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Chemical and Biological Characterization of the 200 West Pump-and-Treat for Performance Assessment and Operational Improvement

September 2019

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Prepared for the U.S. Department of Energy
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Summary

Contaminated groundwater in the 200W Area of the Hanford Site is being treated ex situ by the 200W Pump-and-Treat (P&T) Facility and the subsurface plume is under hydraulic containment. The 200W P&T Facility removes several contaminants of concern from groundwater, as well as nitrate. Groundwater treatment capacity at the facility is currently constrained by the biological treatment of nitrate. In FY19, preparation of a 200W P&T Optimization Plan proposes to bypass all nitrate treatment to (1) allow the facility to operate at its original design capacity of 2500 gal/min, (2) provide the option for increasing capacity to 3750 gal/min, and (3) enable reliable injection of treated effluent water back into the subsurface. Nitrate will continue to be treated through blending of water within the aquifer.

In a previous report,[1] Thomle et al. (2017) provided a comprehensive engineering assessment of biofouling at the 200W P&T Facility. In that report, both operational changes and facility modifications were recommended to help mitigate the symptoms and consequences of biofouling by expanding treatment processes in the plant. The 200W P&T Facility's fluidized bed reactor (FBR) is a biological treatment system that has been identified as a principal source of biofouling. Carbon and nutrient inputs to the FBR are needed to sustain high rates of activity but can also be responsible for microbial proliferation in the effluent water distribution pipeline, and loss injection well capacity. Some of the research objectives described in this report were intended to fill critical knowledge gaps associated with the FBR that can be used to optimize the biological system for continuous operation and sustained performance.

The impact of potential and actual changes to the treatment system is needed to inform decision making for the 200W P&T operations. To identify the role granular activated carbon (GAC) provides in the currently configured treatment process, archived samples were analyzed to provide a more complete description of the sorbed inorganic constituents and how these concentrations have responded over time. Since the 200W P&T system is used to treat a variety of waste streams, the influent conditions will change from the original 200-ZP-1 contaminant conditions. As influent conditions change, treatment efficiency must be maintained to meet the required discharge standards. Thus, potential modifications to the treatment processes need to be considered to address the complex nature of co-mingled groundwater plumes.

The main findings of this report are as follows:

1. **Iodine accumulation on FBR GAC.** Iodine is accumulating on the GAC in the 200W P&T FBR. Levels of total iodine measured were 857 µg/g GAC. Appreciable levels (160 µg/mL) of I were also measured from stored sludge samples. Relatively low levels (6 µg/g) of Tc were associated with GAC.
2. **GAC actively sorbs groundwater contaminants.** GAC in the FBR is accumulating a variety of contaminants from Hanford groundwater. Analysis excluded micronutrients known to be added to the FBR (e.g., Al, Fe, Mn), and accumulating groundwater constituents included U (0.31 µg/g wet wt), Cr (159 µg/g wet wt), As (0.7 µg/g wet wt), Sr (9 µg/g wet wt), Ba (41 µg/g wet wt), Ca (1070 µg/g wet wt), and Mg (145 µg/g wet wt).
3. **Dissimilatory nitrate reduction to ammonia.** In laboratory studies, FBR enrichment cultures were found to attenuate nitrate by a process called dissimilatory nitrate reduction to ammonia (DNRA). This result is important because DNRA does not eliminate nitrate from the system, but rather converts it to a biologically available form that can lead to microbial contamination in the effluent distribution pipelines and contribute to biofouling at the injection wells. Lab studies were followed up by direct

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analysis of P&T groundwater, which confirmed that ammonia is being produced in the membrane bio-reactor and accumulating in the P&T effluent. No ammonia was detected from FBR samples.

4. **Ferrocyanide impact on FBR activity.** Ferrocyanide was not inhibitory to the FBR enrichment cultures at concentrations below 50 μM (FeCN in Hanford groundwater is 0.5 μM), but exposure did slow growth in a concentration-dependent manner under aerobic and anaerobic conditions. Such a result could eventually negatively affect FBR activity and sustained performance.
5. **Options for treatment of contaminants in changing P&T system influent streams.** Currently, technetium is removed from groundwater using Purolite A530E ion exchange resin. The A530E resin outperforms other materials in its ability to sorb technetium as TcO_4^-, but it is not selective, and the use of alternative materials may be required if changes in the groundwater influent create conditions that interfere with TcO_4^- sorption on A530E. Commercially available hybrid anion exchange resins from ResinTech Inc., with the potential to sorb multiple contaminants of concern, were evaluated for their ability to sorb TcO_4^-**.** These included the following hybrid anion exchange resins: (1) CHM-20, a cerium oxide/hydroxide hybrid; (2) ASM-125, a hydrated iron oxide hybrid; (3) ASM-HP-10, a hydrated iron oxide; and (4) Bi form, a bismuth oxide chloride hybrid. All hybrid resins performed well, with virtually complete removal of TcO_4^- achieved within 24 hours at solution-to-solid ratios of 200 and 1000. The ASM-125 and Bi form hybrid resins, consisting of a Type 2 (trimethylamine) strong base anion resin, had the highest affinity for TcO_4^- with a K_d of > 600,000 mL/g for the higher solution-to-solid ratio.

These results provide operational support to the current and potential future configurations of 200W P&T chemical and biological treatment systems. The analyses support the optimization plan that bypasses the biological treatment for nitrate by demonstrating that biofouling issues should be minimized and the groundwater treatment capacity can be increased with minimal to no impact on effective removal of priority contaminants.

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Acronyms and Abbreviations

200W P&T	200 West Pump-and-Treat
ASME	American Society of Mechanical Engineers
BSA	bovine serum albumin
CFR	Code of Federal Regulations
DNRA	dissimilatory nitrate reduction to ammonia
FBR	fluidized bed reactor
GAC	granular activated carbon
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectroscopy
MBR	membrane bio-reactor
NQAP	Nuclear Quality Assurance Program
P&T	pump-and-treat
PNNL	Pacific Northwest National Laboratory
SGW	synthetic groundwater

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1.0 Introduction

Groundwater beneath the Central Plateau at Hanford contains a complex mixture of contaminants. Groundwater in the 200W Area of the Hanford Site is being treated ex situ by the 200W Pump-and-Treat (P&T) Facility to remove key contaminants of concern (uranium, technetium, nitrate, carbon tetrachloride) from groundwater by a combination of chemical and physical processes, as well as a biological treatment for primary removal of nitrate. Treated water is then reinjected into the subsurface to contain and diminish groundwater contaminant plumes. However, the flow capacity of the injection wells decreases over time due to biofouling. The work summarized in this report addresses some immediate challenges associated with the 200W P&T biological system and provides results on the testing of commercial ion exchange resins for removal of Tc-99 from Hanford groundwater. It is intended to provide new information that could help guide decision making and optimization of various treatment systems without compromising activity or performance of the facility.

As part of the currently configured 200W P&T Facility, the biological treatment system consists of twin fluidized bed reactors (FBRs) that are operated under anoxic conditions to reduce nitrate, and provide additional capacity for the removal of carbon tetrachloride and hexavalent chromium from groundwater (Byrnes et al. 2013). The FBR contains granular activated carbon (GAC), which provides a substrate for bacterial colonization and biofilm formation but can also provide a reactive surface(s) for inorganic contaminant adsorption. Prior to the work presented in this report, GAC samples from the 200W P&T Facility had not been fully characterized to identify the complete suite of groundwater contaminants that are actively removed from Hanford groundwater by the GAC in the FBR.

As part of standard operations of the 200W P&T FBR biological treatment system, incoming groundwater is pH titrated with phosphoric acid and supplemented with a proprietary carbon source (MicroC® 4100) and micronutrient blend. Carbon and nutrient dosages are determined by a set of calculations that assume complete reduction of groundwater nitrate (Carlson et al. 2016; Lee and Lee 2016). Respiratory denitrification, defined as microbiological reduction of nitrate to gaseous products, is considered to be the primary pathway for groundwater nitrate removal in the 200W P&T Facility. Effluent water can contain excess carbon and nutrients, which can lead to microbial contamination throughout the effluent water distribution pipeline and contribute to the fouling of injection wells. The 200W P&T Facility injection wells were designed for 150 gpm effluent water, but regular cleaning (well re-habilitation and re-development) is required to maintain injection operations (see Thomle et al. 2017). Current trends suggest a consistent decline in the efficiency of the 200W P&T Facility injection wells.

Thomle et al. (2017) prepared a comprehensive engineering assessment of biofouling at the 200W P&T Facility and recommended operational changes and facility modifications to mitigate the consequences of biofouling. This investigation focused on identifying how the FBR is contributing to biofouling, and how modifying its operation can reduce biofouling at the injection wells. Since specific and diagnostic information is needed for reliable process optimization, this effort focused specifically on the conditions that contribute to or exacerbate biofouling at the 200W P&T Facility. The overarching goal is to fill critical knowledge gaps in the understanding of how the FBR biological treatment system functions and to more accurately define the biological requirements for continuous operation and sustained performance, while minimizing the factors that contribute directly to biofouling.

