Characterization and Well Remediation Strategies for Improved Long-term Pump and Treat System Injection Well Performance – 17272

Hope Lee *, Brady Lee *, Mark Carlson **, James Geiger ***, Danielle Saunders *, Kevin Bral ****, Elsa Cordova *, Mike Niebauer ****, Beren Christiansen *

* Pacific Northwest National Laboratory

** CH2M Hill PRC

*** CH2M HILL Plateau Remediation Company

**** CH2M Hill

ABSTRACT

In June of 2012 the CH2M Hill Plateau Remediation Company completed construction of a 2,500 gallon-per-minute (gpm) pump and treat (P&T) system designed to remove technetium-99, uranium, carbon tetrachloride, trichloroethene, nitrate, and total and hexavalent chromium from incoming groundwater. The entire pump and treat system consists of the following primary components: 1) extraction wells (20+); 2) a radionuclide removal facility; 3) a biological system; 4) sludge processing; 5) vapor processing; and 6) injection wells.

Overall efficiency of the 200 West P&T has been adversely impacted due to fouling in vertical injection wells where treated water is being reinjected into the subsurface. Fouling of the wells is thought to be caused by bacteria producing extracellular polymeric substances (EPS), as well as high manganese and iron in the biofilm. Due to decreased specific injectivity, all injection wells are on a maintenance schedule requiring rehabilitation. During rehabilitation, the wells are out of service affecting the overall capacity of the P&T, plus the rehabilitation process adds hundreds of thousands of dollars of cost to operate the P&T each year.

Chemical and biological foulants may be coming from the P&T or are already present in the subsurface and active pumping has stimulated growth of the microbes present. Chemical and microbiological characterization efforts are in progress for the biological plant and injection well system to determine causative agents in the biofouling. P&T effluent tank samples, injection well distribution tanks, bailed samples from the fouled well, and post-rehabilitation samples were analyzed. Chemical constituents analyzed include, sulfate, phosphorus, chemical oxygen demand, total organic carbon, and iron. Microbial characterization included total heterotrophs, anaerobic heterotrophs, nitrate reducing bacteria, iron reducing bacteria and sulfate reducing bacteria. Molecular biological tools employed included 16S rRNA metagenomes, and functional gene analysis using qPCR.

Chemical analysis of the P&T effluent and injection well tanks showed the presence of ~6.5 mg/L nitrate, 96 mg/L sulfate and phosphorus levels below 1 mg/L. Bailed samples from the injection well showed similar concentrations for each chemical, but following cleaning, TOC, total iron, manganese and other constituents were higher than prior to cleaning. These data show that bacterial nutrients and metals that may precipitate are present in the well water and are likely the causative agent in the biofouling.

INTRODUCTION

In June of 2012 the CH2M Hill Plateau Remediation Company completed construction of a 2,500 gallon-per-minute (gpm) pump and treat system designed to remove technetium-99, uranium, carbon tetrachloride, trichloroethene, nitrate, and total and hexavalent chromium from incoming groundwater. The 200 West Area Pump and Treat Facility consist of two buildings; a RAD building, and a biological treatment building. Technetium and uranium containing groundwater is pumped into the RAD building at a maximum flow rate of 600 gpm, where ion exchange is used to remove these contaminants. Treated groundwater is then mixed with groundwater pumped from wells with no radioactive constituents in an equalization tank. Mixed groundwater is then pumped into two parallel, fluidized bed biofilm reactors (FBBRs) containing microbes that transform nitrate, hexavalent chromium, as well as the chlorinated solvents. From the FBBRs the groundwater is pumped through a carbon separation tank, and then into four membrane bioreactors where total suspended solids (TSS) are removed, and carbon are degraded under aerobic conditions. Water from the MBR then goes to an air stripper to remove remaining volatile compounds, and sludge from the MBR is thickened in a rotary-drum thickener and centrifuge. Sludge is then treated with lime to kill bacteria and reduce odor, and the product is disposed of at the Environmental Restoration Disposal Facility at Hanford. Treated groundwater is pumped to a series of 20 injection wells for reintroduction into the aquifer at rates of up to 240 gal/min per well [3].

