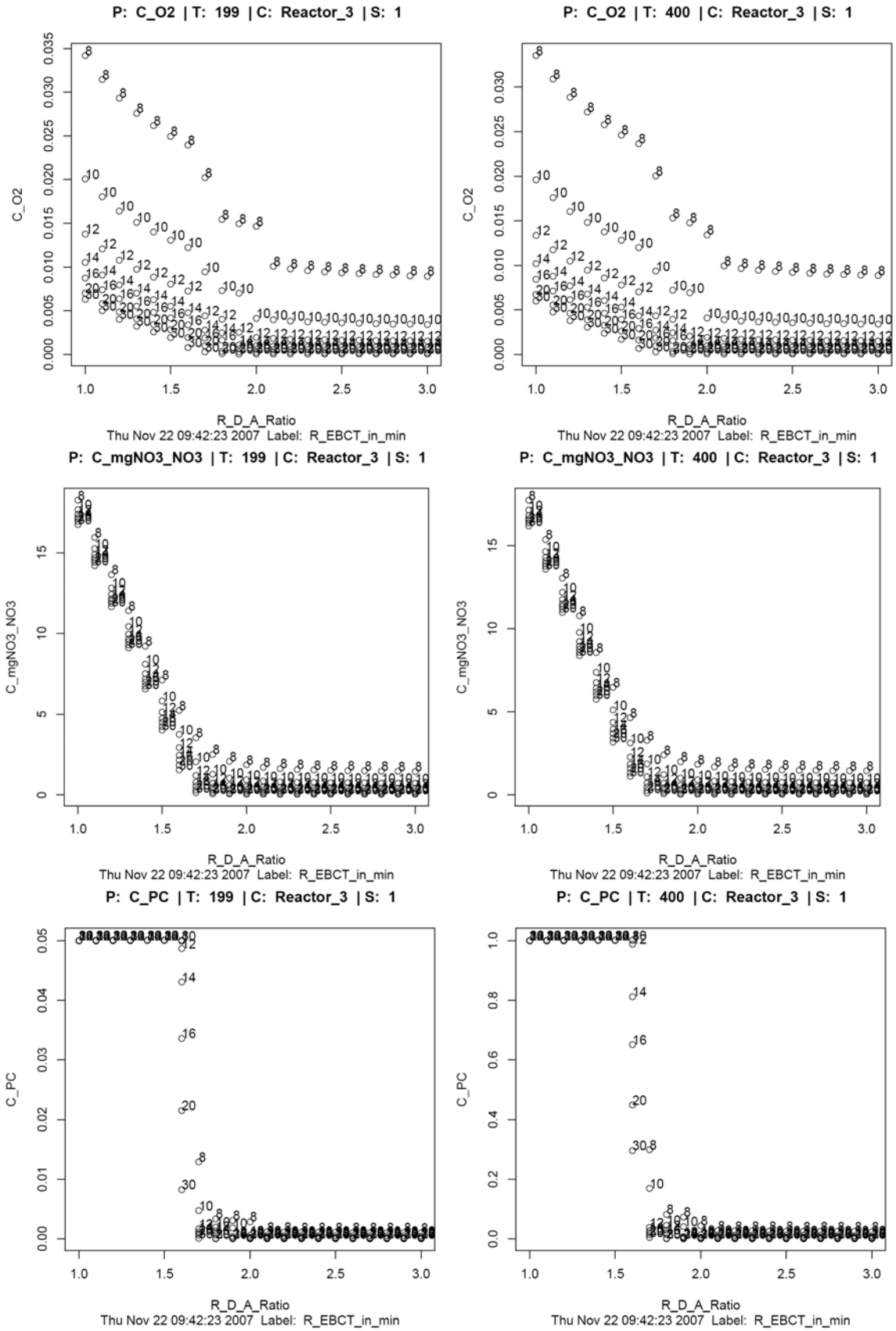

Figure E.5 - Influence of [D/A] (x-axis) and EBCT (numbers in graphs in minutes) on Steady State Effluent Oxygen (top), nitrate (middle), and Perchlorate (bottom) for Influent Perchlorate Concentrations of 50 µg/L (left) or 1 mg/L (right).

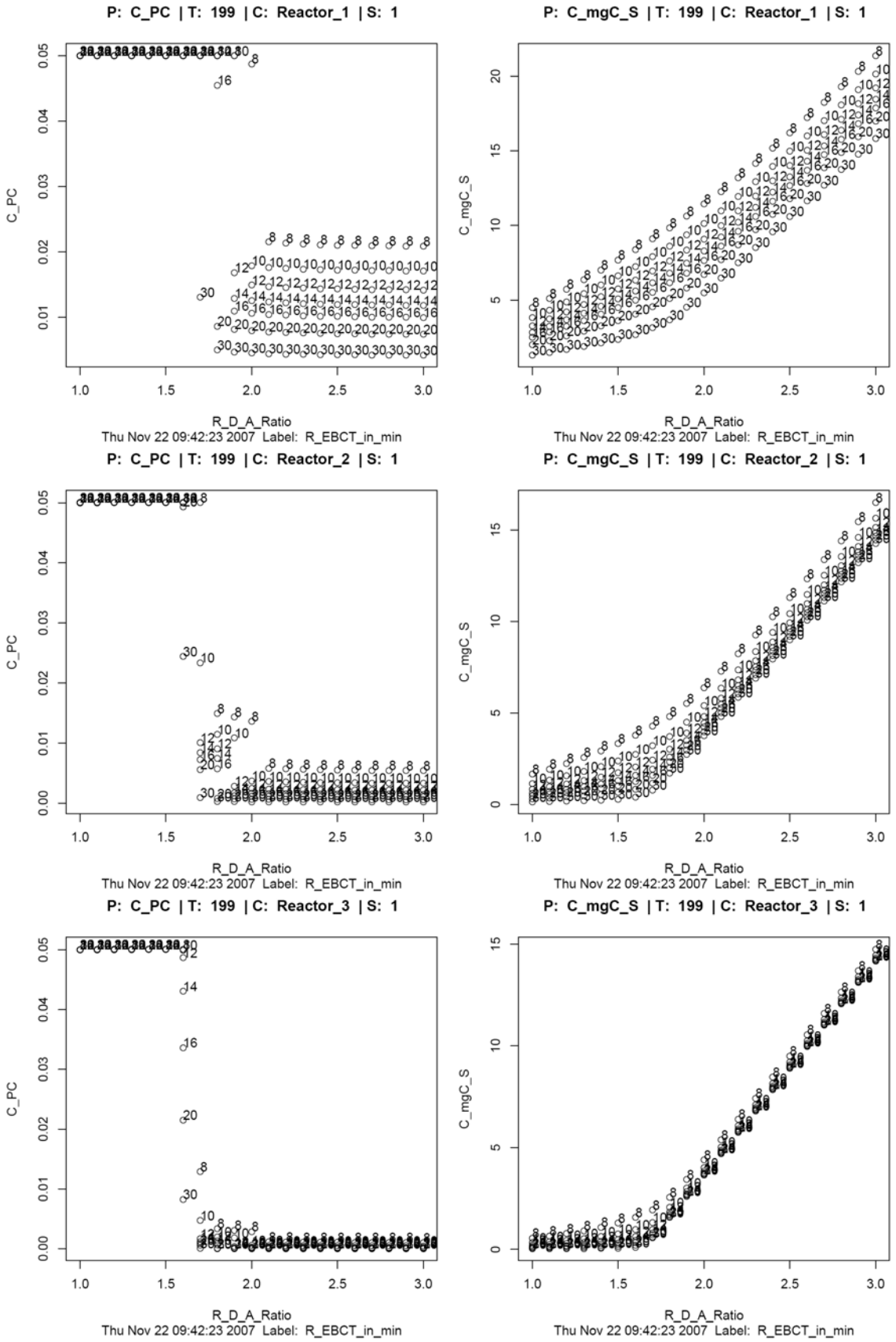

Figure E.6 - Effluent Perchlorate Concentrations (left) and Substrate Concentrations (right) as a Function of [D/A] (x-axis) and EBCT (numbers in plot in min) in the First (top), Second (middle), and Third (bottom) Reactor for Influent Perchlorate Concentrations of 50 µg/L.

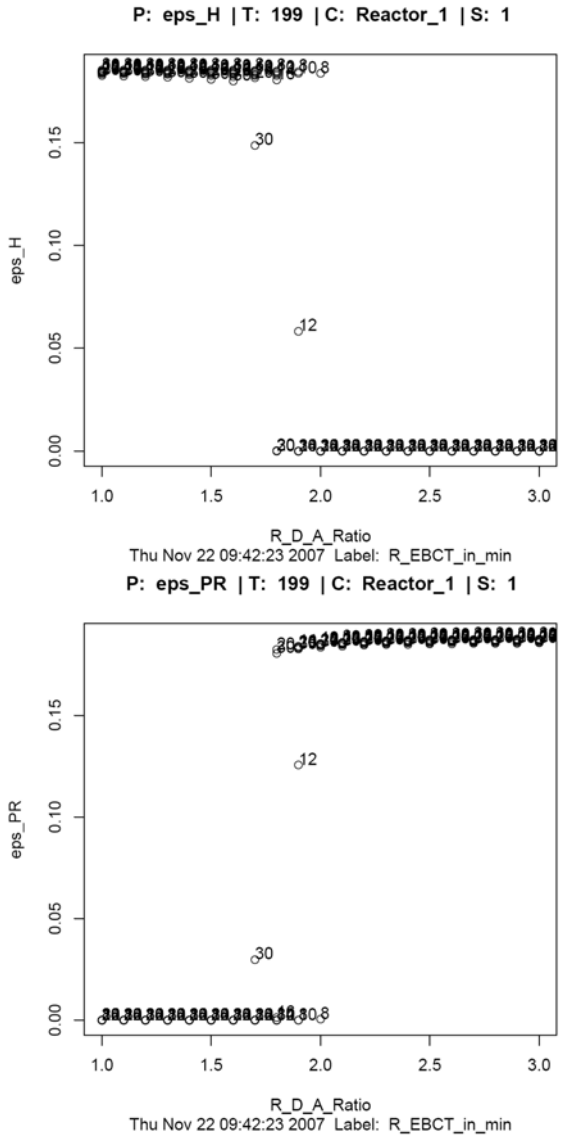

Figure E.7 - Volume Fraction of Heterotrophic Bacteria (top) and Perchlorate Reducing Bacteria (bottom) at the Surface of the Biofilm as a Function of [D/A] (x-axis) and EBCT (numbers in plot in min). Note that the Model Assumes the Biofilm to be Composed of Water (80%) and Biomass (20%) so that a Volume Fraction of 0.20 is the Maximum Possible Volume Fraction for a Bacterial Species.