The 200W P&T system is being used to treat a variety of waste streams with influent conditions changing from the original 200-ZP-1 contaminant conditions. As influent conditions change, treatment efficiency must be maintained to meet the required discharge standards. Thus, potential modifications to the treatment processes need to be considered to address the complex nature of the co-mingled groundwater plumes. To this end, a series of commercially-available hybrid anion exchange resins from ResinTech Inc., which have the potential to treat multiple contaminants of concern, were screened for their ability to remove Tc-99 from Hanford groundwater. Their performance was compared against that for the resin current used in the P&T system, Purolite A530E.

The objectives of this task were to (1) provide new information regarding the fate and distribution of iodine and technetium in the 200W P&T Facility; (2) provide specific information about groundwater treatment by GAC in the FBR; (3) provide specific information about nitrate attenuation and nutrient conditions; (4) explore nitrate attenuation process tolerance to ferrocyanide concentrations, which may be toxic to microbial populations associated with the FBR, and finally; (5) provide expanded information on the performance of commercial ion exchange resins for the removal of Tc-99 from Hanford groundwater.

2.0 Inorganic Contaminant Partitioning at 200W P&T

Hanford groundwater treated by the 200W P&T Facility contains, among other contaminants, radioiodine (I-129) and technetium (Tc-99). The RAD building of the P&T Facility is expected to remove the majority of Tc-99 in the groundwater by ion exchange chromatography. Aqueous iodine species have been assumed to be sequestered onto the GAC substrate in the FBR biological treatment system (Parker and Wellman 2017). Elemental analysis of the ion exchange resin, however, revealed significantly higher concentrations of I-127 (and Fe) than either I-129 or Tc-99 (Levitskaia et al. 2017). The fate of I-129 in the 200W P&T Facility is not fully known. Objective 1 of this investigation was to analyze archived 200W P&T samples, specifically solids, filtrates, and concentrates, not only to demonstrate the retention of I-129 and Tc-99, but more specifically, to quantify the mass and distribution of both contaminants through the 200W P&T Facility.

To mitigate biofouling constraints and increase treatment capacity at the 200W P&T Facility, bypassing the biological treatment of nitrate treatment altogether is under evaluation. As modifications to the existing P&T Facility are explored, it is important to identify potential impacts to operations and the extant system. To this end, GAC samples obtained from the twin FBRs were analyzed to characterize its function as a sorbent for the treatment of Hanford groundwater and identify potential impacts from FBR removal.

2.1 Experimental Methods

GAC and concentrated solids (sludge, filtrate) from the 200W P&T Facility were retrieved from cold storage and analyzed for total Tc-99 and iodine (I). Sample locations within the facility are shown in Figure 1, and were collected on 04/02/2013, 08/04/2015, and 03/27/2017.

Briefly, nitric acid (8M) extractions were performed at a 1:5 solid-to-solution ratio according to method ASTM D5198. Samples were heated for 2 hours at 90°C and filtered (0.45 µm) prior to analysis. Acid digestion combined with high temperature is known to volatilize iodine from the sample; therefore, a mathematical correction was applied to iodine values based on a previous analysis of pure standards and spiked controls. Analytical controls were performed in duplicate and included a sample blank (solutions only), a spiked blank (solution spiked with contaminant of interest), and a matrix spike. All filtrates were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) (Thermo Scientific X-Series II) for total technetium and iodine concentration. Preparations from GAC samples were also analyzed by inductively coupled plasma optical emission spectroscopy for cations. Organic carbon analysis was conducted on pre weighted GAC using a total organic carbon analyzer.

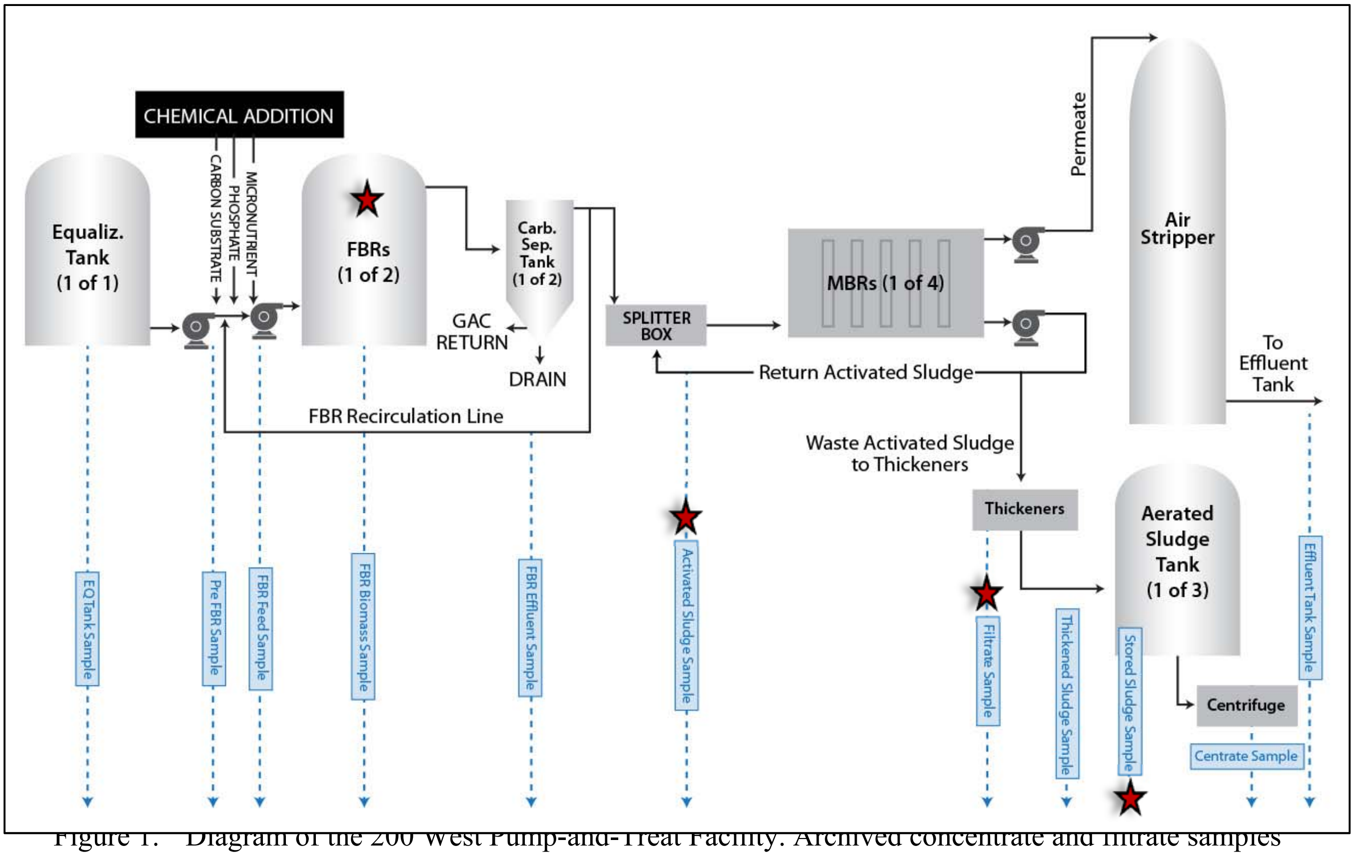

Figure 1. Diagram of the 200 West Pump-and-Treat Facility. Archived concentrate and filtrate samples collected throughout the plant (labeled by a red star) were submitted in duplicate for analysis of total technetium (Tc) and iodine (I) by ICP-MS.

2.2 Results

Iodine was detected from all of the GAC samples, one of the activated sludge samples (V16-Y52A; collected 08/04/2015), and both stored sludge samples (V10-Y71B; collected on 08/04/2015) (Table 1). Concentrations increased from 24.3–161.4 µg I / g GAC [corrected values 30.6–203.3 µg I / g GAC] (collected on 04/02/2013) to 680.1 µg of I / g GAC [corrected values, 857.0 µg I / g GAC] (collected on 03/27/2017) in approximately 4 years. Total iodine was detectable in a single activated sludge sample (V16-Y52A), though the concentration was low (6.4 µg I / g; corrected value of 8.0 µg I / g). Iodine concentrations in stored sludge samples (V10-Y71B) ranged from 120.5–130.6 µg I / g (corrected values, 151.8–164.0 µg I / g). Iodine was not detected from either of two filtrate samples (V-12-Y70A; collected on 08/04/2015).