The Biological Plant (BP) consists of the equalization tanks, and all processes through the injection wells, including sludge handling processes. Since onset of operation in 2012, two primary problems have persisted in the biological plant: 1) FBBR operational challenges associated with nutrient delivery and formation of extracellular polymeric substance (EPS), leading to excessive buoyancy and loss of the GAC, as well as fouling of pump and treat components downstream of the FBBRs; and 2) Fouling of re-injection wells, decreasing the overall capacity of the entire Pump and Treat Facility. Since groundwater beneath the Central Plateau at Hanford contains a range of co-mingled organic and inorganic contaminants, a fairly complex system for complete treatment, especially with regard to the biological component of the plant, was required.

In an effort to provide data that will help with optimization of the plant, for both contaminant removal and re-injection of groundwater, a sampling campaign was done at various points in the plant with samples being taken from the equalization tank and unit processes up to and including the effluent tank. In addition, injection well and injection well distribution samples were taken to provide insight into causes of biofouling. Samples were analyzed using chemical, microbiological and molecular techniques in an effort to understand how chemical constituents and microbiology fluctuates at different points throughout the plant.

MATERIALS AND METHODS

Plant Sampling

An assortment of samples that included water, sludge, and solids suspended in water were taken from various points over the biological treatment train at the 200 West

Pump and Treat Facility (Fig. 1). Samples were retrieved and shipped directly to

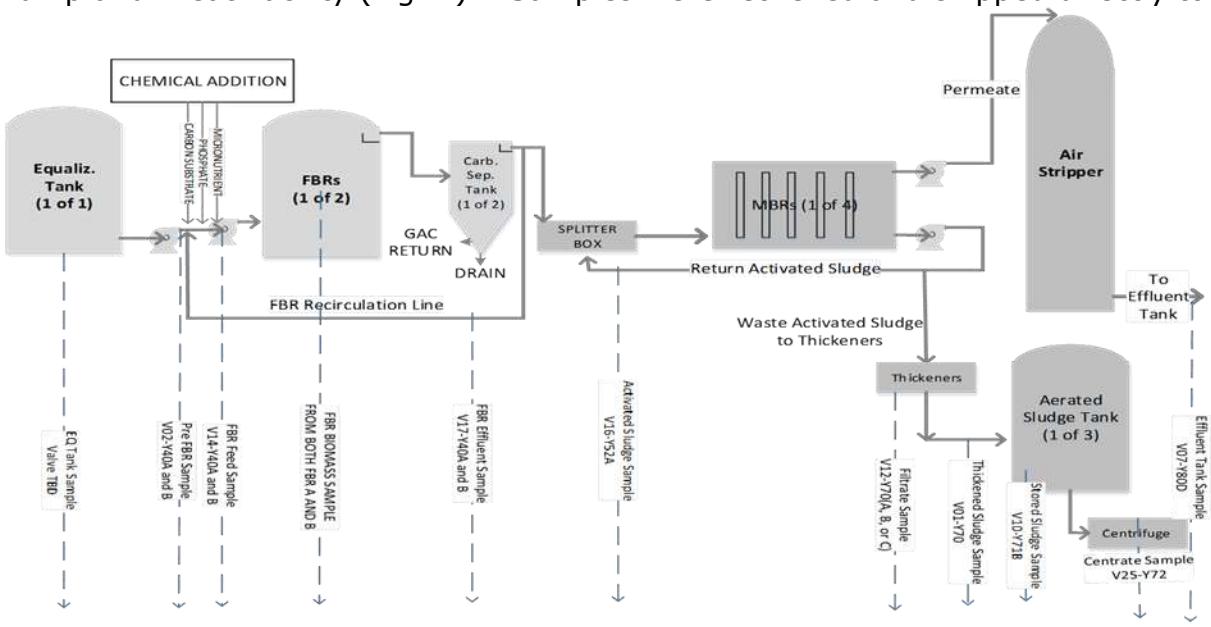

Fig. 1. Diagram of biological component of pump and treat system and location of sampling points used during the optimization study.

the laboratory on ice and processed the same day to assure that samples were fresh. Processing included: determination of physical parameters of the samples, extraction of the biomass from solid/suspended solid samples, chemical analysis and microbiological and molecular analysis.