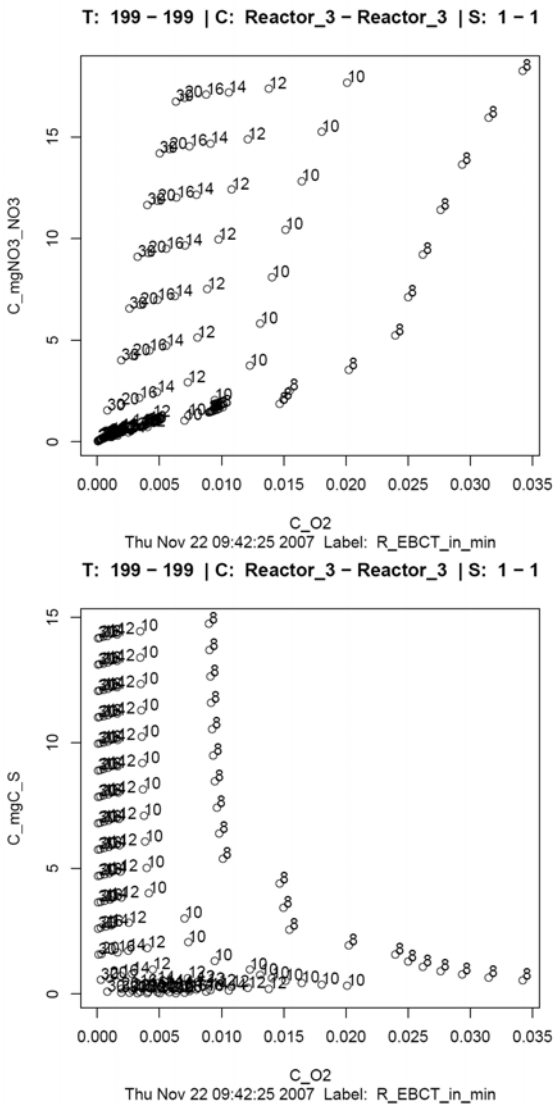

Figure E.8 - Correlation of Effluent Nitrate (top) and Organic Substrate (bottom) Concentrations as a Function of Effluent Oxygen Concentrations for all [D/A] with EBCT (numbers in plot in min) for Influent Perchlorate Concentrations of 50 µg/L.

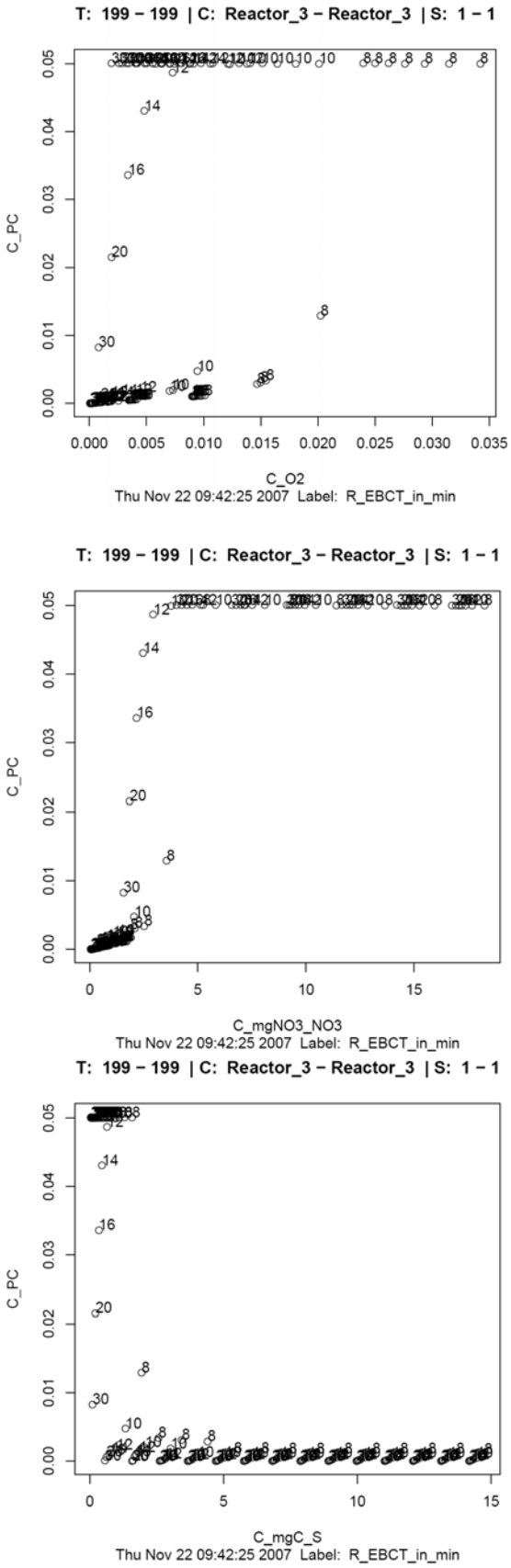

Figure E.9 - Correlation of Effluent Perchlorate Concentration with Effluent Oxygen (top), Nitrate (middle), and Organic Substrate (bottom) Concentrations for all [D/A] with EBCT (numbers in plot in min) for Influent Perchlorate Concentrations of 50 µg/L.

1.8 Influence of Biofilm Thickness and Backwashing

Backwashing intervals of 1 and 7 d were evaluated. Results are shown in Figure E.10 (base thickness after backwashing of 500 µm) and Figure E.11 (base thicknesses after backwashing of 100 µm). After backwashing, especially with a backwashing interval of 7 d, effluent concentrations increased temporarily. However, the increase of effluent perchlorate concentrations were small for all evaluated cases and backwashing does not appear to be a major factor as long as sufficient biomass (e.g., more than 200 or 300 µm) remains in the system after backwashing (see also Figure E.4 and associated discussion of the minimum biofilm thickness required for efficient perchlorate removal). A detailed comparison of a base thickness after backwashing of 100 or 500 µm with backwashing intervals of 7 d are shown in Figure E.12. It can be seen that a smaller base thickness results in a more significant increase of effluent concentrations after backwashing and it requires a longer period until effluent perchlorate concentrations decrease again.

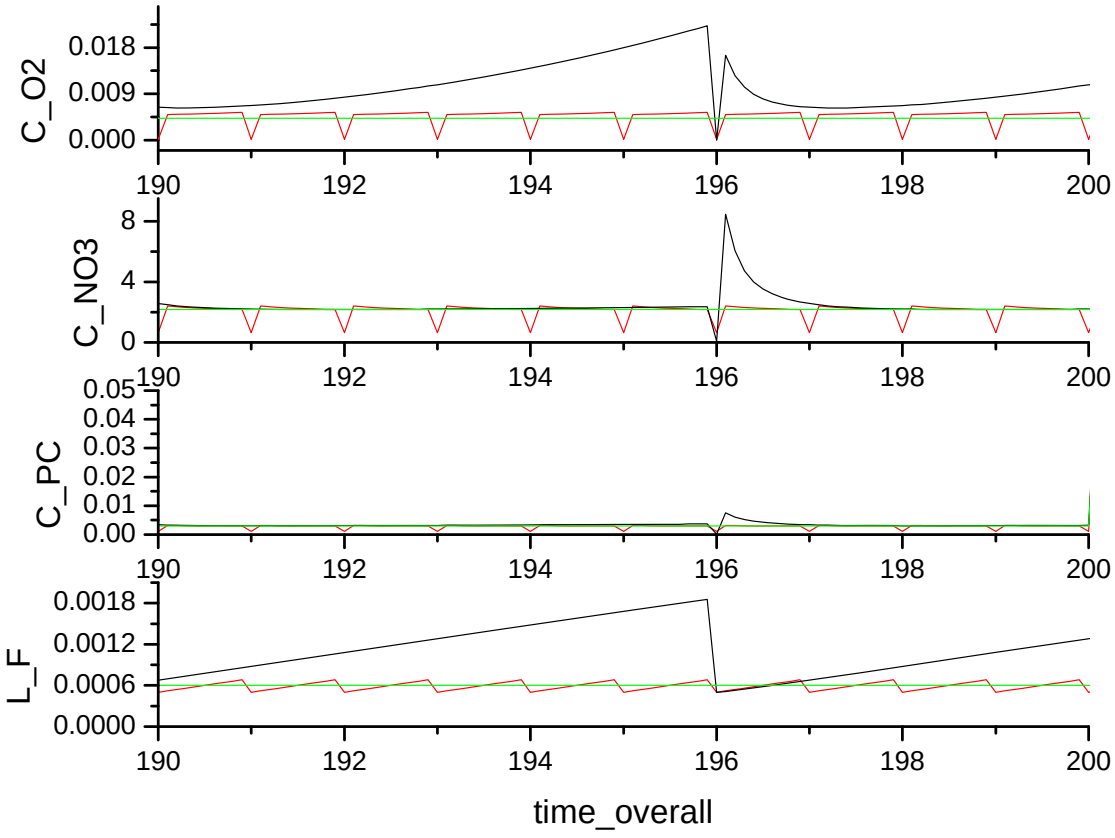

Figure E.10 - Influence of Backwashing Interval on Effluent Oxygen, Nitrate and Perchlorate Concentrations and the Corresponding Biofilm Thickness Development for a Base Thickness of 500 µm. Results are Shown for Constant Thickness (green), Daily Backwashing (red), and Weekly Backwashing (black).

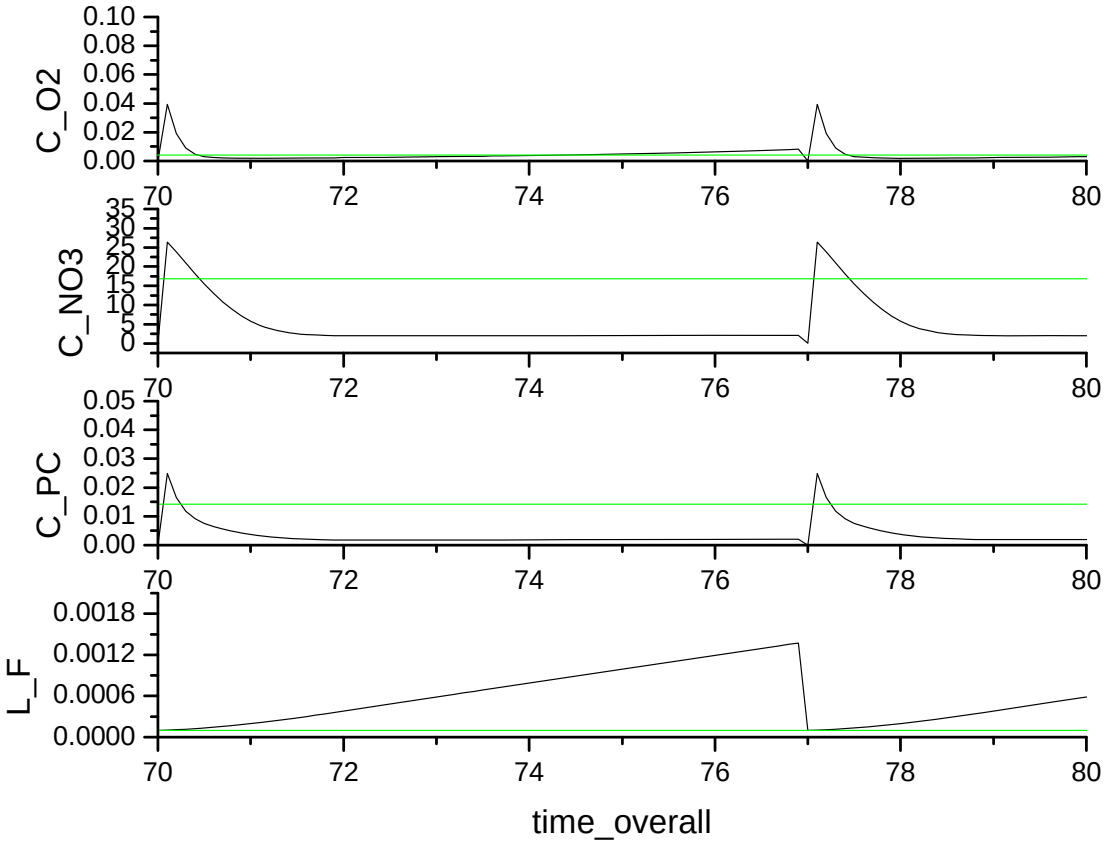

Figure E.11 - Influence of Backwashing, Similar to Figure E.10, But With a Base Thickness of 100 µm. Results are Shown for Constant Thickness (green) and Weekly Backwashing (black).