Technetium was detected in all of the GAC samples but was not detected in any of the other concentrate samples. Concentrations increased from 0.2–0.9 µg Tc / g GAC (collected on 04/02/2013) to 6.1 µg of Tc / g GAC (collected on 03/27/2017) in approximately 4 years. For comparison, Levitskaia et al. (2017) analyzed contaminant loading (3 yr) onto spent 200W P&T Purolite A530E IX resin and measured 58 µg I-127 / g resin, 0.06 µg I-129 / g resin, and 22 µg Tc-99 / g resin.

Table 1. Summary of total iodine (I) and technetium (Tc) concentrations from samples at the 200W P&T Facility.

ID	Sample Description	Location	Sampled Date	Units	Total I	Corrected Total I[(a)]	Total Tc
B2P0M9	GAC from FBR-A	Top of Bed	04/02/2013	µg / g	161.36	203.31	0.85
B2P0N1	GAC from FBR-A	Bottom of Bed	04/02/2013	µg / g	24.31	30.63	0.17
B39HP1	GAC from FBR-A	Top, Grab	03/27/2017	µg / g	680.17	857.01	6.05
B325P2-1	Activated Sludge	V16-Y52A	08/04/2015	µg / mL	ND	ND	ND
B325P2-2	Activated Sludge	V16-Y52A	08/04/2015	µg / mL	6.38	8.04	ND
B325K0-1	Filtrate	V-12-Y70A	08/04/2015	µg / mL	ND	ND	ND
B325K0-2	Filtrate	V-12-Y70A	08/04/2015	µg / mL	ND	ND	ND
B352P6-1	Stored Sludge	V10-Y71B	08/04/2015	µg / mL	120.50	151.83	ND
B352P6-2	Stored Sludge	V10-Y71B	08/04/2015	µg / mL			ND

(a) Corrected iodine concentrations take into account a 26% loss by I by volatilization during sample preparation.

Analysis of GAC samples from the FBR helps characterize its function as a sorbent during the treatment of Hanford groundwater. The complete lists of accumulated cations and metals are provided in Tables 2 and 3. No significant accumulation of lanthanides was measured (Table 4). Uranium (^{238}U) was also measured to be accumulating on GAC; concentrations were 0.13 µg / g (wet wt.) for the 2013 sample and 0.31 µg / g (wet wt.) for the 2016 sample. While some priority contaminants are accumulating on the GAC (e.g., U, Cr), concentrations were generally low and the majority of constituents measured are derived from micronutrients added to the FBR to sustain microbial reduction of nitrate.

Table 2. Elemental analysis of 200W P&T GAC. Values are reported as means. Standard deviation was less than 10% for all analytes.

Cation (µg / g wet wt)	2013	2017
Aluminum[(a)] (Al)	43	106
Barium (Ba)	10	41
Calcium (Ca)	282	1070
Chromium (Cr)	69	166
Cobalt[(a)] (Co)	3	6
Copper[(a)] (Cu)	29	174
Iron[(a)] (Fe)	485	1390
Magnesium (Mg)	48	145
Manganese[(a)] (Mn)	3	8
Nickel[(a)] (Ni)	1	3
Phosphorous (P)	290	582
Sodium (Na)	16	20
Strontium (Sr)	3	9
Sulfur (S)	194	414
Vanadium (V)	6	14
Zinc[(a)] (Zn)	11	18
Zirconium (Zr)	ND	ND

(a) Denotes a micronutrient added to the FBR.
ND indicates 'not detected.'

Table 3. Analysis of 200W P&T GAC for RCRA metals. Values are reported as means. Standard deviation was less than 10% for all analytes.

Metal (µg / g wet wt.)	2013	2017
Antimony (^{121}Sb)	ND	ND
Arsenic (^{75}As)	0.3	0.7
Cadmium (^{111}Cd)	ND	ND
Cesium (^{133}Cs)	ND	ND
Chromium (^{52}Cr)	67.0	159.0
Lead (^{208}Pb)	0.02	0.02
Mercury (^{200}Hg)	ND	ND
Molybdenum(a) (^{95}Mo)	1.0	1.0
Rhenium (^{185}Re)	0.01	0.03
Selenium(a) (^{82}Se)	ND	0.6
Silver (^{107}Ag)	ND	N

(a) Denotes a micronutrient added to the FBR.
ND indicates 'not detected.'

Table 4. Analysis of 200W P&T GAC for lanthanides. Values are reported as means. Standard deviation was less than 10% for all analytes.

Lanthanide (µg / g wet wt)	2013	2017
Cerium (^{140}Ce)	0.007	0.017
Dysprosium (^{163}Dy)	ND	ND
Erbium (^{166}Er)	ND	ND
Gadolinium (^{157}Gd)	ND	ND
Holmium (^{165}Ho)	ND	ND
Lanthanum (^{139}La)	ND	ND
Lutetium (^{175}Lu)	ND	ND
Neodymium (^{146}Nd)	ND	ND
Praseodymium (^{141}Pr)	ND	ND
Samarium (^{147}Sm)	ND	ND
Terbium (^{159}Tb)	ND	ND
Thulium (^{169}Tm)	ND	ND
Ytterbium (^{172}Yb)	ND	ND

ND indicates 'not detected.'

2.3 Summary

Limited samples were available from the P&T Facility for analysis. Analysis of archived samples revealed that iodine is widely distributed throughout the 200W P&T Facility, and that technetium is not being fully captured by the ion chromatography (IC) resin in the RAD building.

The GAC in the 200W P&T FBR biological treatment system is retaining the most I and Tc relative to other facility samples that were available, and their concentrations are increasing over time. Additional groundwater constituents that are accumulating on GAC include U, Cr, As, Sr, Ba, Ca, and Mg. The mechanisms that determine binding capacity are still unknown. Identifying processes (adsorption, chemical, biological) that are involved in sequestering contaminants and quantifying their susceptibility for release will help to determine the GAC binding capacity across a range of future groundwater chemistries that may be routed to the 200W P&T Facility.

3.0 FBR Culture Characterization: Denitrification Activity

The 200W P&T FBR biological treatment system has been operating under the assumption that groundwater nitrate is being reduced and eliminated by a process of denitrification. Denitrification is a respiratory process where nitrate is the terminal electron acceptor that is sequentially reduced to nitrogenous gases (NO, N_2O, N_2). The process results in a net loss of N from the 200W P&T Facility. Denitrification is expected to be the primary nitrate attenuation pathway in the 200W P&T Facility because (1) the FBR biological treatment system was originally inoculated with a denitrifying seed culture, (2) analyses of grab samples taken from the FBR biological treatment system showed a high abundance of *nirS* and *nirK* functional genes that are specific to denitrifying bacteria, and (3) laboratory experimentation consistently demonstrated the rapid decrease in nitrate concentration following supplementation with a carbon source (Lee et al. 2014; Lee et al. 2015a, b; Lee et al. 2015c; Lee et al. 2016; Lee et al. 2017; Morad et al. 2017). Once oxygen has been consumed from the FBR biological treatment system, denitrification is the highest energy yielding anaerobic respiratory process (Strohm et al. 2007), and thus should be the predominant mechanism for NO_3^- attenuation from groundwater.

The objective of this investigation was to identify nitrate attenuation mechanisms in the 200W P&T FBR biological treatment system and the potential impacts of groundwater chemistry on activity.

3.1 Experimental Methods

An FBR denitrifying enrichment culture was resuscitated from -80°C cold storage and maintained in synthetic groundwater. A series of experiments was conducted to quantitatively couple denitrification activity to groundwater composition and concentrations of micronutrients, iodine, and ferrocyanide. By providing accurate laboratory data that more narrowly define nitrate attenuation, the nutritional requirements for sustained activity and culture maintenance, the FBR can be optimized to potentially reduce biofouling at the injection wells.

Synthetic groundwater medium (Table 5) was prepared using the recipe detailed below using previously established procedures outlined by Truex et al. (2017).

Table 5. Synthetic groundwater medium.

Constituent	Conc. (mg/L)	Mass for 1 L (g)
$H_2SiO_3*nH_2O$, silicic acid	15.3	0.0153
KCl, potassium chloride	8.20	0.0082
$MgCO_3$, magnesium carbonate	13.0	0.0130
NaCl, sodium chloride	15.0	0.0150
$CaSO_4$, calcium sulfate	67.0	0.0670
$CaCO_3$, calcium carbonate	150	0.1500

To mimic Hanford groundwater, the synthetic groundwater was composed of excess $CaCO_3$ (solubility is 14 mg/L) and stirred for 1 week to reach equilibrium. Prior to use, the groundwater was filtered by passage through a 0.2-µm pore size filter. The synthetic groundwater medium did not require pH adjustment (approximately 7.8), though pH was routinely recorded throughout experimentation.