Chemical Analysis

Upon arrival in the lab, pH, temperature, redox, conductivity and dissolved oxygen measurements were taken on the sludge, mixed phase, and water samples. For sludge samples, sludge was added to nanopure water at a ratio of 1:4, and then the measurements were taken. Samples were also analyzed to determine the dry weight of each sample. Water samples from the plant, injection well system, as well as supernatant from the extractions described below were sent to Test America, Inc. for analysis of total metals by inductively coupled plasma (ICP), hexavalent chromium, total suspended solids, total volatile solids, anions by ion chromatograph (IC), alkalinity, ammonia, total Kjeldahl nitrogen, sulfide and chemical oxygen demand.

Sample Processing

Solid/sludge and liquid samples from the plant were analyzed for total heterotrophic bacteria using most probable number (MPN) and heterotrophic plating using nutrient agar. For MPN analysis, samples were added to phosphate buffered saline (PBS) and then serially diluted in nutrient broth. Similarly, samples were diluted in PBS and then dilutions were plated on nutrient agar and incubated at room temperature for up to one week. Liquid samples were filtered through a 0.2 µm filter and then frozen at -20 °C until analyzed. Sludge samples and suspended GAC samples from FBBR were prepared by adding 20 mL of FBBR water, containing cellular material, to

approximately 20 g (assumed 1 g = 1 mL) of solid carbon/biomass sample in sterile 50 mL conical centrifuge tubes. Sample volumes were measured using sterile 50-mL serological pipettes. Five replicates were prepared for each sample, for a total of 100 g of solid material and 100 mL of overlying water processed for analysis. Following a brief test to determine optimum conditions for maximum biomass recovery, samples were sonicated for 2 hours at the highest setting in a sonicating water bath. Supernatant was removed immediately from the solid and placed into new sterile 50-mL conical centrifuge tubes (approximately 25 mL). Once the supernatant was removed, 10 mL of phosphate buffered saline (PBS) was added to the solid material. The sample was then vortexed for 1 to 2 minutes to separate the biological materials from the GAC solids. After vortexing, the resulting solution was removed and this solution was combined with the liquid from the initial extraction. This process was repeated and the supernatant was combined with the first two extractions for an approximate volume of 225 mL in five separate conical centrifuge tubes. These samples were weighed, balanced, and then centrifuged at 10,000 relative centrifugal force (RCF) for 10 minutes. The supernatants were removed and combined into a collection bottle and stored. The resulting pellet was washed twice with 10 mL of PBS, and the final pellet from each tube was resuspended in 50% glycerol, aliquoted into 1-mL cryogenic vials, and stored at -80 °C until analyses were performed.

DNA Extraction

Frozen samples were thawed and 0.5 mL of the cell slurry was extracted using both a MoBio Ultraclean Soil DNA kit (MoBio Laboratories, Carlsbad, CA) and a Fast DNA Spin Kit for Soil (MP Biomedical, Santa Ana, CA). For filters, approximately ¼ of a filter was processed using the same kits. Manufacturer protocols were followed during the extraction process, with the exception that a Disruptor Genie (Scientific Industries, Inc, Bohemia, NY) set at 3,000 rpm for 10 minutes was used for mechanical lysis of microbial cells in the sample. An equal volume of each extraction was combined and the results were quantified using a NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE).