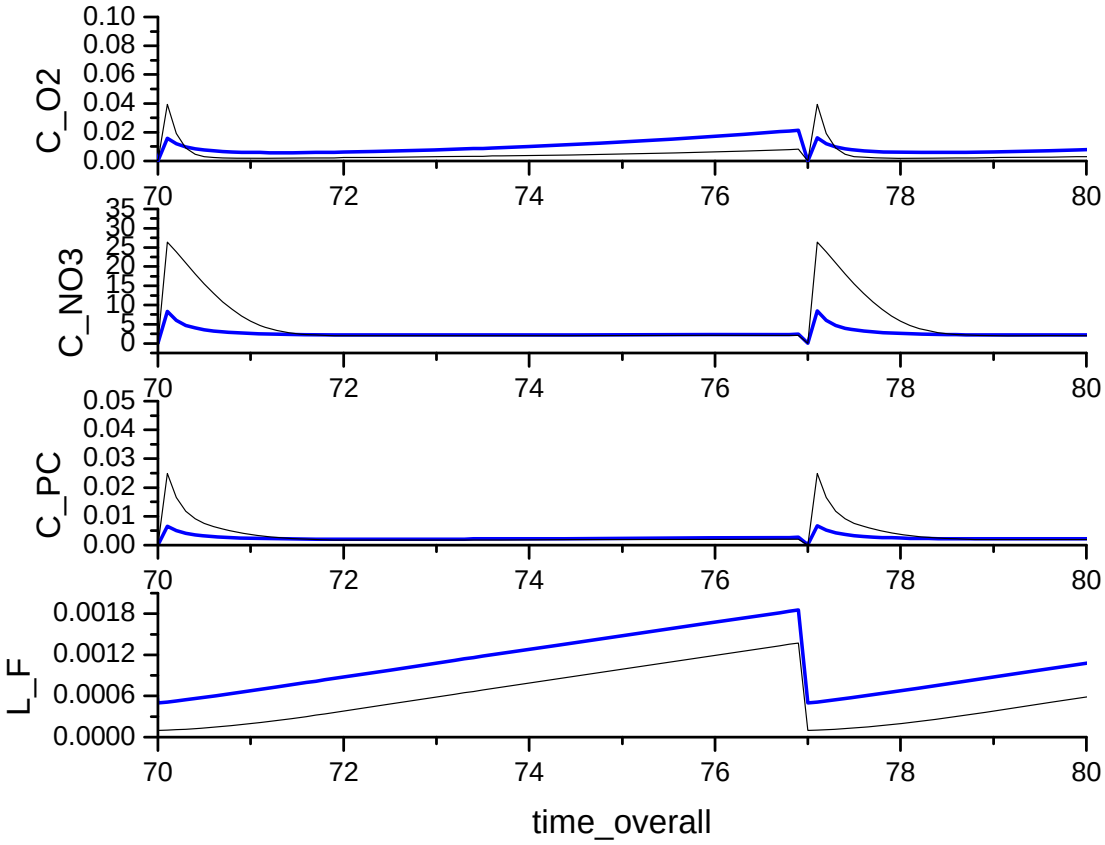

Figure E.12 - Comparison of the Influence of the Base Thickness of 100 µm (black line) and 500 µm (thick blue line) for a Backwashing Interval of 7 d.

1.9 Influence of Influent Perchlorate Concentrations

The influence of influent perchlorate and nitrate concentrations were evaluated for influent perchlorate concentrations up to 10,000 µg/L (Figure E.13). Increased influent perchlorate and influent nitrate concentrations do results in increased effluent perchlorate concentrations. However, with an EBCT of 8 min and [D/A] = 1.8 effluent perchlorate concentrations were still below 0.9 mg/L for influent perchlorate concentrations of 10 mg/L.

In Figure E.14 heterotrophic and perchlorate reducing biomass fractions are shown for the evaluated influent perchlorate and nitrate concentrations. It can be seen that perchlorate reducing bacteria start to dominate the biofilm with influent perchlorate concentrations. Comparing the different biofilm reactor compartments it can be seen that heterotrophic bacteria dominate in the first compartment for influent perchlorate concentrations up to 5,000 µg/L while perchlorate reducers are dominant in the second and the third compartment for all evaluated influent perchlorate concentrations.

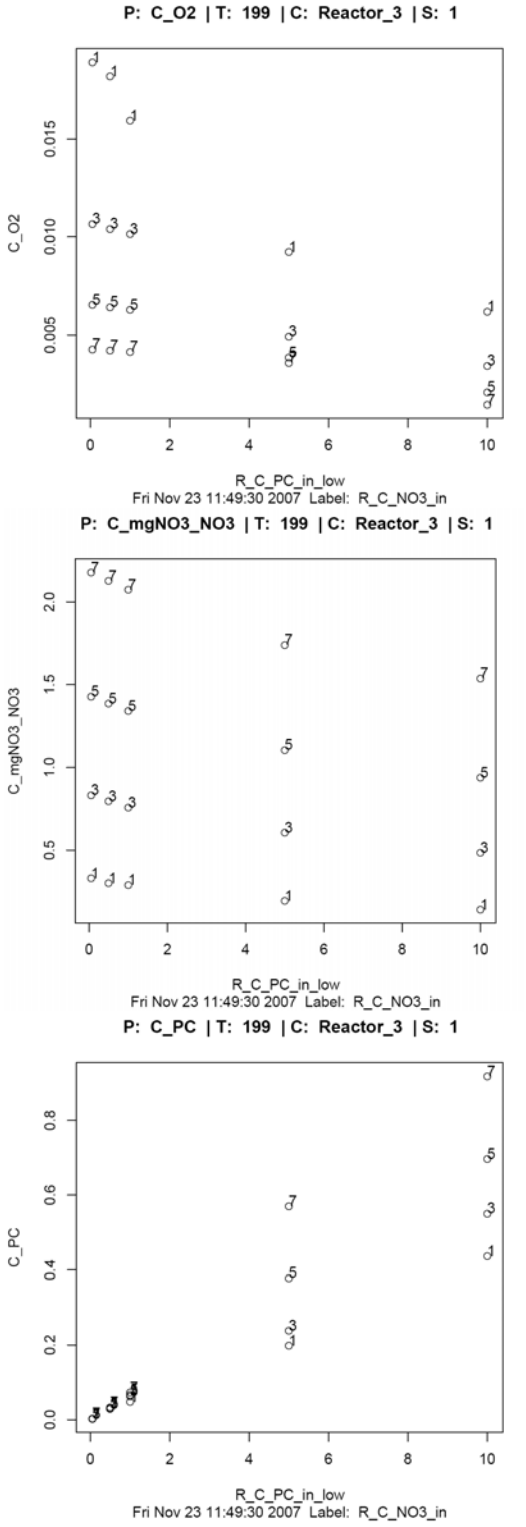

Figure E.13 - Effluent Oxygen, Nitrate, and Perchlorate Concentrations for Different Influent Perchlorate Concentrations (x-axis) and Influent Nitrate Concentrations (numbers in plot in mg N/L) at a Fixed EBCT of 8 min.

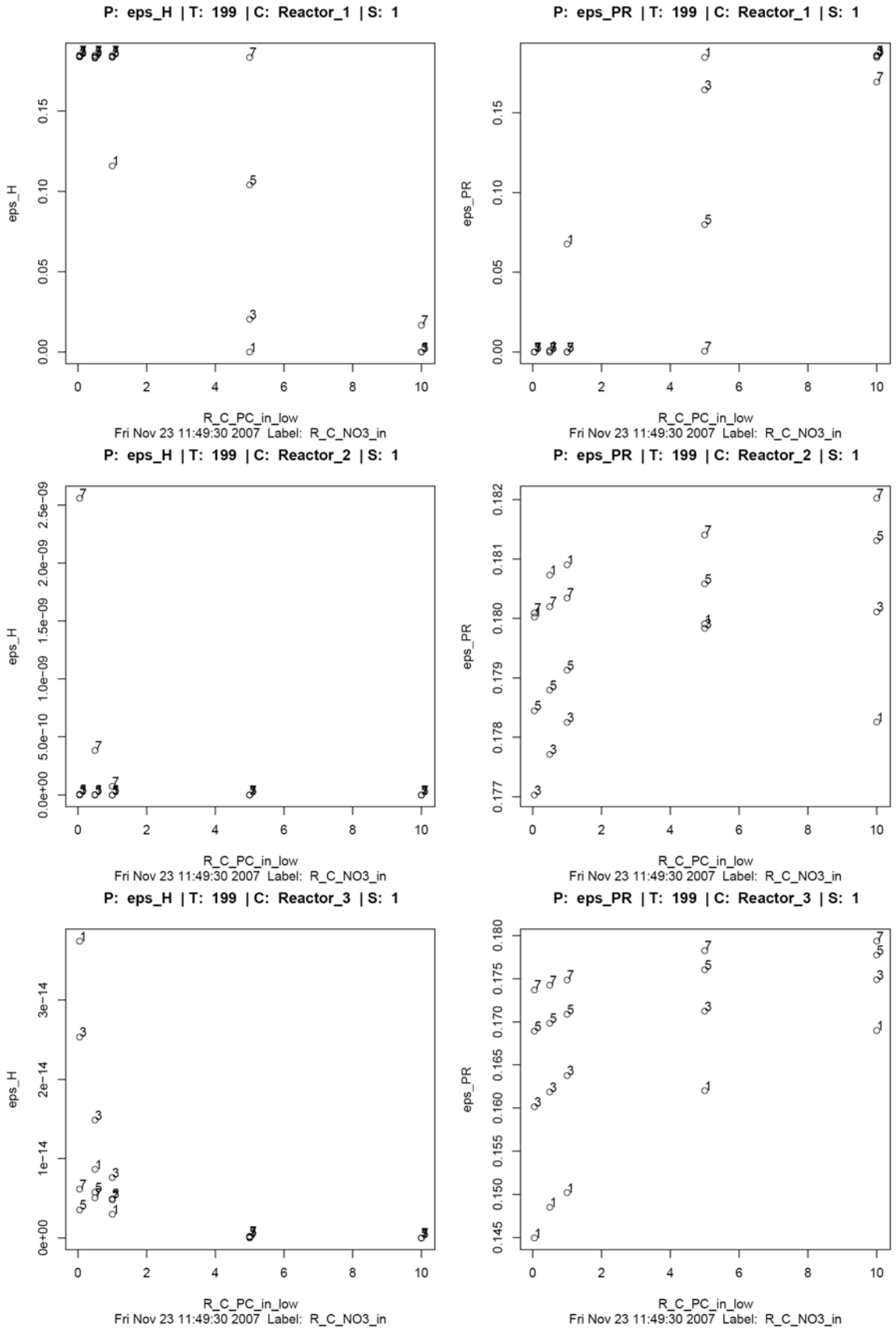

Figure E.14 - Influence of Influent Perchlorate (x-axis) and Nitrate (numbers in plot as mg N/L) on the Volume Fraction of Heterotrophic (left) and Perchlorate Reducing Bacteria (right) in Reactors 1 (top), 2 (middle), and 3 (bottom).