Synthetic groundwater medium was supplemented with the carbon source (MicroC® 4100, 116 mL/L) and micronutrient blend (7.73 µL/L) used at the P&T Facility, and nitrate to be within range of influent groundwater concentrations (100 mg/L; 1.6 mM - final). Sodium lactate was also used as a defined, fermentable carbon source (1 mM final concentration). A stock FBR denitrifying enrichment culture was grown and propagated in fully composed synthetic groundwater medium until baseline denitrification activity was established for five consecutive medium exchange cycles. Once baseline activity was established with the stock culture, five independent experimental bioreactors were inoculated to measure nitrate attenuation in response to groundwater composition. Semi-continuous (batch) cultivation techniques were used for these experiments in an attempt to most closely mimic FBR operations while permitting the necessary experimental throughput.

Experimental bioreactors maintained a 1:50 aspect ratio for settled GAC to composed groundwater height. This ratio is much less than the actual FBR biological treatment system at the 200W P&T Facility; however, higher amounts of GAC could not be effectively mixed in the lab-scale reactors. Bioreactors were maintained in the dark at 25°C on a five-position magnetic stirrer to ensure efficient mixing of the groundwater and to keep the GAC fully suspended. All five bioreactors were identically prepared on fully composed synthetic groundwater and inoculated from a common stock culture.

Bioreactors were continuously operated until microbial biomass and denitrification activity stabilized, at which time experimental manipulations were initiated. Composed synthetic groundwater (100 mL) was exchanged weekly to maintain a consistent concentration of carbon and nitrate. Individual bioreactor treatments were assigned to evaluate the effects of (1) trace nutrient composition, (2) iodine exposure (250 ppb), (3) ferrocyanide exposure (200 ppb), and (4) the combined effects of iodine and ferrocyanide (250 and 200 ppb, respectively). The remaining bioreactor served as the no treatment control.

Aqueous sampling was performed daily for chemical analysis. Common anions were analyzed on a Dionex™ ICS-2000 ion chromatograph equipped with an IonPac™ AS185 column. Chromatography conditions maintained an eluent KOH concentration of 22 mM for 10 minutes, then increased to 40 mM for an additional 15 min. Prepared and purchased (SPEX CertiPrep) chemical standards were used for peak identification for the following compounds: lactate, acetate, nitrite, nitrate, phosphate, chloride, sulfate, and fluoride. Low range, Test ‘N Tube Direct Hach® kits were used to measure nitrate, ammonia, and total organic carbon per the manufacturer’s instructions. Microbial biomass was measured using the Pierce BCA Protein Assay kit following the manufacturer’s instructions (Thermo Scientific). Standard curves were prepared using bovine serum albumin (BSA), the dynamic range was determined to be between 25 and 2000 ug/mL ($R^2 = 0.99$).

Experiments were conducted with the 200W P&T nitrate attenuating enrichment culture that was originally obtained directly from the FBR biological treatment system and has since been maintained as a stable frozen stock in the laboratory. Preliminary growth studies were performed to ensure consistent results and performance with previous reports. Lab cultures were scaled up using MicroC® 4100 as the sole carbon source. MicroC® 4100 is a carbohydrate-based liquid feedstock formulated by Environmental Operating Solutions, Inc., for use in biological water and wastewater treatment plants. MicroC® 4100 is a custom formulation that is prepared for the 200W P&T Facility to support anaerobic nitrate-reduction by denitrifying bacteria. MicroC® 4100 did not produce consistent growth between bioreactors and biomass yields were low. As a complex carbon source, MicroC® 4100 is intended to support many diverse microbial and metabolic groups, thus establishing the development of a highly active microbial community in the FBR biological treatment system. In actuality, complex carbon sources can decrease operational control and reproducibility of complex biological systems (like the FBR) because it encourages the growth of metabolic competitors that unpredictably change the carbon substrate profile of the system. Heterotrophic and fermentative bacteria, for example, have been shown to strongly influence NO_3^- attenuation activity by quickly altering the quantity and quality of carbon supplies available for nitrate reducing bacteria (van den Berg et al. 2016; van den Berg et al. 2017a; van den Berg et al. 2017b). Poor growth performance of GAC samples on MicroC® 4100 has been shown prior (Morad et al. 2017). Subsequent experiments adopted lactate as the sole carbon/energy source.

To specifically evaluate nitrate attenuation at the 200W P&T Facility, groundwater samples were collected and immediately acidified on March 27, 2019, by CH2M Hill Plateau Remediation Company and received by Pacific Northwest National Laboratory (PNNL) in April 2019. Groundwater was sampled at locations before and after both FBRs and exiting the membrane bioreactor (MBR) treatment units at the 200W P&T Facility. Prepared groundwater samples were pH neutralized and chemically analyzed. Ammonia was quantified using the HACH® kit for Low Range Ammonia Nitrogen by the AmVer™ Salicylate Test 'N Tube™ Method following the manufacturer's instructions.

3.2 Results

Dense biomass was quickly established for the 200W P&T denitrifying enrichment culture in five independent bioreactors containing artificial groundwater medium with lactate as the sole carbon/energy source. Iodate and ferrocyanide treatments (250 and 200 ppb, respectively) had no discernable effects on cell density or nitrate attenuation activity during repeated oxic-anoxic growth cycles. Representative IC results from nitrate attenuation experiments are summarized in Table 6. Available nitrate (5–10 mM) was quickly consumed (8–16 hours), following the onset of anaerobic conditions (N_2 sparging). As lactate levels decreased, peaks for a number of co-eluting products (2–3) steadily increased. Co-elution of acetate and formate reference standards with lactate was validated by IC; suggesting possible lactate fermentation to a mixture of short chain fatty acids. By 8 hours of anaerobic growth on nitrate containing artificial groundwater, a consistent increase in pH coincided with the accumulation of ammonia. Eventually, alkaline conditions became inhibitory (~pH 10), resulting in the accumulation of organic carbon (fermentation products) and NO_2^-. Activity could be fully restored by air sparging, whereby the ammonia would be utilized for growth, or partially restored by medium exchange (i.e., ammonia dilution). This result has not been previously reported because all prior experimentation with the FBR enrichment culture was conducted in buffered growth medium and only nitrate depletion was measured, not the conversion to reduced nitrogenous products. These experiments show that nitrate attenuation by the 200W P&T FBR enrichment culture includes a respiratory process called dissimilatory nitrate reduction to ammonia (DNRA), not solely denitrification as previously assumed.

It is important to acknowledge that the nitrogen mass conversions presented in Table 6 do not balance. Not all of the ammonia produced will accumulate in the system. Some fraction was assimilated, as indicated by the sharp increase in microbial biomass measured as total protein. Moreover, nitrite was detected in all of the bioreactors, though inefficient peak resolution did not permit quantitation. Finally, because GAC was incorporated into these experiments, solid phase chemistry measurements should be considered. We only measured water chemistry in these experiments.

Although the composition of the 200W P&T FBR biological treatment system may have changed relative to the enrichment culture, the enrichment culture is the most reliable and accurate representation of the FBR microbial community available. These findings are also corroborated by previous lines of inquiry, analyses, and the most current effluent water chemistry data from the 200W P&T Facility. Therefore, this analysis is presumed to be pertinent to the current state and operation of the biological treatment system.

Analysis of P&T groundwater samples confirmed the laboratory experiments that demonstrated the production and accumulation of NH_3 during the biological treatment of Hanford groundwater (Table 7). Ammonia was only detected in effluent water leaving the MBR system, indicating unexpectedly that conditions remain conducive to nitrate reduction to ammonia. At this stage in the treatment process, groundwater aeration and active mixing would effectively prevent further anaerobic transformations of nitrate and could promote the rapid uptake and utilization of ammonia by bacteria already present in the treated groundwater. The concentrations for ammonia presented here are not high, though previous reports have documented high concentrations of ammonia from the injection transfer buildings and injection well head water samples (Lee et al. 2017), suggesting variable inputs over time. Nonetheless, the continuous production of ammonia becomes magnified when considering the large volumes of water being treated over time, and ultimately promotes the establishment of nuisance bacterial growth in groundwater and throughout the effluent distribution pipeline.

Table 6. Nitrate attenuation by the 200W P&T enrichment culture. Groundwater treatments included iodate (250 ppb) and/or ferrocyanide (200 ppb). Outcomes are presented as the measured change (delta, Δ) in values over the course of a 64-hour experiment.