16S rRNA Metagenome Analysis

DNA extracted from each of the samples quantified, and approximately 30 µl was sent to Argonne National Laboratory for sequencing of the 16S rRNA metagenome. DNA barcodes and linkers were added using polymerase chain reaction, and the resulting amplicons were sequenced at the Institute for Genomics and Systems Biology Next Generation Sequencing Core Facility at Argonne National Laboratory using an Illumina MiSeq instrument. Raw sequence reads were demultiplexed with using EA-Utils with zero mismatches allowed in the barcode sequence [2]. Reads were quality filtered with BBDuk2 to remove adapter sequences and PhiX with matching kmer length of 31 bp at a hamming distance of 1 [4]. Reads shorter than 51 bp were discarded. Reads were merged using USEARCH with a minimum length threshold of 175 bp and maximum error rate of 1% [7]. Sequences were dereplicated (minimum sequence abundance of 2) and clustered using the distance-based, greedy clustering method of USEARCH at 97% pairwise sequence identity among operational taxonomic unit (OTU) member sequences [8]. De novo prediction of chimeric sequences was performed using USEARCH during clustering. Taxonomy was assigned to OTU sequences using BLAST alignments followed by least common ancestor assignments

across SILVA database version 123 clustered at 99% [5, 11]. OTU seed sequences were filtered against RDP Gold reference database version 9 to identify chimeric OTUs using USEARCH [6].

Quantitative Polymerase Chain Reaction

Functional dynamics of the microbial community in samples from the plant was determined using qPCR and primers targeting specific functional genes or for16S rRNA of specific functional guilds of bacteria or archaea. Table I shows PCR primers

TABLE I. List of primers used during qPCR and bacteria that was source of DNA for making of standard curve.

Target	Functional Gene	Primer Name	Annealing Temp (°C)	Standard
Nitrate Reductase	narG	1960m2f	63	*Psuedomonas stutzeri*
		2050m2r	63-58	
Nitrate Reductase	narG	narG 1960F	55	*Psuedomonas stutzeri*
		narG 2659R		
Periplasmic Nitrate Reductase	napA	napA V17F	61	*Psuedomonas stutzeri*
		napA 4R		
Nitrite	nirS	nirS1F	63	*Psuedomonas stutzeri*
		nirS6R		
Nitrite	nirK	nirK1F	58	*Achromobacter xylosoxidans*
		nirK5R		*Desulfovibribo vulgaris*
Nitric Oxide	cnor	cnorB2F	57	*Psuedomonas stutzeri*
		cnorB6R		
Bacteria	16S rRNA	27F	55	*Burkholderia cepacia F1*
		519R		*Methanococcus maripaludis*
Archaea	16S rRNA	A8F	55	Plasmid

used, along with bacteria or archaea used as positive controls and to develop standard curves for quantification purposes. DNA concentration in each sample was normalized to10 ng/µL and separated into aliquots, which were used for each set of qPCR reactions. Concentration and purity of DNA was determined using the Nanodrop 8000 micro-spectrophotometer (Thermo Fisher Scientific; Waltham, MA).

All qPCR assays were performed using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). A SsoAdvanced SYBR Green supermix kit (Bio-Rad Laboratories, Hercules, CA) was used for amplification and real-time fluorescence measurement. Each PCR reaction was 20 µL final volume and contained 1X of hot-start Sso7d-fusion polymerase, SYBR Green dye, dNTPs, $MgCl_2$, and stabilizers. Amplifications conditions included an extended denaturation step of 2 minutes at 98 °C, followed by 30 or 40 cycles (primer set dependent) of denaturation at 98 °C (30 sec), annealing (See Table for temperature) for 30 seconds, and a 30 second extension. Fluorescence was read at the end of each extension step. Primer concentration and thermocycler conditions for each primer pair is given in Table II.

Following amplification, a melt analysis was performed over a temperature range of 65 to 95 °C at a rate of 0.5 °C per second to confirm that the melting temperature of the unknown matched the melting temperature of the control. In addition, amplicon size was confirmed using a DNA 1000 Chip Kit, which was run on a 2100 Bioanalyzer (Agilent Technologies; Santa Clara, CA).