1.10 EBCT Requirements Depend on Influent Perchlorate or Nitrate Concentrations

As was shown above (E.13Figure E), increased influent perchlorate and nitrate concentrations result, for a fixed EBCT, in increased effluent perchlorate concentrations. In the current section the influence of EBCT is evaluated for increased perchlorate and increased nitrate concentrations.

1.10.1 Effect of Influent Perchlorate Concentrations

The influence of EBCT on effluent oxygen and nitrate (Figure E.15) and perchlorate (Figure E.16Figure E.) concentrations is shown for influent perchlorate concentrations ranging from 50 µg/L to 10 mg/L. It can be seen that effluent oxygen and nitrate concentrations and not influenced by influent perchlorate concentrations (Figure E.16). This can be explained with both normal heterotrophs and also perchlorate reducing bacteria prefer oxygen and nitrate over perchlorate as electron acceptor. In Figure E.16 effluent perchlorate concentrations are shown for three different sections within the biofilm reactor. The influent perchlorate concentrations has a significant influence on effluent perchlorate concentrations but increasing EBCT allows to reduce effluent perchlorate concentrations to very low levels. In Figure E.16 it can be seen that the entire filter bed is necessary to remove the influent perchlorate. When effluent perchlorate concentrations are plotted on a logarithmic scale (right side of Figure E.16) it can be seen that increased influent perchlorate concentrations are associated with increased effluent concentrations even with large EBCT.

The amount of perchlorate reducing bacteria are shown for the three reactor sections in Figure E.Figure E.17. Increasing influent perchlorate concentrations allows for a complete shift of active bacteria from normal heterotrophs to perchlorate reducers in the first section of the biofilm reactor. Increasing EBCT reduces effluent perchlorate concentrations (Figure E.16) but, as can be seen especially in the third section, increasing EBCT and decreased surface loading reduces the extent of perchlorate reducing bacteria (Figure E.17).

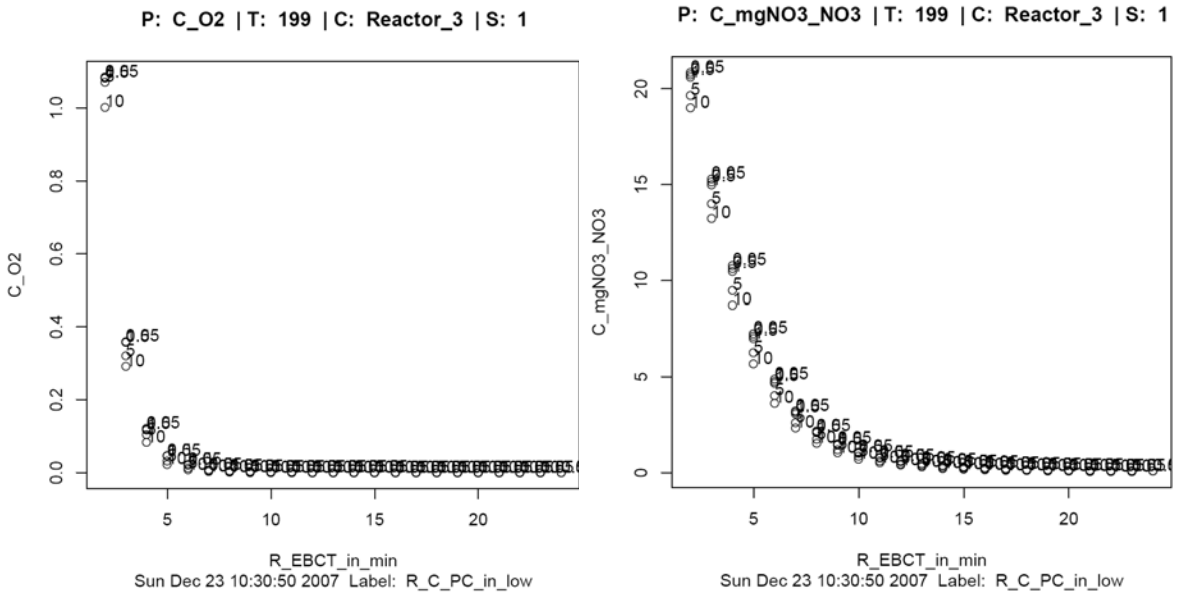

Figure E.15 - Effluent Oxygen and Nitrate Concentrations for Different EBCT (x-axis) Simulated for a Range of Influent Perchlorate Concentrations (number in plot in mg/L, 0.05 – 10 mg/L). Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

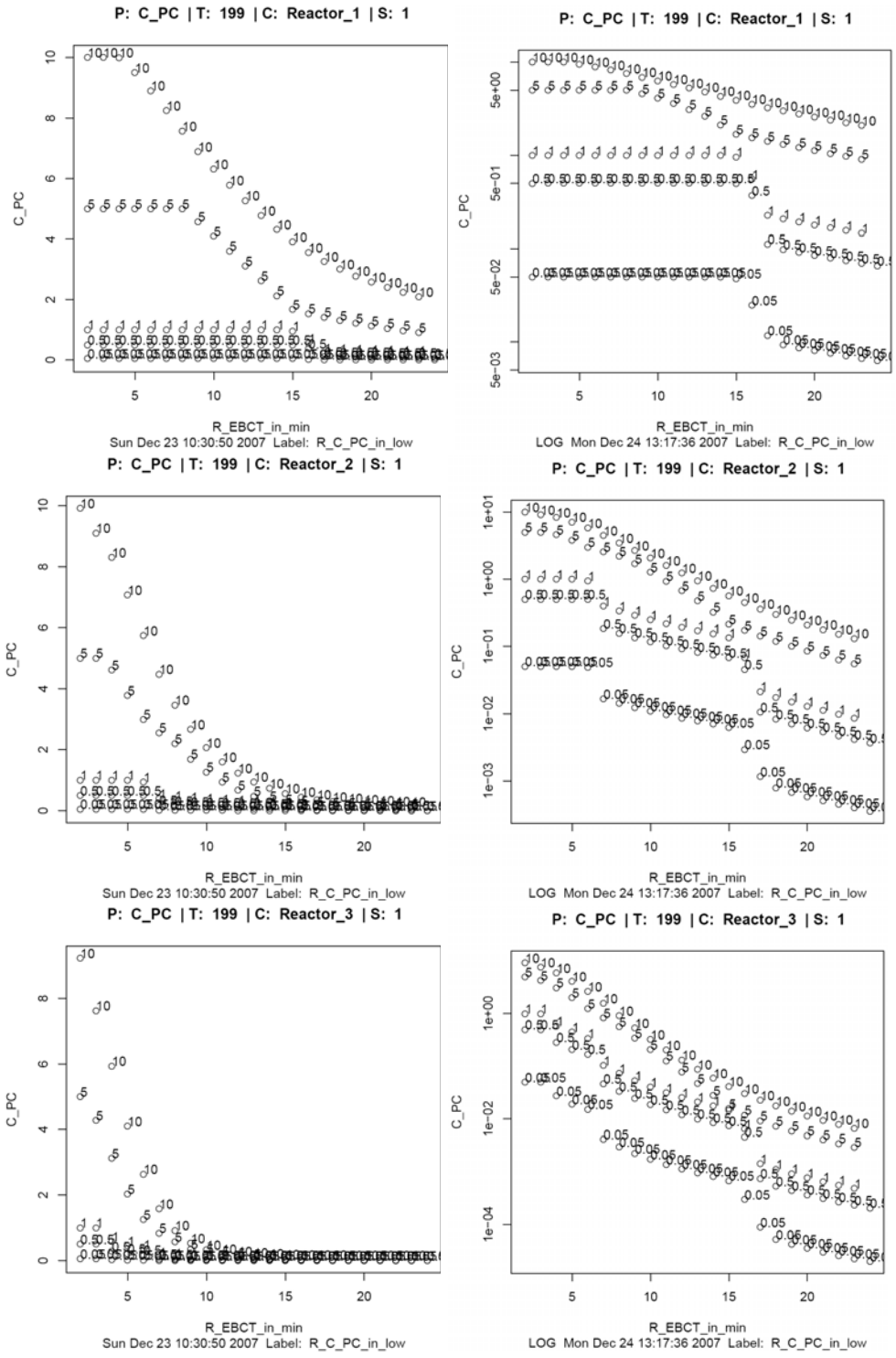

Figure E.16 - Effluent Perchlorate Concentrations in the First (top), Second (middle), and Third (bottom) Compartment for Different EBCT (x-axis) Simulated for a Range of Influent Perchlorate Concentrations (number in plot in mg/L, 0.05 – 10 mg/L). Plots on Left and Right have Linear or Logarithmic y-axis, Respectively. Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

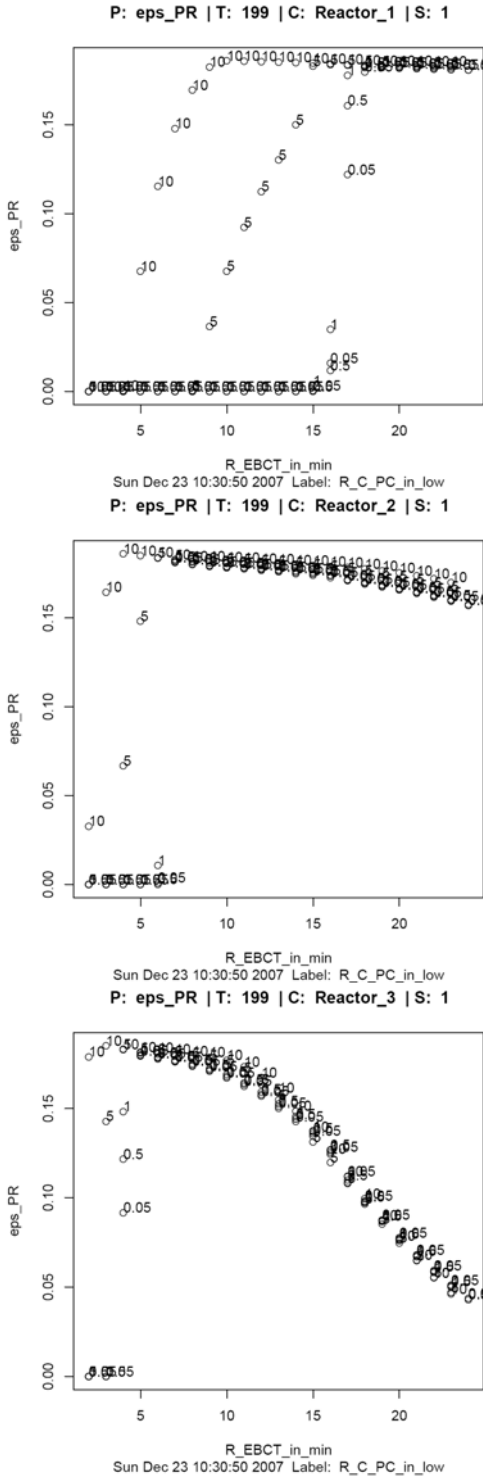

Figure E.17 - Influence of EBCT (x-axis) and Influent Perchlorate Concentration (numbers in plot as mg/L) on the Volume Fraction of Perchlorate Reducing Bacteria in the First (top), Second (middle), and Third (bottom) Compartment. Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

1.10.2 Effect of Influent Nitrate Concentrations

The effect of influent nitrate concentration of up to 56 mg NO_3^-/L (= 21 mg N/L) on reactor performance are presented below. Effluent nitrate concentrations decrease to below 5 mg NO_3^-/L with EBCT larger than 15 min (Figure E.18). With EBCT larger than 15 min complete perchlorate removal can also be achieved (Figure E.19). Regardless of increased influent nitrate concentrations, the perchlorate reducing bacteria dominate in the 2nd and 3rd section of the biofilm reactor (Figure E.20). In Figure E..21 the correlation of effluent oxygen, nitrate, and perchlorate are shown. It can be seen that low effluent oxygen concentrations are a necessary requirement for complete perchlorate removal. However, low effluent perchlorate concentrations (< 5 µg/L) can be achieved with effluent nitrate concentrations of up to 5 mg/L.