$\Delta_{(t=64\ hr)}$

Bioreactor	Treatment	pH (U)	Protein (μg/mL)	NO_3^- (mg/L)	NO_3^- (mg/L)	NH_3 (mg/L)
1	Control	+1.5	+120.0	-3.94	+0.37	+2.12
3	IO_3	$^+$2.0	+292.9	-2.93	+0.35	+1.59
4	$Fe(CN)_6^{4-}$	$^+$2.0	+246.4	-3.08	+0.42	+1.04
5	IO_3^-/ $Fe(CN)_6^{4-}$	$^+$2.0	+127.1	-3.38	+0.31	+1.59

Experiments were conducted at 5 ppm NO_3^-. Nitrite (NO_2^-) was detected for all bioreactors though the peak co-eluted and could not be reliably quantified.

Table 7. Ammonia concentrations 200W P&T groundwater.

Location	Ammonia (N-NH_3, mg/L)
Water in FBR-A	0.0
Water in FBR-B	0.0
Water out FBR-A	0.0
Water out FBR-B	0.0
Water out MBR	0.67
Detection range 0.02–2.5 mg/L NH_3-N.	

3.3 Summary

Lee et al. (2017) and Thomle et al. (2017) described changes in groundwater chemistry as it moves through the 200W P&T Facility. Ammonia levels in untreated (native) groundwater are typically very low, around 8 μg /L, and ammonia is not consistently measured during routine monitoring at the 200W P&T Facility. Ammonia levels do, however, spike in the FBR biological treatment system and concentrations decrease as effluent water moves through the facility. Biologically significant levels of ammonia have been measured in effluent water collected from transfer buildings and injection wells. Any available ammonia in effluent water will be aggressively utilized for microbial growth and activity. Thus, sources of ammonia can contribute to well fouling and need to be minimized to control biofouling of the effluent distribution pipeline and injection wells.

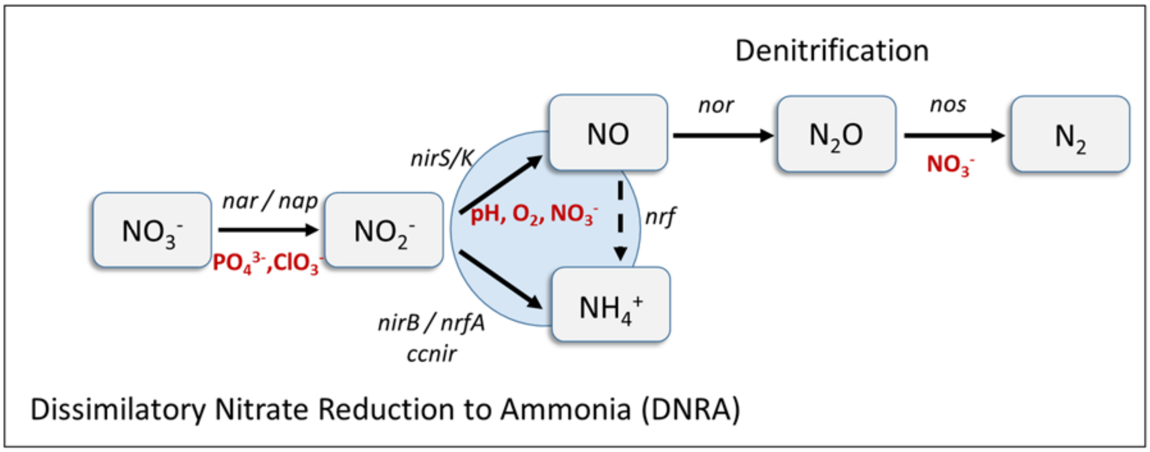

Figure 2. Competing anaerobic nitrate reduction pathways. The enzymes that catalyze specific reactions are listed in italics. Relevant environmental regulators of key reactions are in red and the pathway bifurcation (blue halo) is tightly controlled by a number of enzymatic and metabolic regulators.

DNRA, or ammonification, describes one pathway for the anaerobic reduction of nitrate. DNRA and denitrification (reduction of nitrate to nitrogenous gases) are distinct metabolic processes that compete for nitrate in the environment (Figure 2). The results reported here are relevant to the FBR biological treatment system because unlike denitrification, which results in the net loss of nitrogen, DNRA retains nitrogen in the system by converting it to a highly accessible (bioavailable) form, as soluble ammonia. DNRA in the FBR biological treatment system could be an important and continual source of ammonia at the 200W P&T Facility, contributing to biofouling of injection wells.

Denitrification is a more energetically favorable reaction than DNRA but the enzymatic pathway is energetically expensive, giving a competitive advantage for DNRA bacteria. Strohm et al. (2007) illustrated this point through a series of growth studies on simple substrates that showed that DNRA produced 2x higher cell mass per mol of NO_3^- than denitrification. Denitrifying bacteria typically have a high affinity for organic carbon substrates, but DNRA bacteria have a higher affinity for NO_3^-. The published literature has clearly established the high frequency with which denitrifying activity (communities) in natural and engineered systems are overcome and replaced by DNRA activity (communities) over time (Tiedje et al. 1983; Rivett et al. 2008; van den Berg et al. 2017a; van den Berg et al. 2017b; Kessler et al. 2018), particularly in conditions when the organic carbon / nitrate ratio is high (Strohm et al. 2007; Giblin et al. 2013). The likelihood that DNRA is the predominant nitrate attenuation pathway occurring in the FBR biological treatment system and is providing a consistent supply of ammonia in the 200W P&T Facility would need to be confirmed using reliable and accurate molecular tools and additional effluent testing.

Environmental conditions that tend to favor DNRA over denitrification include a consistent supply of OC, carbon quality (fermentable substrates), low concentrations of nitrate, high C/N ratio, high temperatures, and variable pH, nitrite, and sulfide concentrations. Moreover, DNRA populations (and activity) are generally much less sensitive to changing environmental conditions than denitrifying bacteria (Rivett et al. 2008; van den Berg et al. 2016). It is improbable to assume that all DNRA activity can be eliminated, but it is conceivable that conditions in the FBR and MBR biological treatment systems could be effectively managed to better control nitrate attenuation pathways in favor of denitrification to prevent unwanted production of ammonia. If implemented, though, the P&T Optimization Plan for eliminating the biological treatment of nitrate will clearly reduce if not eliminate the cause and consequences of biofouling that currently constrain treatment capacity at the 200W P&T Facility.

4.0 FBR Culture Sensitivity to Cyanide

During the 1950s, ferrocyanide containing waste generated by the U Recovery Plant was discharged to the BY cribs and to a trench in the northern part of the 200 East Area (Hartman and Dresel 1998). Resulting groundwater now contains significant levels of cyanide, with other co-located contaminants (e.g., Tc-99 and nitrate).

200 East Area groundwater is being considered for processing at the 200W P&T Facility; however, cyanide is a potent toxin and has the potential to interfere with biological treatment processes at the 200W P&T Facility. These processes are principally responsible for removing nitrate from the groundwater.

The objective of this investigation was to provide a preliminary investigation into the inhibitory effects of ferrocyanide on the growth and activity of the FBR denitrifying enrichment culture. Aerobic and anaerobic experiments were performed to assess toxicity, and to discern the potential ferrocyanide interactions.

4.1 Experimental Methods

The 200W P&T FBR denitrifying enrichment culture (described by Szecsody et al. 2017) was resuscitated from -80°C cold storage and maintained on synthetic groundwater medium for aerobic and anaerobic growth experiments. The base synthetic groundwater medium was prepared following the recipe described elsewhere in this report (Truex et al. 2017). For growth experiments, synthetic groundwater medium was supplemented with lactate (sodium lactate, 1 mM final concentration), phosphate (KH_2PO_4, 100 µM final concentration), and nitrate ($NaNO_3$, 1.6 mM final concentration) to be within range of influent groundwater concentrations to the 200W P&T Facility. Fully composed synthetic groundwater medium was pH 7.8.

Aerobic growth studies were performed in sterile culture tubes with plastic snap-caps. Anaerobic growth studies were performed in glass serum bottles with butyl rubber septa and crimp seals. Serum bottles were flushed with sterile N_2 for 5 minutes to decrease O_2 levels prior to inoculation. All experiments were performed in triplicate at 25°C. A filter sterilized (0.5 mM) ferrocyanide stock solution was diluted to establish an experimental concentration range of 0 to 200 µM. Ferrocyanide additions did not affect the final pH of the composed synthetic groundwater medium. Bacterial biomass and ferrocyanide was determined spectrophotometrically by measuring optical density at 610 nm, and absorbance at 320 nm, respectively, using the Eon™ microplate spectrophotometer (BioTek).

4.2 Results

The results for the aerobic dose-response experiments are shown in Figure 3. The growth responses between the no ferrocyanide positive control cultures and cultures exposed to the lowest concentration of ferrocyanide (1 µM) were fairly consistent; however, higher concentrations of ferrocyanide (>10 µM) exhibited a long lag phase before the onset of growth. In fact, no growth was measured for any of these treatments for the first 3 days of incubation. Following this extended lag phase, the growth rate and biomass yield for the all cultures between 1 and 50 µM ferrocyanide were consistent with the no ferrocyanide positive control cultures. Growth was severely impeded by ferrocyanide at the highest concentrations tested, 100 and 200 µM (graphs are overlapping).