RESULTS AND DISCUSSION

Chemical Analysis of BP Samples

Common measurements for wastewater treatment such as total suspended solids (TSS), total volatile solids (TVS), Kjeldahl nitrogen, and chemical oxygen demand (COD) were relatively low in the equalization tank and FBBR feed solutions, but increased through the FBBR and in sludge generated at the back end of the BP (Table II). TVS in the FBBR averaged 4,346 and 3,576 mg/kg bed for FBBR A and FBBR B, respectively. These values correspond with the dry weights which were slightly lower for samples taken from FBBR B. The highest TVS values for the plant on the day sampling took place, was in the centrate, which contained 2,000 mg/L TVS.

Analytical values for nitrogen through the BP, indicated that multiple reductive processes may be occurring as the FBBR receive nitrate. When samples were taken, nitrate in the equalization tank was at 28 mg/L, within the FBBRs, concentrations dropped to approximately 70 µg/kg bed. At the same time, nitrite levels increased from 3 µg/L in the equalization tank to approximately 1 mg/kg bed in the FBBRs, indicating nitrate reduction to nitrite. Nitrite concentrations in FBBR A were slightly higher than those in FBBR B. Ammonia results indicate that dissimilatory nitrate reduction to ammonia is also occurring. Ammonia levels jumped from 8 µg/L in the equalization tank to averages of 13 mg/kg bed in FBBR A and 8.6 mg/kg bed in FBBR B. Ammonia levels increased through sludge processing and the most ammonia was once again found in the centrate samples (160 mg ammonia/L). Kjeldahl nitrogen which measures organic nitrogen, ammonia and ammonium showed increasing concentrations when comparing the FBBR units to values for the equalization tank.

Sulfur balance through the plant also shows interesting trends. Sulfate concentrations in the equalization tank and the FBBRs typically remained between 40 and 45 mg/L or kg bed, respectively. Concentrations dropped in the activated sludge, stored sludge and the concentrated sludge. Supporting the trend noted for other analytes, sulfate concentrations in the centrate increased to 170 mg/L, and concentrations in the effluent tank were approximately 80 mg/L. Sulfide and total sulfide in the equalization tank was ~0.45 mg/L when the samples were taken. Sulfide levels were just under 7.8 mg/kg bed in FBBR A and approximately 7.5 mg/kg bed in FBBR B. Sulfide levels decreased in to below 7 mg/kg solid in samples from the activated sludge, and sludge processing units, including the centrate, which had concentrations similar to the equalization tanks. Total sulfide averaged 185 mg/kg bed in FBBR A and 139 mg/kg bed in FBBR B, and decreased through the remainder of the BP. Together these results indicate that some level of sulfate reduction is occurring within the FBBR. There is currently no explanation for the elevated sulfate in the plant effluent tank.

TABLE II. Analytical results from processes throughout the biological plant; including the equalization tank, FBBR feed tanks, both FBBRs, the activated sludge, thickened sludge, stored sludge, the centrate and the plant effluent.