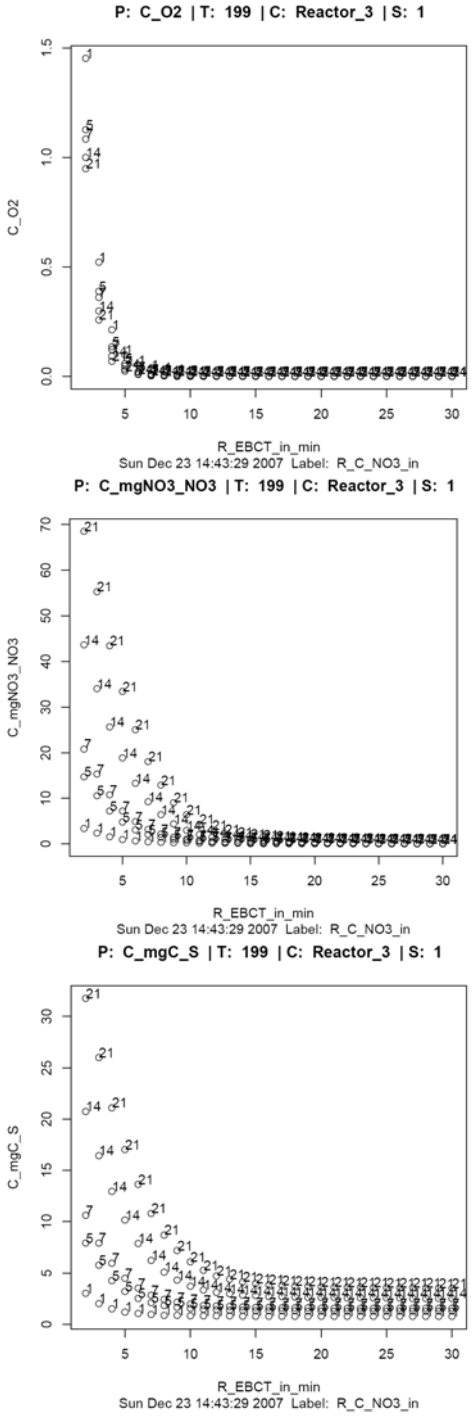

Figure E.18 - Effluent Oxygen, Nitrate, and Acetate Concentrations for Different EBCT (x-axis) Simulated for a Range of Influent Nitrate Concentrations (number in plot in mg N/L, 2 – 21 mg/L). Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

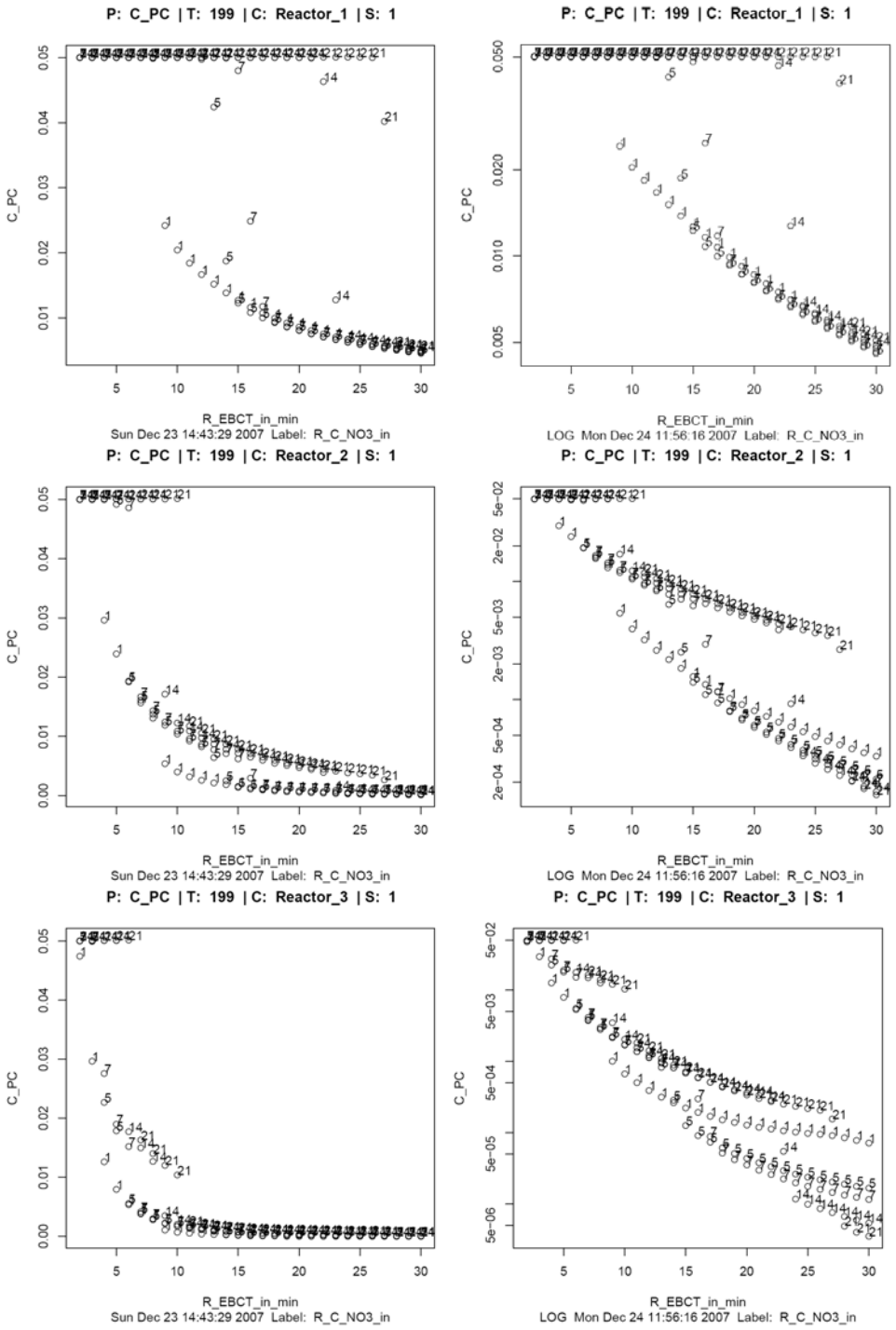

Figure E.19 - Effluent Perchlorate Concentrations in the First (top), Second (middle), and Third (bottom) Compartment for Different EBCT (x-axis) Simulated for a Range of Influent Nitrate Concentrations (number in plot in mg N/L, 2 – 21 mg/L). Plots on Left and Right have Linear or Logarithmic y-axis, Respectively. Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

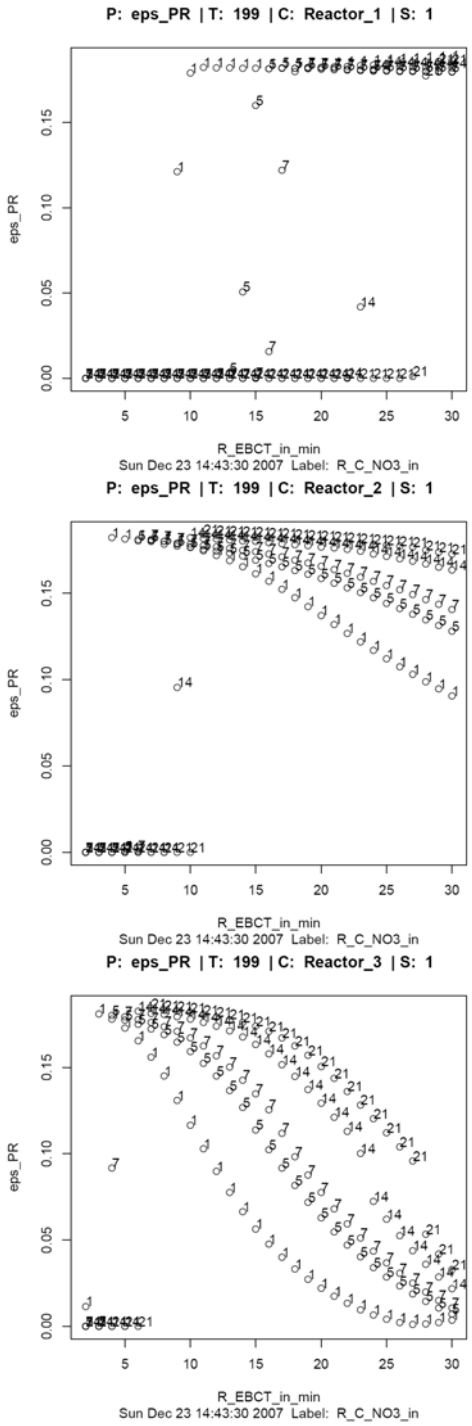

Figure E.20 - Influence of EBCT (x-axis) and Influent Nitrate Concentration (numbers in plot as mg N/L) on the Volume Fraction of Perchlorate Reducing Bacteria in the First (top), Second (middle), and Third (bottom) Compartment. Otherwise Standard Conditions (D/A ratio = 1.8, parameter combination #14).

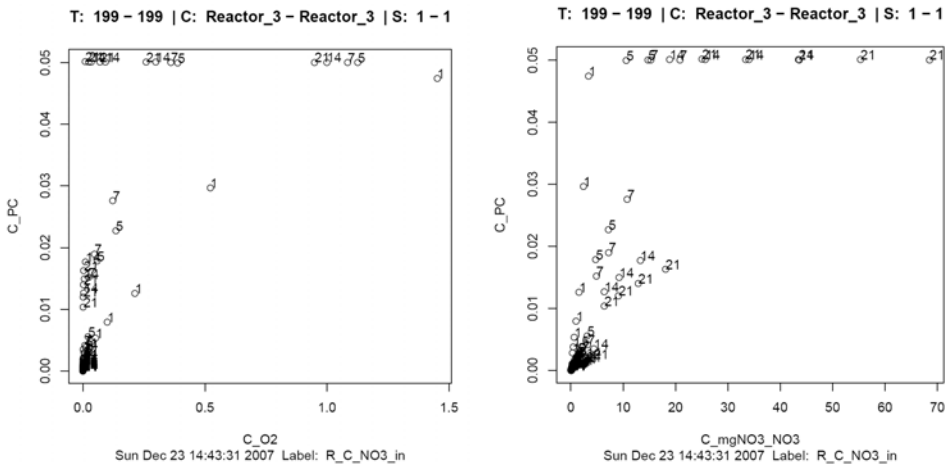

Figure E.21 - Correlation of Effluent Perchlorate Concentrations for all Simulations with Effluent Oxygen Concentrations (left) and Effluent Nitrate Concentrations (right) for EBCT Ranging from 2 to 30 min. Numbers in Plot are Influent Nitrate Concentration in mg N/L.