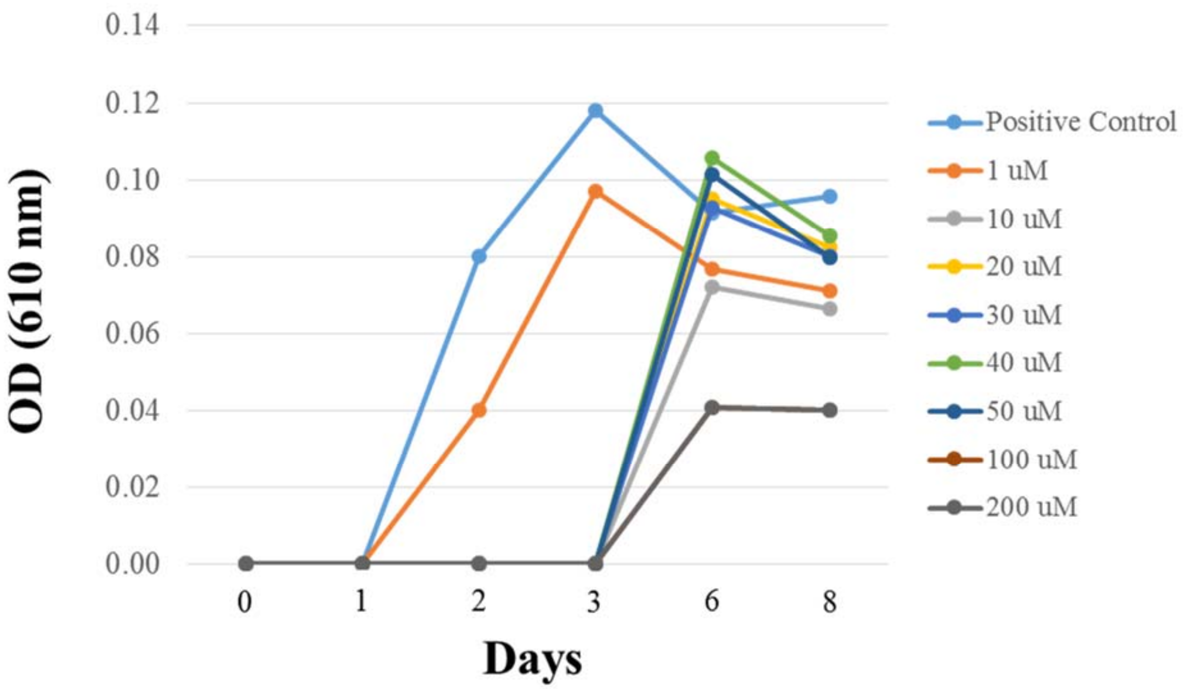

Figure 3. Aerobic growth of the FBR enrichment culture at increasing concentrations of ferrocyanide. Growth experiments were conducted in triplicate. Standard deviation was < 10%.

The results for the anaerobic dose-response experiments are shown in Figure 4. The growth responses between the no ferrocyanide positive control cultures and cultures exposed to the lower concentrations of ferrocyanide (1 to 50 µM) were fairly consistent. The growth rate of the ferrocyanide-treated cultures was negatively impacted, but growth yields were consistent and generally greater than those produced under aerobic conditions. The highest concentrations of ferrocyanide (100 and 200 µM) became inhibitory.

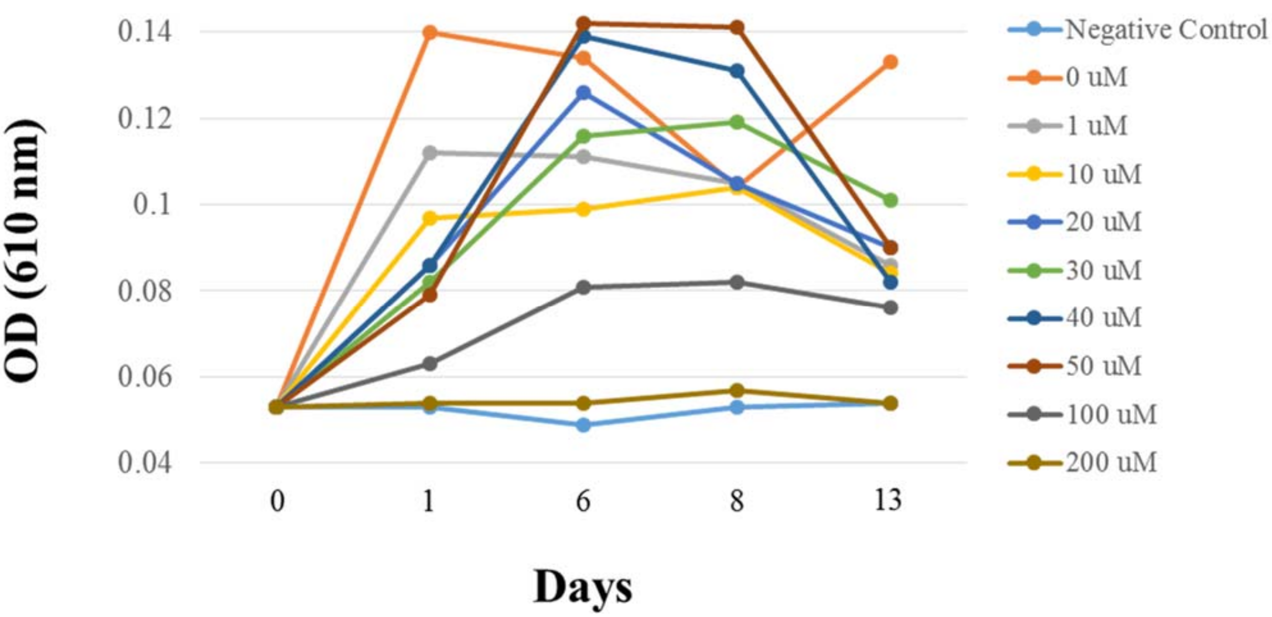

Figure 4. Anaerobic growth of the FBR enrichment culture at increasing concentrations of ferrocyanide. growth experiments were conducted in triplicate. Standard deviation was < 10%.

Ferrocyanide concentrations were measured spectrophotometrically at the end of the anaerobic growth experiments (Figure 5). The analysis was limited to the concentration range from 1 to 50 µM because these levels were the most relevant, and higher concentrations were inhibitory. The absorbance spectra between filter sterilized spent medium (0.2 µm to remove biomass) and un-inoculated controls were not statistically different (t-test; $P < 0.05$). These results indicate that ferrocyanide was not degraded during anaerobic growth of the FBR denitrifying enrichment culture.

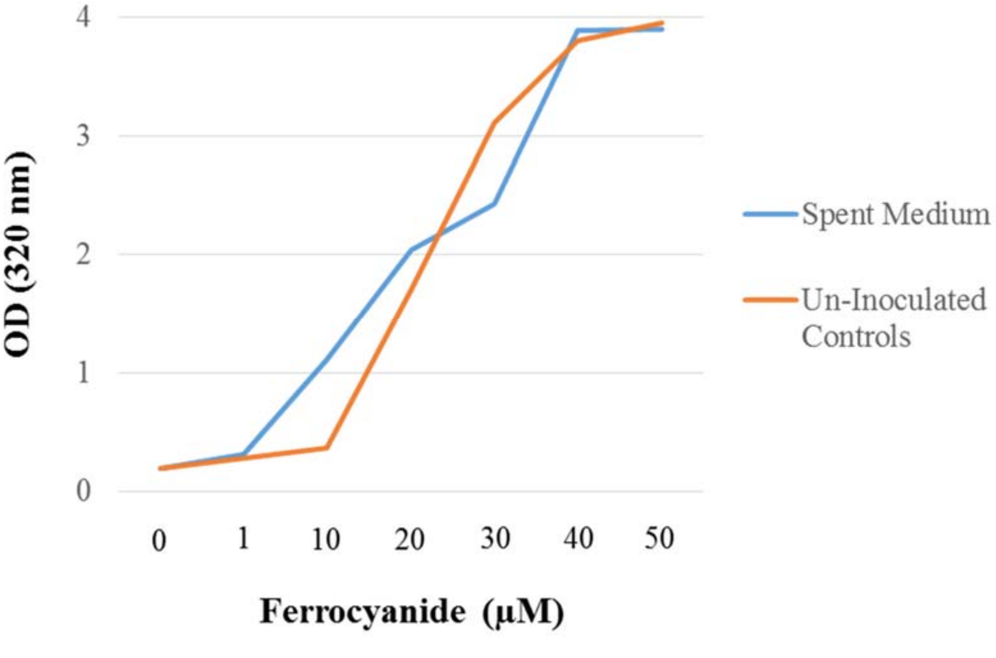

Figure 5. Ferrocyanide measurements pre- and post-anaerobic growth of the FBR enrichment culture.