Analyte	EQ TANK V12-T30 (mg/L)	EQ TANK V12-Y30 (mg/L)	FBR Feed A (mg/L)	FBR A Top (mg/kg)	FBR A Middle (mg/kg)	FBR A Bottom (mg/kg)	FBR Feed B (mg/L)	FBR B Top (mg/kg)	FBR B Middle (mg/kg)	FBR B Bottom (mg/kg)	AS V16-Y52A (mg/kg)	TS RDT A (mg/kg)	TS RDT C (mg/kg)	SS V10-Y71B (mg/kg)	Centrate (mg/L)	Eff Tank (mg/L)
Total Suspended Solids	4.000	4.000	4.000	3116.880	4530.349	5390.195	4.000	1909.853	4627.552	4189.355	227.842	1238.891	893.729	229.751	1200.000	4.000
Total Volatile Solids	180.000	190.000	160.000	4155.840	4530.349	5727.082	180.000	2387.316	4926.103	4588.341	165.703	1566.414	1675.742	1330.140	2000.000	130.000
Nitrate as N	28.000	28.000	28.000	0.069	0.067	0.067	28.000	0.064	0.084	0.080	0.041	0.057	0.056	0.048	45.000	9.400
Nitrite as N	0.003	0.003	0.003	1.039	1.007	1.011	0.003	0.955	0.896	1.197	0.311	0.427	0.419	0.363	41.000	0.060
Orthophosphate	0.078	0.078	0.078	1714.284	1543.674	1515.992	0.078	1161.827	1462.903	1476.249	1025.289	683.526	670.297	519.964	0.480	1.300
Sulfate	45.000	47.000	46.000	207.792	162.757	144.861	45.000	238.732	149.276	129.671	62.139	19.936	19.550	36.277	170.000	79.000
Alkalinity	240.000	110.000	110.000	1904.760	1845.698	1684.436	110.000	1750.699	2089.862	1994.931	569.605	1352.813	1396.452	1571.983	130.000	89.000
Ammonia	0.008	0.008	0.008	14.892	12.752	13.307	0.019	9.549	8.509	8.977	8.803	48.416	75.408	290.212	160.000	0.008
Nitrogen, Kjeldahl	0.220	0.220	0.220	259.740	21.813	21.898	0.220	55.704	283.624	10.374	11.392	356.003	377.042	278.120	110.000	0.220
Nitrogen, Kjeldahl	0.220	0.220	0.220	25.974	352.360	539.019	0.220	8.913	13.136	141.640	12.428	270.563	363.077	435.318	190.000	0.220
Sulfide	0.460	0.460	0.460	7.792	7.718	7.917	0.460	7.321	7.016	9.376	4.764	6.550	6.424	5.562	0.460	0.460
Total Sulfide	0.470	0.450	0.450	242.424	109.064	202.132	0.450	238.732	164.203	13.366	14.499	6.550	6.284	5.562	0.450	0.450
Chemical Oxygen Demand	6.500	6.500	6.500	311.688	352.360	370.576	6.500	190.985	403.045	129.671	134.634	2990.428	4189.355	1571.983	1100.000	6.500
Aluminum	0.056	0.056	0.056	1.905	2.181	2.358	0.056	1.751	2.538	1.536	1.553	3.133	3.351	1.330	0.690	0.056
Arsenic	0.005	0.005	0.005	0.078	0.076	0.076	0.005	0.072	0.067	0.090	0.145	0.157	0.209	0.115	0.006	0.005
Boron	0.046	0.035	0.047	0.606	0.503	0.488	0.035	0.398	0.269	0.359	0.911	0.513	0.503	0.544	0.045	0.052
Calcium	65.000	67.000	69.000	259.740	234.907	269.510	63.000	238.732	238.841	259.341	103.565	142.401	139.645	55.624	53.000	61.000
Chromium	0.029	0.033	0.031	1.506	1.661	2.021	0.033	1.496	1.493	1.137	0.176	0.570	0.489	0.206	0.120	0.010
Cobalt	0.007	0.007	0.007	0.118	0.114	0.115	0.007	0.108	0.102	0.136	0.145	0.441	0.391	0.169	0.075	0.041
Copper	0.005	0.007	0.005	4.848	5.705	7.917	0.005	2.546	5.374	4.588	0.290	1.851	1.536	0.593	0.660	0.005
Iron	0.032	0.032	0.071	22.511	23.491	26.951	0.032	20.690	25.377	15.760	83.887	384.484	363.077	22.975	54.000	0.093
Magnesium	19.000	20.000	21.000	84.848	77.184	85.906	19.000	73.211	79.116	77.802	28.998	35.600	34.911	25.394	14.000	18.000
Manganese	0.003	0.003	0.003	0.468	0.470	0.539	0.003	0.398	0.493	0.459	0.435	1.182	1.173	0.157	0.240	0.250
Molybdenum	0.005	0.005	0.005	0.242	0.201	0.202	0.005	0.334	0.194	0.150	0.099	0.399	0.405	0.786	0.140	0.032
Nickel	0.007	0.008	0.008	0.111	0.107	0.118	0.007	0.102	0.104	0.128	0.135	0.983	0.978	0.181	0.071	0.018
Selenium	0.008	0.007	0.005	0.090	0.159	0.202	0.008	0.119	0.078	0.136	0.104	0.142	0.140	0.121	0.005	0.006
Strontium	0.280	0.290	0.270	1.229	1.124	1.263	0.280	1.082	1.209	1.177	0.507	0.812	0.796	0.230	0.270	0.250
Uranium	0.064	0.064	0.064	1.108	1.074	1.078	0.064	1.019	0.955	1.277	1.346	1.851	1.815	1.572	0.064	0.064
Zinc	0.021	0.021	0.021	0.693	0.722	0.910	0.021	0.700	0.776	0.738	0.601	1.709	1.676	0.508	0.580	0.021
Cr (VI)	0.019	0.017	0.019	0.045	0.067	0.044	0.020	0.041	0.039	0.052	0.027	0.037	0.168	0.031	0.003	0.003
pH	7.919	7.721	7.802	7.501	7.336	7.314	7.840	7.633	7.316	7.822	7.801	7.517	7.621	8.389	8.151	7.543
Temp (°C)	8.600	9.833	5.700	10.733	9.067	9.533	7.533	23.000	10.300	15.900	7.300	14.800	12.233	5.567	8.700	9.533
Redox (RmV)	296.167	329.833	321.500	178.733	206.433	203.867	298.467	105.000	142.633	182.867	171.000	79.667	166.667	-205.000	242.567	289.067
Conductivity (µS/cm)	690.500	685.033	687.133	605.400	613.933	595.633	687.233	1263.867	643.333	595.267	607.867	462.700	447.833	2568.000	2157.000	673.767
DO (%)	79.367	69.133	64.233	56.333	51.633	38.267	69.433	42.133	32.467	74.033	56.700	26.533	44.867	0.867	64.833	76.133
Dry Weight (mg/g)	271.209	275.270	278.051	443.985	446.204	455.348	274.504	359.791	452.063	420.361	2.445	55.377	56.981	33.560	271.119	271.875