Conclusions

A mathematical model was developed to simulate the competition of heterotrophic and perchlorate reducing bacteria in a biofilm reactor. The model was calibrated by adjusting the effective surface to volume ratio in the reactor based on observed perchlorate removal in pilot and bench-scale experiments. Using the mathematical model the effect of EBCT, electron donor addition ([D/A]), backwashing, and influent perchlorate and nitrate concentrations were evaluated. To the extent that the model describes redox conditions within the biofilm and microbial competition, these simulations allow for the extrapolation of predicted reactor performance and reactor design based on current pilot and bench-scale experiments. Modeling results demonstrate expected performance but results should be used with caution. Further pilot-scale testing is recommended with a larger range of EBCT, electron donor additions, and influent concentrations to evaluate conditions leading to reactor failure. The current modeling results provide a systematic evaluation of how operating conditions influence reactor performance and results can be used to guide future testing and design.

References

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Morgenroth, E. and Wilderer,P.A. (2000): Influence of Detachment Mechanisms on Competition in Biofilms. *Water Resources*, **34** (2), 417-426.

R development core team (2008): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (http://www.r-project.org/). http://www.R-project.org.

Reichert, P. (1998): *Aquasim 2.0 - User manual. Computer Program for the Identification and Simulation of Aquatic Systems*. Swiss Federal Institute for Environmental Science and Technology (EAWAG). CH 8600 Dübendorf, Switzerland.

Wanner, O., H.J. Eberl, E. Morgenroth, D.R. Noguera, C. Picioreanu, B.E. Rittmann, and M.C.M. van Loosdrecht (2006): *Mathematical Modeling of Biofilms*. IWA Publishing, London, UK. Series: Scientific and Technical Report Series Report No. 18.

Additional Model Information

1.11 Estimated Parameter Combinations in Choi, 2005

Table E.2 - The Final 33 Parameter Combinations Based on Choi, 2005.

Number	K_PC_PR	b_PR	K_O2_PR	K_S_O2_PR	mue_PC_PR
1	0.0233	0.1203	0.005	1.6485	2.3121
2	0.0222	0.2462	0.0028	1.68	3.0282
3	0.030396	0.10189	0.0045247	3.8379	2.5454
4	0.073778	0.17872	0.011824	1.726	2.1273
5	0.14449	0.10069	0.0043004	3.8793	3.2245
6	0.031785	0.23155	0.0026212	3.8468	4.0863
7	0.1164	0.12447	0.0040953	3.1452	3.0053
8	0.15226	0.14082	0.018836	2.0375	2.2254
9	0.1142	0.24509	0.007351	1.9251	3.3074
10	0.094974	0.21934	0.015054	2.6638	2.0716
11	0.15822	0.21803	0.0068767	3.1065	3.6913
12	0.11328	0.16731	0.0086007	3.9062	2.4753
13	0.15006	0.14457	0.015106	3.1272	2.3807
14	0.17045	0.17074	0.018541	2.0024	2.7736
15	0.019852	0.24322	0.0061974	3.5105	2.1427
16	0.13858	0.14883	0.011549	3.9155	3.1404
17	0.097369	0.22216	0.01062	3.3788	2.6317
18	0.004532	0.19929	0.0020637	3.2488	2.4937
19	0.12511	0.14255	0.012241	2.1002	2.0472
20	0.054094	0.14686	0.0080624	3.829	2.5042
21	0.13369	0.11525	0.0058513	2.0806	2.3937
22	0.12801	0.19216	0.0092865	3.1911	3.6383
23	0.18317	0.13522	0.015426	2.971	2.8876
24	0.13649	0.23912	0.0094916	3.965	2.8904
25	0.1594	0.17769	0.0065178	2.7006	3.0338
26	0.071413	0.17151	0.0044285	3.3963	3.2901
27	0.072008	0.24116	0.0057039	3.4436	2.0874
28	0.046983	0.11153	0.0064089	2.7268	2.2373
29	0.14435	0.15558	0.011267	3.0777	3.2179
30	0.053957	0.19607	0.007524	3.3786	2.0797
31	0.10573	0.2313	0.0077227	3.1388	3.8936
32	0.10837	0.21536	0.010793	2.649	2.9056
33	0.23469	0.069263	0.012664	3.9321	3.5655