4.3 Summary

Iron complexes with cyanide are extremely stable and are presumed to exhibit low toxicity to biological systems and high recalcitrance in environments above pH 2 (Dzombak et al. 2006; Gensemer et al. 2006). It is not clear, however, if the ferrocyanide complex could dissociate to form free CN- over long residence times in Hanford groundwater.

A variety of bacteria and fungi can degrade ferri- and ferro-cyanide, particularly at more alkaline conditions (Dursun and Aksu 2000; Luque-Almagro et al. 2005; Morris et al. 2005). While CN^- is primarily inhibitory to aerobic metabolic processes, there are reports of CN^- inhibition of anaerobic microbial processes, such as denitrification (Kim et al. 2011; Kapoor et al. 2016). The short-term growth experiments performed here, while preliminary, suggest the ferrocyanide complex was stable during short-term microbial growth studies. The highest concentrations of ferrocyanide tested (100 and 200 μM) did inhibit culture growth, but the mode of action in these experiments was not specifically investigated.

Aerobic and anaerobic growth responses to increasing concentrations of ferrocyanide were not consistent with the expected response for chemical toxicity of free cyanide. The extended lag phase during aerobic growth conditions slightly improved biomass yields under anaerobic growth conditions, and slower growth rates imply that ferrocyanide may be reacting chemically, possibly with O_2. While preliminary, it is clear that ferrocyanide does affect the growth of the 200W P&T FBR denitrifying enrichment culture, but more work is needed to resolve the biological mode of action under different O_2 regimes.

5.0 Tc-99 Resin Testing

Currently, Tc-99 is a risk driver at the Hanford Site because of its long half-life and high mobility in the environment. At the 200W P&T Facility, Tc-99 is removed from groundwater using Purolite A530E ion exchange resin. Although the A530E resin has been demonstrated to effectively remove TcO_4^- from groundwater, it is not selective, and the use of alternative materials may be required if changes in the groundwater influent create conditions that interfere with TcO_{4-} sorption on A530E (Levitskaia et al. 2017). Alternatively, optimization of P&T operations may lead to the use of a mixed bed that simultaneously treats multiple contaminants. As a result, commercially available hybrid anion exchange resins from ResinTech Inc., with the potential to sorb multiple contaminants of concern, were evaluated for their ability to sorb TcO_4^-. These included the following hybrid anion exchange resins: (1) CHM-20, a cerium oxide/hydroxide hybrid; (2) ASM-125, a hydrated iron oxide hybrid; (3) ASM-HP-10, a hydrated iron oxide; and (4) Bi form, a bismuth oxide chloride hybrid. All hybrid resins performed well, with virtually complete removal of TcO_4^- achieved within 24 hours at solution-to-solid ratios of 200 and 1000. The ASM-125 and Bi form hybrid resins, consisting of a Type 1 (trimethylamine) strong base anion resin, had the highest affinity for TcO_4^- with a K_d of > 600,000 mL/g for the higher solution-to-solid ratio.

5.1 Experimental Methods

5.1.1 Active Sorbent Deployment in Hybrid Ion Exchange Resins

Several ion exchange resins (Resin Tech Inc., West Berlin, NJ) were analyzed and compared against the performance of Purolite A530E. CHM-20 is a gel hybrid Type 2 strong base anion resin (dimethylethanolamine) with cerium oxide/hydroxide. ASM-125 is a chloride form gel hybrid Type 1 strong base anion resin (trimethylamine) containing hydrated iron oxide, used to treat PWR (pressurized water reactor) water for antimony and silica. ASM-HP-10 is a chloride form gel hybrid Type 2 strong base anion resin (dimethylethanolamine) with hydrated iron oxide, used to remove arsenic, uranium, and other trace level contaminants from treat drinking water. Bi-form is a gel hybrid Type 1 strong base anion resin (trimethylamine) with bismuth oxide chloride (BiOCl). The active sorbent is homogeneously distributed throughout the resins. Corrections were made for moisture content for each resin according to assays provided by the manufacturer (see Table 8).

Table 8. ResinTech resin characteristics.

Material	Type	Metal	Moisture Content (%)
CHM-20	Type 2 (dimethylethanolamine)	La = 2.69-3.13 g/kg Ce = 55.4 g/kg	-
ASM-10-HP	Type 2 (dimethylethanolamine)	Fe = 115 g/kg	45.1
ASM-125	Type 1 (trimethylamine)	Fe = 122 g/kg	45.9
Bi Form	Type 1 (trimethylamine)	Bi = 114g/kg	-

5.1.2 Batch Sorption

Batch sorption experiments were conducted to evaluate the materials' performance under aerobic conditions. The stock solution consisted of synthetic groundwater (SGW) and ammonium pertechnetate (NH_4TcO_4) to obtain a concentration of 1.0 mg/L technetium. The SGW was made by addition of the reagents to deionized water in the order identified in Table 9 (Truex et al. 2017). Once the chemicals were dissolved, an excess of calcium carbonate ($CaCO_3$) was added, to reproduce the Hanford carbonate-saturated groundwater, and the solution was stirred and refrigerated. After ~1 week, the solution was filtered to remove excess $CaCO_3$ using a 0.45-µm filter. The pH of the stock solution prior addition of materials was 7.96.

Table 9. Synthetic Hanford Groundwater (Truex et al. 2017)

Constituent	Conc. (mg/L)
$H_2SiO_3*nH_2O$, silicic acid	15.3
KCl, potassium chloride	8.20
$MgCO_3$, magnesium carbonate	13.0
NaCl, sodium chloride	15.0
$CaSO_4$, calcium sulfate	67.0
$CaCO_3$, calcium carbonate	150[a]

(a) Above $CaCO_3$ solubility, groundwater filtered prior to use.

Two ratios of material to contaminant were targeted in the batch loading tests (i.e., 200 and 1000). 0.100 ± 0.001 g of resin was targeted and was placed into a 125-mL bottle or 50-mL falcon tube with the appropriate amount of TcO_4^- stock solution (Parker 2016). The batch sorption tests included solution blanks (initial TcO_4^- solution with no material) and material blanks (material in SGW with no TcO_4^-). Process blanks were prepared and handled in the same manner as all other loading tests. To ensure appropriate mixing conditions between the material and the solution, the bottles were sealed and placed on a shaker table set at 125 rpm at room temperature, under aerobic conditions for 24-hours. After the 24-hour contact time, the bottles were removed from the shaker table, the solid materials settled for less than 15 minutes. A 10-mL aliquot was collected from each bottle and filtered using 0.45-µm polyethersulfone syringe filter. Total Tc was analyzed using a ThermoFisher Scientific X series 2 inductively coupled plasma-mass spectrometer (ICP-MS, analytical method detection limit, considering calibration data = 0.01 µg/L).

The distribution coefficient, K_d (mL/g), was calculated using the following equation, defining the relationship between the volume of materials

$$Kd = \frac{Ci - Ct}{Ct} \times \frac{V}{W}$$

where C_i is the initial concentration of analyte in the contact solution, C_t is the concentration of analyte in the solution at time t, V is the volume of the contact solution in mL, and W is the weight of the dry material in grams.

To assess the competitive effects of other constituents, present in SGW, samples were also analyzed for total calcium, magnesium, potassium, sodium, and sulfur using a PerkinElmer Optima 8300 dual view inductively coupled plasma-optical emission spectrometer and a PerkinElmer S-10 auto-sampler interface.

5.2 Results

The initial and final total technetium concentrations are shown in Table 10 at a solution-to-solid ratio of 200, with a solution volume of ~20 mL and ~0.1 g of dry resin, and at a solution-to-solid ratio of 1000, with a solution volume of ~100 mL and ~0.1 g of dry resin.

Table 10. Technetium loading results for resins.

Material Tested	Solution to Solid Ratio	Initial Technetium Concentration (mg/L)	Final Technetium Concentration (mg/L)	Technetium Loading (mg/g)	K_d after 1 day (mL/g)
A530E	200	1.34	0.00011	0.27	2,420,000
CHM-20	200	1.08	0.00055	0.22	392,000
ASM-10-HP	200	1.08	0.00053	0.22	408,000
ASM-125	200	1.08	0.00038	0.22	563,000
Bi-form	200	1.08	0.00028	0.22	775,000
A530E	1000	1.34	0.00017	1.33	7,990,000
CHM-20	1000	1.08	0.00247	1.08	437,000
ASM-10-HP	1000	1.08	0.00269	1.08	401,000
ASM-125	1000	1.08	0.00176	1.08	614,000
Bi Form	1000	1.08	0.00168	1.08	642,000

Potential uptake of calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), sodium (Na^+), and sulfur (S) from the synthetic groundwater by the materials (Table 11 with a solution-to-solid ratio of 200) was calculated as the difference between the initial concentration of the cations and the final concentration after 24-hour incubation period. These results provide an indication of the selectivity of the materials for iodine. A negative number indicates release of the element into solution from the material.