Orthophosphate concentration in the equalization tank was 78 µg/L, including levels in each feed stream for the FBBR. Concentrations in the FBBR were in the range of 1,500 mg/kg of bed material, indicating ample phosphorus for growth of bacteria in the bed. Orthophosphate in the sludge samples was in the range of 650 mg/kg sludge, with minimal amounts in the centrate, and effluent tank concentrations of 1.3 mg/L.

In general, metals were concentrated in the GAC bed material of the FBBRs; thereby, were also present at elevated levels in sludge through the rest of the plant, but not in the plant effluent. Metals concentrations in the equalization tank were in the low part per billion range, with the exception of magnesium which was present at 19 mg/L. Metals concentration in the GAC bed material were in the high µg/kg to low mg/kg range. Copper was present in the FBBR bed material and were present at concentrations of 6.2 and 4.2 mg/kg bed in FBBR A and FBBR B, respectively. Iron was concentrated to levels of 24.3 mg/kg bed (FBBR A) and 20.6 mg/kg bed (FBBR B). Iron concentrations were also high in the activated and concentrated sludge samples. Magnesium which was high in the equalization tank was present in the FBBR bed materials at concentrations near 80 mg/kg bed. Magnesium levels in the effluent tank returned to concentrations similar to the equalization tank. Manganese was present in the equalization tank at concentrations near 5 µg/L, and at average concentrations of 200 µg/kg bed material. Magnesium concentrations remained elevated in the plant effluent at a level of 250 µg/kg. Hexavalent chromium was low in all samples taken, however in the FBBR beds, concentrations averaged 1.5 mg/kg bed material. Strontium and uranium also appeared to concentrate in the FBBR beds. Strontium was relatively high in the equalization tank (~280 µg/L), and at levels just above 1 mg/kg bed material in the FBBRs. Equilization tank concentrations of uranium were near 64 µg/L, while levels were near 1.1 mg/kg bed in the FBBRs.