1.12 Model Definitions and Parameters in AQUASIM

```
**********************************************************************
AQUASIM Version 2.1e (win/mfc) - Listing of System Definition
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Date and time of listing:  11/23/2007  13:41:26

**********************************************************************
Variables
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b_H:          0.2
b_PR:         0.17074
Calc_A_vs_Q_In:R_A_total/Q_in
Calc_COD_eAcceptor_In:
          1*C_O2_in_VarList+2.85714*R_C_NO3_in+0.643216*C_PCin_VarL
          ist
Calc_COD_eDonor_In:
          C_Sin
Calc_eDonor_vs_eAcceptor_In:
          Calc_COD_eDonor_In/Calc_COD_eAcceptor_In
C_mgC_S:      C_S*0.375
C_mgNO3_NO3:   C_NO3*4.42857
C_NO3:        Dyn. Volume State Var.
C_NO3_z_099_R1:C_NO3(Reactor_1,Biofilm Matrix,0.99,rel.space)
C_NO3_z_100_R1:C_NO3(Reactor_1,Biofilm Matrix,1,rel.space)
C_NO3_z_100_R2:C_NO3(Reactor_2,Biofilm Matrix,1,rel.space)
C_NO3_z_100_R3:C_NO3(Reactor_3,Biofilm Matrix,1,rel.space)
C_NO3_z_100_R4:C_NO3(Reactor_4,Biofilm Matrix,1,rel.space)
C_NO3_z_100_R5:C_NO3(Reactor_5,Biofilm Matrix,1,rel.space)
C_NO3_z_100_R6:C_NO3(Reactor_6,Biofilm Matrix,1,rel.space)
C_O2:         Dyn. Volume State Var.
C_O2_in_VarList:
          Variable List Variable (time_overall)
C_O2_z_099_R1: C_O2(Reactor_1,Biofilm Matrix,0.99,rel.space)
C_O2_z_100_R1: C_O2(Reactor_1,Biofilm Matrix,1,rel.space)
C_O2_z_100_R2: C_O2(Reactor_2,Biofilm Matrix,1,rel.space)
C_O2_z_100_R3: C_O2(Reactor_3,Biofilm Matrix,1,rel.space)
C_O2_z_100_R4: C_O2(Reactor_4,Biofilm Matrix,1,rel.space)
C_O2_z_100_R5: C_O2(Reactor_5,Biofilm Matrix,1,rel.space)
C_O2_z_100_R6: C_O2(Reactor_6,Biofilm Matrix,1,rel.space)
C_PC:         Dyn. Volume State Var.
C_PCin_VarList:Variable List Variable (time_overall)
C_PC_z_099_R1: C_PC(Reactor_1,Biofilm Matrix,0.99,rel.space)
C_PC_z_100_R1: C_PC(Reactor_1,Biofilm Matrix,1,rel.space)
C_PC_z_100_R2: C_PC(Reactor_2,Biofilm Matrix,1,rel.space)
C_PC_z_100_R3: C_PC(Reactor_3,Biofilm Matrix,1,rel.space)
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C_PC_z_100_R4: C_PC(Reactor_4,Biofilm Matrix,1,rel.space)
C_PC_z_100_R5: C_PC(Reactor_5,Biofilm Matrix,1,rel.space)
C_PC_z_100_R6: C_PC(Reactor_6,Biofilm Matrix,1,rel.space)
C_S: Dyn. Volume State Var.
C_Sin: R_D_A_Ratio*(1*C_O2_in_VarList+2.85714*R_C_NO3_in+0.64321
6*C_PCin_VarList)
C_S_z_099_R1: C_S(Reactor_1,Biofilm Matrix,0.99,rel.space)
C_S_z_100_R1: C_S(Reactor_1,Biofilm Matrix,1,rel.space)
C_S_z_100_R2: C_S(Reactor_2,Biofilm Matrix,1,rel.space)
C_S_z_100_R3: C_S(Reactor_3,Biofilm Matrix,1,rel.space)
C_S_z_100_R4: C_S(Reactor_4,Biofilm Matrix,1,rel.space)
C_S_z_100_R5: C_S(Reactor_5,Biofilm Matrix,1,rel.space)
C_S_z_100_R6: C_S(Reactor_6,Biofilm Matrix,1,rel.space)
Detach_dyn_rate:
1e+008
D_NO3: 0.00016
D_O2: 0.000219
D_PC: 0.0001792
D_S: 0.000104
eps_H: X_H/rho_X
eps_Hini: 0.1
eps_H_average: (1/11)*(eps_H_z_000+eps_H_z_010+eps_H_z_020+eps_H_z_030+e
ps_H_z_040+eps_H_z_050+eps_H_z_060+eps_H_z_070+eps_H_z_08
0+eps_H_z_090+eps_H_z_100)
eps_H_z_000: eps_H(Reactor_1,Biofilm Matrix,0,rel.space)
eps_H_z_010: eps_H(Reactor_1,Biofilm Matrix,0.1,rel.space)
eps_H_z_020: eps_H(Reactor_1,Biofilm Matrix,0.2,rel.space)
eps_H_z_030: eps_H(Reactor_1,Biofilm Matrix,0.3,rel.space)
eps_H_z_040: eps_H(Reactor_1,Biofilm Matrix,0.4,rel.space)
eps_H_z_050: eps_H(Reactor_1,Biofilm Matrix,0.5,rel.space)
eps_H_z_060: eps_H(Reactor_1,Biofilm Matrix,0.6,rel.space)
eps_H_z_070: eps_H(Reactor_1,Biofilm Matrix,0.7,rel.space)
eps_H_z_080: eps_H(Reactor_1,Biofilm Matrix,0.8,rel.space)
eps_H_z_090: eps_H(Reactor_1,Biofilm Matrix,0.9,rel.space)
eps_H_z_100: eps_H(Reactor_1,Biofilm Matrix,1,rel.space)
eps_I: X_I/rho_X
eps_I_z_100: eps_I(Reactor_1,Biofilm Matrix,1,rel.space)
eps_L: 1-(eps_H_z_100+eps_I_z_100+eps_PR_z_100)
eps_PR: X_PR/rho_X
eps_PRini: 0.2-eps_Hini
eps_PR_average:(1/11)*(eps_PR_z_000+eps_PR_z_010+eps_PR_z_020+eps_PR_z_0
30+eps_PR_z_040+eps_PR_z_050+eps_PR_z_060+eps_PR_z_070+ep
s_PR_z_080+eps_PR_z_090+eps_PR_z_100)
eps_PR_z_000: eps_PR(Reactor_1,Biofilm Matrix,0,rel.space)
eps_PR_z_010: eps_PR(Reactor_1,Biofilm Matrix,0.1,rel.space)
eps_PR_z_020: eps_PR(Reactor_1,Biofilm Matrix,0.2,rel.space)
eps_PR_z_030: eps_PR(Reactor_1,Biofilm Matrix,0.3,rel.space)

eps_PR_z_040: eps_PR(Reactor_1,Biofilm Matrix,0.4,rel.space)
eps_PR_z_050: eps_PR(Reactor_1,Biofilm Matrix,0.5,rel.space)
eps_PR_z_060: eps_PR(Reactor_1,Biofilm Matrix,0.6,rel.space)
eps_PR_z_070: eps_PR(Reactor_1,Biofilm Matrix,0.7,rel.space)
eps_PR_z_080: eps_PR(Reactor_1,Biofilm Matrix,0.8,rel.space)
eps_PR_z_090: eps_PR(Reactor_1,Biofilm Matrix,0.9,rel.space)
eps_PR_z_100: eps_PR(Reactor_1,Biofilm Matrix,1,rel.space)
eta: 0.8
Flux_NO3_BoundaryLayer:
    D_NO3*(C_NO3-C_NO3_z_100_R1)/R_L_L
Flux_NO3_BoundaryLayer_R2:
    D_NO3*(C_NO3-C_NO3_z_100_R2)/R_L_L
Flux_NO3_BoundaryLayer_R3:
    D_NO3*(C_NO3-C_NO3_z_100_R3)/R_L_L
Flux_NO3_BoundaryLayer_R4:
    D_NO3*(C_NO3-C_NO3_z_100_R4)/R_L_L
Flux_NO3_BoundaryLayer_R5:
    D_NO3*(C_NO3-C_NO3_z_100_R5)/R_L_L
Flux_NO3_BoundaryLayer_R6:
    D_NO3*(C_NO3-C_NO3_z_100_R6)/R_L_L
Flux_NO3_Surface:
    D_NO3*(C_NO3_z_100_R1-C_NO3_z_099_R1)/(0.01*L_F)*eps_L
Flux_O2_BoundaryLayer:
    D_O2*(C_O2-C_O2_z_100_R1)/R_L_L
Flux_O2_BoundaryLayer_R2:
    D_O2*(C_O2-C_O2_z_100_R2)/R_L_L
Flux_O2_BoundaryLayer_R3:
    D_O2*(C_O2-C_O2_z_100_R3)/R_L_L
Flux_O2_BoundaryLayer_R4:
    D_O2*(C_O2-C_O2_z_100_R4)/R_L_L
Flux_O2_BoundaryLayer_R5:
    D_O2*(C_O2-C_O2_z_100_R5)/R_L_L
Flux_O2_BoundaryLayer_R6:
    D_O2*(C_O2-C_O2_z_100_R6)/R_L_L
Flux_O2_Surface:
    D_O2*(C_O2_z_100_R1-C_O2_z_099_R1)/(0.01*L_F)*eps_L
Flux_PC_BoundaryLayer:
    D_PC*(C_PC-C_PC_z_100_R1)/R_L_L
Flux_PC_BoundaryLayer_2:
    D_PC*(C_PC-C_PC_z_100_R2)/R_L_L
Flux_PC_BoundaryLayer_3:
    D_PC*(C_PC-C_PC_z_100_R3)/R_L_L
Flux_PC_BoundaryLayer_4:
    D_PC*(C_PC-C_NO3_z_100_R4)/R_L_L
Flux_PC_BoundaryLayer_5:
    D_PC*(C_PC-C_PC_z_100_R5)/R_L_L
Flux_PC_BoundaryLayer_6:
    D_PC*(C_PC-C_PC_z_100_R6)/R_L_L
Flux_PC_Surface:

D_PC*eps_L*(C_PC_z_100_R1-C_PC_z_099_R1)/(0.01*L_F)
Flux_S_BoundaryLayer:
D_S*(C_S-C_S_z_100_R1)/R_L_L
Flux_S_BoundaryLayer_R2:
D_S*(C_S-C_S_z_100_R2)/R_L_L
Flux_S_BoundaryLayer_R3:
D_S*(C_S-C_S_z_100_R3)/R_L_L
Flux_S_BoundaryLayer_R4:
D_S*(C_S-C_S_z_100_R4)/R_L_L
Flux_S_BoundaryLayer_R5:
D_S*(C_S-C_S_z_100_R5)/R_L_L
Flux_S_BoundaryLayer_R6:
D_S*(C_S-C_S_z_100_R6)/R_L_L
Flux_S_Surface:D_S*eps_L*(C_S_z_100_R1-C_S_z_099_R1)/(0.01*L_F)
k_H: 0.2
K_NO3_H: 0.5
K_NO3_PR: 0.15
K_O2_H: 0.1
K_O2_PR: 0.018541
K_PC_PR: 0.17045
k_PR: 0.109477
K_S: 1
K_S_O2_PR: 2.0024
K_S_PC_PR: 1
L_F: Biofilm Thickness
mue_O2_H: 5
mue_O2_PR: 4.8
mue_PC_PR: 2.7736
Num_ParComb: 1
Num_Sim: 1
Q_in: (R_A_total/R_a_SurfVol)/(R_EBCT_in_min/(24*60))
rate_detatch: max(if u_F>0 then u_F*R_DetachBetweenBackwash else 0 end
if ,Detach_dyn_rate*(time_backwashCycle-(R_Backwash_Int-R
_Backwash_Duration))*(R_Backwash_Int-time_backwashCycle)*
if L_F>R_L_Fdetach then L_F-R_L_Fdetach else 0 endif)
rho_X: 25000
R_a_SurfVol: 1000
R_A_total: 3.4
R_Backwash_Duration:
0.02
R_Backwash_Int:0
R_C_NO3_in: 7
R_C_O2_in_high:8
R_C_O2_in_low: 8
R_C_PC_in_high:1
R_C_PC_in_low: 0.05
R_DetachBetweenBackwash:
0.8
R_D_A_Ratio: 1.8
R_EBCT_in_min: 8

R_L_Fdetach: 0.0001
R_L_Fini: 0.0006
R_L_L: 1e-005
R_n: 3
R_YesNo_HetGrowth:
1
R_YesNo_PRBgrowth:
1
time_backwashCycle:
((time_overall/R_Backwash_Int) mod 1)*R_Backwash_Int
time_overall: Time
u_F: Growth Velocity of Biofilm
X_H: Dyn. Volume State Var.
X_I: Dyn. Volume State Var.
X_PR: Dyn. Volume State Var.
Y_H: 0.4
Y_O2_PR: 0.4
Y_PC_PR: 0.4

****
Processes
****
HetGro: R_YesNo_HetGrowth*mue_O2_H*C_O2/(K_O2_H+C_O2)*C_S/(K_S+C_S)*X_H
X_H : 1
C_S : -1/Y_H
C_O2 : -(alp_H-Y_H)/Y_H
HetGro_nitrate:R_YesNo_HetGrowth*eta*mue_O2_H*K_O2_H/(K_O2_H+C_O2)*C_S/(K_S+C_S)*C_NO3/(K_NO3_H+C_NO3)*X_H
X_H : 1
C_S : -1/Y_H
C_NO3 : -(1-Y_H)/2.86/Y_H
HetInact: b_H*X_H
X_H : -1
X_I : 1
HetResp: b_H*C_O2/(K_O2_H+C_O2)*X_H
X_H : -1
C_O2 : -1
PRGro_nitrate: R_YesNo_PRBgrowth*eta*mue_O2_PR*K_O2_PR/(K_O2_PR+C_O2)*C_S/(K_S_O2_PR+C_S)*C_NO3/(K_NO3_PR+C_NO3)*X_PR
X_PR : 1
C_S : -1/Y_O2_PR
C_NO3 : -(1-Y_H)/2.86/Y_H
PRGro_O2: R_YesNo_PRBgrowth*mue_O2_PR*C_O2/(K_O2_PR+C_O2)*C_S/(K_S_O2_PR+C_S)*X_PR
X_PR : 1
C_S : -1/Y_O2_PR

C_O2 : -(1-Y_O2_PR)/Y_O2_PR
PRGro_PC: R_YesNo_PRBgrowth*mue_PC_PR*eta*C_PC/(K_PC_PR+C_PC)*K_O2_PR/(K_O2_PR+C_O2)*K_NO3_PR/(K_NO3_PR+C_NO3)*C_S/(K_S_PC_PR+C_S)*X_PR
X_PR : 1
C_S : -1/Y_PC_PR
C_PC : -(alp_H-Y_PC_PR)/Y_PC_PR/0.64
PRInact: b_PR*X_PR
X_PR : -1
X_I : 1
PRInactPC: b_PR*X_PR*K_O2_PR/(K_O2_PR+C_O2)
X_PR : -1
X_I : 1
PRResp_O2: b_PR*C_O2/(K_O2_PR+C_O2)*X_PR
X_PR : -1
C_O2 : -1
PRResp_PC: b_PR*C_PC/(K_PC_PR+C_PC)*X_PR*K_O2_PR/(K_O2_PR+C_O2)
X_PR : -1
C_PC : -1/0.64

****
Compartments
****
Reactor_1: Biofilm Reactor Compartment
Active Variables: X_PR, X_I, C_O2, C_S, C_PC, X_H, C_NO3
Active Processes: PRGro_O2, PRResp_PC, PRResp_O2, PRInact, HetInact, HetResp, HetGro, HetGro_nitrate, PRGro_nitrate, PRGro_PC
Reactor_2: Biofilm Reactor Compartment
Active Variables: X_PR, X_I, C_O2, C_S, C_PC, X_H, C_NO3
Active Processes: PRGro_O2, PRResp_PC, PRResp_O2, PRInact, HetGro, HetInact, HetResp, HetGro_nitrate, PRGro_nitrate, PRGro_PC
Reactor_3: Biofilm Reactor Compartment
Active Variables: X_PR, X_I, C_O2, C_S, C_PC, X_H, C_NO3
Active Processes: PRGro_O2, PRResp_PC, PRResp_O2, PRInact, HetGro, HetInact, HetResp, HetGro_nitrate, PRGro_nitrate, PRGro_PC

****
Links
****
R_1_2: Reactor_1 -> Reactor_2
R_2_3: Reactor_2 -> Reactor_3

From EM_PerchloratNitrate_28apr07.AQU on 23. November 2007

1.13 Stoichiometric and Kinetic Matrix

Table E.3 - Stoichiometric and Kinetic Matrix (based on Choi, 2005)**.**

Component, i →	1	2	3	4	5	6	7	
↓ Process, j	S_s	S_{O2}	S_{NO3}	S_{ClO4}	X_H	X_{PR}	X_I	Process Rate
Aerobic, Heterotrophic bacteria								
Heterotrophic growth using oxygen	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$			1			$\mu_{\max,H}\left(\frac{S_s}{K_{s,H}+S_s}\right)\left(\frac{S_{O2}}{K_{O2,H}+S_{O2}}\right)X_H$
Heterotrophic growth using nitrate	$-\frac{1}{Y_H}$		-(1-Y_H) /2.86Y_H		1			$\eta\cdot\mu_{\max,H}\left(\frac{S_s}{K_{s,H}+S_s}\right)\left(\frac{S_{NO3}}{K_{NO3,H}+S_{NO3}}\right)\left(\frac{K_{O2}}{K_{O2,H}+S_{O2}}\right)X_H$
Aerobic H Respiration		-1			-1			$b_H\cdot X_H\left(\frac{S_{O2}}{K_{O2,H}+S_{O2}}\right)$
Aerobic H Inactivation					-1		1	$k_{O2H}\cdot X_H$
Aerobic or anoxic, Perchlorate reducers growth on oxygen								
PRB growth using oxygen	$-\frac{1}{Y_{O2,PR}}$	$-\frac{1-Y_{O2,PR}}{Y_{O2,PR}}$				1		$\mu_{\max O2,PR}\left(\frac{S_s}{K_{S\_O2,PR}+S_s}\right)\left(\frac{S_{O2}}{K_{O2,PR}+S_{O2}}\right)X_{PR}$
PRB growth using nitrate	$-\frac{1}{Y_{O2,PR}}$		-(1-Y_H) /2.86Y_H			1		$\eta\cdot\mu_{\max O2,PR}\left(\frac{S_s}{K_{s,PRB}+S_s}\right)\left(\frac{S_{NO3}}{K_{NO3,PRB}+S_{NO3}}\right)\left(\frac{K_{O2}}{K_{O2,PRB}+S_{O2}}\right)X_{PR}$
Aerobic PRB Respiration		-1				-1		$b_{PR}\cdot X_{PR}\left(\frac{S_{O2}}{K_{O2,PR}+S_{O2}}\right)$
Aerobic PRB Inactivation						-1	1	$k_{O2PR}\cdot X_{PR}$
Perchlorate reducers growth on Perchlorate								