Table 11. Groundwater constituent loading results for resins (solution-to-solid ratio = 200).

Active Sorbent	Deployment Mechanism	Ca Uptake (mg/L)	Mg Uptake (mg/L)	K Uptake (mg/L)	Na Uptake (mg/L)	S Uptake (mg/L)
A530E	Ion exchange resin	0.3	0.1	0.1	0.1	16.4
CHM-20	Hybrid ion exchange resin	-0.1	0.1	0.0	-12.0	16.6
ASM-125	Hybrid ion exchange resins	-8.8	-0.1	0.0	-1.7	16.6
ASM-10-HP	Hybrid ion exchange resins	-3.1	0.0	0.0	-0.5	16.6
Bi-Form	Hybrid ion exchange resins	-2.2	0.0	0.0	0.1	16.6

5.3 Summary

As expected, the baseline material currently deployed at the Hanford Site P&T Facility, Purolite A530E ion exchange resin, was very efficient at removing TcO_4^- from solution. Complete removal was achieved within 24 hours at both solution-to-solid ratios, indicating that the resin had not reached its capacity under the conditions tested. The technetium loading was 0.27 mg/g, with a batch K_d of >2,000,000 mL/g, at a solution to solid ratio of 200 (Table 10). The ResinTech hybrid anion exchange resins were also very efficient at removing TcO_4^- from solution, with virtually complete removal achieved within 24 hours at both the solution-to-solid ratios.

The best-performing resins were the Type 1 trimethylamine strong base resins with iron (oxy)hydroxide (ferrihydrite), ASM-125, and bismuth oxide chloride (BiOCl), Bi form, which have a higher affinity for the pertechnetate oxyanion (TcO_4^-). The lower initial technetium concentration than that for A530E (1.08 mg/L versus 1.34 mg/L) meant that the loading (0.22 and 1.08 mg/g for solution-to-solid ratios of 200 and 1000, respectively) and K_d (> 600,000 mL/g for the higher solution-to-solid ratio) were slightly lower.

The Type 2 dimethylethanolamine strong base resin with cerium oxide/hydroxide, CHM-20, and iron (oxy)hydroxide (ferrihydrite), ASM-10-HP, still performed well, with similar technetium loadings at both solution-to-solid ratios, but with a slightly lower K_d of ~400,000 mL/g (Table 10). Conversely, the Type 2 dimethylethanolamine strong base resins performed better for the removal of iodate (IO_3^-) from Hanford 200W Area groundwater, with CHM-20 being the best-performing hybrid resin (Cordova et al. 2009). In comparison, A530E was shown to remove only iodide (I^-), which is a minor species of iodine present in Hanford groundwater (Cantrell et al. 2019).

Table 7 shows that all the sulfur, present as sulfate (SO_4^{2-}) in SGW, is removed by all the ion exchange resins, but the available capacities of the resins are sufficient to remove the TcO_4^-, even in the presence of much higher SO_4^{2-} concentrations (1 mg/L TcO_4^- versus 67 mg/L SO_4^{2-}). The hybrid ion exchange resins also release calcium and sodium into solution during the sorption reaction, which maybe a result of the resin synthesis protocol (Table 7).

The results with these hybrid resins are promising, as they offer the potential to remove Tc-99 with resins that may be better suited to removal of multiple contaminants (e.g., as demonstrated initially for iodine) within one treatment train. Performance of these hybrid resins could be improved by functionalizing the strong base resin with higher amines, such as tributylamine, which should have a higher affinity for TcO_4^- , as well as using cerium oxide/hydroxide as the active sorbent. Future work will involve testing different parent resins, and comparing performance with the hybridized forms, to determine the mechanism of uptake for key contaminants of concern, including TcO_4^-, and uranium species, from complex, co-mingled groundwater plumes.

6.0 Quality Assurance

The results presented in this report originate from work governed by the PNNL Nuclear Quality Assurance Program (NQAP). The NQAP implements the requirements of U.S. Department of Energy Order 414.1D (DOE 2011), *Quality Assurance*, and 10 CFR 830 Subpart A, *Quality Assurance Requirements*. The NQAP uses ASME NQA-1-2012, *Quality Assurance Requirements for Nuclear Facility Applications* as its consensus standard and NQA-1-2012 Subpart 4.2.1 as the basis for its graded approach to quality.

Two quality grading levels are defined by the NQAP:

Basic Research - The required degree of formality and level of work control is limited. However, sufficient documentation is retained to allow the research to be performed again without recourse to the original researcher(s). The documentation is also reviewed by a technically competent individual other than the originator.

The work supporting the results presented in this report under Section 5 was performed in accordance with the *Basic Research* grading level controls.

Not Basic Research - The level of work control is greater than basic research. Approved plans and procedures govern the research, software is qualified, calculations are documented and reviewed, externally sourced data is evaluated, and measuring instrumentation is calibrated. Sufficient documentation is retained to allow the research to be performed again without recourse to the original researcher(s). The documentation is also reviewed by a technically competent individual other than the originator.

The work supporting the results presented in this report under Sections 1–4 was performed in accordance with the *Not Basic Research* grading level controls.

7.0 Conclusions

This report provides information that can be used to evaluate biological and chemical component aspects related to P&T system performance, biofouling, and in anticipation of future influent streams. Efficient biological treatment of groundwater and process optimization requires a thorough understanding of the fundamental processes involved in contaminant attenuation, as well as the process vulnerabilities to groundwater perturbation. Laboratory analyses of facility samples and experiments can provide valuable support to P&T operations and process optimization.

The main findings are summarized below:

1. A variety of radiocontaminants and groundwater constituents are accumulating on the GAC in the FBR. Results showed temporal accumulation of I and Tc-99 on the GAC in the FBR, as well as U, Cr, As, Sr, Ba, Ca, and Mg. Iodine concentrations were much higher than Tc-99, and iodine was also detected in stored sludge samples. The mechanisms involved in sequestering iodine onto the GAC are not known but would be important to define in order to approximate the binding capacity of I onto the GAC, and to predict the stability of I-GAC complexes against different groundwater chemistries that may be considered for future treatment at the 200W P&T Facility.
2. **Mechanisms of nitrate attenuation in the FBR should be specifically evaluated.** In the laboratory tests, FBR enrichment cultures reduced nitrate by DNRA. This result is important because DNRA does not eliminate nitrate from the system, but rather converts it to a biologically available form that can lead to microbial contamination in the effluent distribution pipelines and contribute to biofouling at the injection wells. P&T water samples confirmed the production of NH_3 in the MBR. Diagnostic molecular tools are available to differentiate and monitor nitrate attenuation processes from FBR samples, specifically DNRA. Molecular biological tools could be used more effectively in combination with routine water quality measurements to provide a more robust measure of facility performance and to isolate sources of microbial contamination in the effluent distribution system.
3. **Ferrocyanide appears to be chemically reactive but short-term experiments suggest biological impacts may be minor.** At typical groundwater concentrations, ferrocyanide was not inhibitory to the FBR enrichment cultures but exposure did retard aerobic and anaerobic growth in a concentration dependent manner. Follow-up studies should consider longer term interactions to specifically evaluate the potential for ferrocyanide sequestration on GAC (preferably using GAC from the FBR), the impacts on nitrate attenuation in an established microbial system (i.e., the extant community in the FBR), and to define the chemical reactivity of ferrocyanide (potentially for the production of reactive oxygen species). All of these interactions could have important implications for the performance and sustained activity of the FBR, but may also influence the stability of I and other elements that are accumulating on the GAC in the FBR.
4. **Deploying hybrid resins in the P&T system has the potential to target multiple contaminants of concern in complex co-mingled groundwater plumes.** The combination of a strong base ion exchange resin with cerium oxide/hydroxide as an active sorbent could offer similar performance to the resin currently used for Tc-99 removal in the P&T, Purolite A530E, and also potential for removing multiple contaminants and evolving influent water chemistry.
5. **Optimization Plan for 200W P&T.** Discussions and planning are underway to consider eliminating the biological treatment of nitrate at the 200W P&T Facility. This report is intended to provide additional information regarding the operational functionality of the biological treatment processes, and in anticipation that facility modifications will be implemented, the potential impacts. Bypassing the biological treatment of nitrate will resolve many of the biofouling issues outlined in this report and should optimize plant performance and allow for increased groundwater treatment capacity.

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