Component, i →	1	2	3	4	5	6	7	
↓ Process, j	S_s	S_{O2}	S_{NO3}	S_{ClO4}	X_H	X_{PR}	X_I	Process Rate
Perchlorate PRBGrowth	$-\frac{1}{Y_{ClO4,PR}}$	$\frac{1-Y_{ClO4,PR}}{0.64 \cdot Y_{ClO4,PR}}$		$-\frac{1-Y_{ClO}}{0.64Y_{ClO}}$		1		$\eta \cdot \mu_{maxO2,PR}\left(\frac{S_s}{K_{S\_ClO4,PRB}+S_s}\right)\left(\frac{S_{ClO4}}{K_{ClO4,PR}+S_{ClO4}}\right)\left(\frac{K_{NO3}}{K_{NO3,PRB}+S_{NO3}}\right.$
Perchlorate PRB Respiration				$-\frac{1}{0.64}$		-1		$b_{PR} \cdot X_{PR}\left(\frac{S_{ClO4}}{K_{ClO4,PR}+S_{ClO4}}\right)\left(\frac{K_{O2,PR}}{K_{O2,PR}+S_{O2}}\right)$
Units	COD	O_2	N	ClO_4^-	COD	COD	COD	

Appendix F - Analytical Methods Supporting the Experimental Design

1) Water quality data were measured using on-site hand-held equipment, in-line analytical instruments, and laboratory analyses. The University of Michigan performed the majority of the laboratory analyses. Montgomery Watson Harza Laboratory measured several parameters and provided quality control checks on analyses performed elsewhere. Table F.1 lists the water quality parameters that were monitored as part of this project along with the associated analytical methods that were used.

Table F.1 - Analytical Methods for the FXB Biological Perchlorate Treatment Demonstration.

Parameter	Analytical Method	Parameter	Analytical Method
Perchlorate	EPA 314.0	Fecal Coliforms	SM 9221B
DO	SM 4500-O G	pH	4500-H+ B
Nitrate	EPA 300.0A	Temperature	SM 2550 B
Nitrite	EPA 300.0A	Turbidity	SM 2130 B
Sulfate	EPA 300.0A	VSS	EPA 160.4
Hydrogen Sulfide	EPA 376.2	TSS	SM 2540D
Phosphate	EPA 300.0A	TDS	SM 2540C
DOC/TOC	SM 5310C	BOD	SM 5210B
BDOC	Servais method		
Ammonia	SM 4500-NH_3 D		
Chlorine (free and total)	SM 4500-Cl G		
TTHMs	EPA 502.2		
HAA_5	EPA 552.2		
HPC	SM 9215		

Appendix G - Quality Assurance Project Plan

1.0 Objectives

The objective of this section is to describe the procedures that were used during demonstration testing to ensure data quality and integrity. Careful adherence to these procedures ensured that data generated during testing would accurately serve as the basis for performance evaluation and the development of design criteria. The components of the QAPP are as follows:

- Measurement of precision and accuracy;
- Outline for duplicate sampling;
- Procedures used to ensure data correctness;
- Data management and reporting.

2.0 Methodology for Measurement of Precision and Accuracy

Flow Meter - Water flow rates were verified prior to the start of testing and every 4 weeks thereafter. The fixed-bed effluent reservoir on the pilot plant skid contained a sight glass, which includes 5-gallon graduations. Following a backwash (when the reservoir is drawn down) a constant flow rate were established and the operator measured the time required to accumulate 5 gallons in the effluent reservoir. Average flow rate could then be calculated and checked against the flow rate as indicated on the data acquisition system of the demonstration plant.

Chemical feed systems - Chemical feed system flow rates were verified prior to the start of testing and once per week thereafter. The stock chemical solution tanks were marked with volumetric increments. The volume of stock chemical solution pumped over a given interval (e.g., 1-3 days) was monitored and recorded.

Pressure Transmitters – Each reactor column pressure transmitter was checked against a redundant gauge, where available. Gauge readings were checked against pressure transmitters to insure proper function. Gauge and transmitter readings were logged once daily.

In-Line Analytical Equipment - The in-line DO, nitrate, and perchlorate analyzers were equipped with a manual injection option. Standard perchlorate and nitrate solutions were prepared and injected once per week to verify calibration. Appropriate measures were taken per Dionex instruction to correct any calibration errors.

3.0 Duplicate and Triplicate Samples

The FXB demonstration skid was equipped with in-line perchlorate, DO, and nitrate analyzers that essentially provide real-time system performance data. Duplicate perchlorate samples (sample taken at the same time a real-time analysis is performed) were also taken daily and sent to the University of Michigan for analysis. Nitrate samples were taken three times per week and were also sent to the University of Michigan for analysis. Approximately twice per month, the perchlorate sample was split and sent both to the University of Michigan and to the Clinical Laboratory of San Bernardino or MWH laboratory for a triplicate analysis. In-line DO data were checked against duplicate samples measured using a hand-held DO meter and probe. When duplicate or triplicate samples differ by > 10%, laboratory operators were informed, so that gaps in QA/QC could be detected and corrected. Travel blanks for perchlorate analysis were also prepared and shipped weekly to the University of Michigan and monthly to the Clinical Laboratory of San Bernardino.

4.0 Data Correctness

4.1 Representativeness

Representativeness of water quality samples was ensured by executing consistent sample collection procedures. Specific procedures included the following:

- Sample locations
- Timing of sample collection
- Sample procedures
- Sample preservation
- Sample packaging
- Sample shipping

4.1.1 Sample Locations

A sampling matrix was presented in Table 3.2 in the main report. Samples were taken from feed, effluent and backwash streams (where applicable) of each of the unit processes in the demonstration unit.

4.1.2 Timing of Sample Collection

Feed water quality sampling was done within one hour of effluent water quality sampling. This ensured that the effluent water sample was representative of the feed water quality.

4.1.3 Sampling Procedures, Preservation, Packaging, and Transport

Prior to the collection of each individual water quality sample, the sample tap was allowed to run a minimum of 30 seconds in order to purge the sample tap and sample line of stagnant water. Samples were then collected. Purge time for the depth wise column sample taps was <5 seconds to avoiding altering the hydraulics through the fixed-bed while allowing for sufficient time to purge the sample tap. Additional considerations and procedures for individual water quality parameters are included below:

pH - pH samples were collected at the sample tap in beakers and immediately tested for pH. The temperature at which the pH reading is made was also recorded.

Total Coliform and HPC - All sample containers were provided by the analytical laboratory. Aseptic sampling techniques were used as follows:

- Sample bottles were kept closed until they are filled.
- Sample taps were removed, allowed to soak in a chlorine solution for a minimum of two minutes, rinsed, and reconnected to the sample valve. Water was then allowed to run through the tap for a minimum of two minutes. The sample tap was flamed prior to sampling
- The cap of the sample container was removed without touching the surface of the cap or neck of the bottle.
- The sample container was filled without rinsing and the cap was replaced immediately.
- Samples were refrigerated immediately after collection and were transported to the laboratory and coolers with frozen blue ice.
- Samples were refrigerated upon receipt at the laboratory analyzed within holding times specified in the standard method.

4.2 Representativeness of Operational Parameters

Representativeness of operational parameters entails collecting a sufficient quantity of data during operation to be able to detect a change in operational parameters. As specified, detecting a plus or minus 10% change in an operating parameter is sufficient for proper QA/QC. Operational parameters include bioreactor column flow rate and chemical dosing rates.

5.0 Data Management and Reporting

Daily checklists and log sheets were maintained to record flow rates, head losses, sampling times, and sampling locations. These tables were also used to document QA/QC procedures performed as well any operational disturbances and subsequent corrective actions. The operator also recorded backwash flowrate and duration, influent and effluent perchlorate concentration, DO concentration, nitrate concentration, turbidity, pH, and temperature. Operational and water quality parameters recorded daily were used to assess system performance.

The demonstration skid loged several operational and water quality parameters continuously. Flowrate, head loss, perchlorate concentrations, DO concentrations, and nitrate concentrations were recorded in the demonstration skid's SCADA system at pre-set intervals. These data were downloaded from the demonstration skid's SCADA system through the internet and were combined with on-site data and data from the University of Michigan and CLSB/MWH laboratories, and transferred to a master spreadsheet daily. The spreadsheet was built so that performance plots (perchlorate removal, etc.) were updated automatically, thereby enabling the rapid observation of system performance trends. These performance plots were prepared by the on-site engineer and sent to Jess Brown three times per week. Regular operational modifications were made based on system performance plots as necessary. These data were formally reported in the Quarterly Progress Reports, and the Draft Final Report.

6.0 Example QA/QC Data

Table G.1 presents QA/QC data gathered for perchlorate. Perchlorate analyses for raw water and Bioreactor F130 effluent samples were often taken in duplicate or triplicate. Duplicate and triplicate samples were stored in a $4^{o}C$ refrigerator until shipment on ice to the University of Michigan or the MWH Laboratory. In-line perchlorate values for the raw water were consistently 10-14 μg/L lower than the values measured at the University of Michigan Laboratory in spite of regular calibrations of both analyzers. It is possible that there was some perchlorate was degrading in the sample lines, though these lines were regularly flushed with chlorine. The duplicate and triplicate analyses performed on the Bioreactor F130 effluent were consistent among the three analysis sites. While this does not reveal anything about the sample line degradation theory, it does confirm consistent perchlorate removal to below detection in the bioreactor.

Table G.1 - Perchlorate Quality Assurance Quality Control Data.

Table G.1 Perchlorate QA/QC Data					
	Feed		F130		
Date	Inline (µg/L)	UM (µg/L)	Inline (µg/L)	UM (µg/L)	MWH (µg/L)
8/3/2007			< 2	< 2	
8/6/2007	40.11	55.52	< 2	< 2	
8/9/2007	39.90	55.77	< 2	< 2	
8/13/2007	39.37	54.46	< 2	< 2	
8/16/2007	36.19	54.60	< 2	< 2	
8/20/2007	38.01	56.76	< 2	< 2	
8/23/2007	38.74	56.30	< 2	< 2	
9/7/2007				< 2	2.9
9/10/2007				12	10
9/17/2007				< 2	< 0.5
9/19/2007	39.10	55.57	< 2	< 2	
9/20/2007			< 2	< 2	
9/21/2007			< 2	< 2	< 0.5
9/24/2007	35.38	57.19	< 2	< 2	< 0.5
10/3/2007			< 2		< 0.5
11/12/2007			< 2	2.63	< 0.5
11/16/2007			< 2	< 2	< 0.5
11/19/2007	38.7792	55.40		< 2	0.64
11/20/2007				< 2	0.56