Chemical Analysis of Injection Well Samples

Injection well samples encompass the effluent tank, injection well tanks, and the injection well samples. Injection well samples can be broken up into bailed samples, samples after plunging the well for mixing, and a post-cleaning sample after chemicals were added. Most parameters tested were similar between the effluent tank, injection well tanks and the bailed samples from the injection wells (TABLE III). The largest changes among this set of samples was the post-cleaning sample taken following addition of chemicals. As expected, the pH was below 3.5 following chemical treatment. Concentrations of most chemical constituents including metals were elevated in the injection well sample following cleaning. Conductivity increased to nearly 3,000 µS/cm, TOC increased to 120 mg/L, and TDS increased to 2,400 mg/L. Nitrate and phosphate were present in this well at concentrations of 3.8 and 4.3 mg/L, respectively. Magnesium increased to 57 mg/L, and the manganese concentration was 32 mg/L.

Microbial and Molecular Analysis of BP Samples

Bacterial Abundance in Samples

Bacteria in the system were analyzed using MPN, which estimates bacterial numbers in liquid media, and using formation of colony forming units (CFU) on solid media. When growing in liquid media, cells density in the biofilm was in the range of

TABLE III. Analytical results from injection well infrastructure. All concentrations mg/L, unless otherwise noted.

	Effluent Tank Pre-Flush	289, Effluent Tank, Valve V07-Y80D	ITB Tank Valve V05-Y91H Pre-Flush	ITB Tank Valve V08-Y91	299-W6-13 Bailed	299-W6-13 Post-Plunge	299-W6-13 Post Clean
Alkalinity	120	90	120	110	110	200	0.54
Bicarbonate Alkalinity as CaCO3	120	90	120	110	110	200	0.54
Calcium	65	70	65	64	69	79	220
Carbonate Alkalinity as CaCO3	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Chloride	44	44	43	44	54	56	270
Copper	0.023	0.17	0.013	0.01	0.28	0.18	0.51
Hydroxide Alkalinity	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Iron (Ferrous)	0.19	0.04	0.19	0.03	0.07	0.14	0.74
Iron (Total)	0.12	0.62	0.075	0.06	10	3.2	7.3
Magnesium	22	23	22	22	22	26	57
Manganese	2.6	11	0.037	0.017	7.5	4.2	32
Nitrate as N	6.3	7.3	6.8	7.8	9.2	1.3	3.8
Orthophosphate	0.76	2.4	0.75	1.6	2.7	3.2	4.3
Phenolphthalein Alkalinity	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Potassium	5.7	15	5.7	5	6.2	6.2	15
Silicon	19	21	19	20	18	18	42
SiO2, Silica	40	44	40	42	38	39	90
Sodium	27	21	27	21	28	30	180
Sulfate	94	92	93	92	97	85	91
Total Dissolved Solids (TDS)	400	450	400	440	440	500	2400
Total Hardness	250	270	250	250	260	300	800
Total Organic Carbon	1.1	0.78	5.88	0.72	13	52	120
pH	7.54	7.34	7.56	7.42	7.85	8.13	3.44
Temp (°C)	22.07	22.30	22.30	21.47	22.77	23.60	22.40
Redox(RmV)	460.83	435.23	453.43	443.23	455.20	351.67	491.07
Conductivity (µS/cm)	729.40	675.35	743.12	1158.00	765.86	952.33	2918.41
DO (%)	127.33	88.17	102.43	91.97	75.77	101.30	88.40

107 to 108 cells/ml in FBBR A, and 108 to 109 cells/ml in FBBR B. Solid media yielded approximately an order of magnitude lower than the MPN numbers, but the trend of FBBR A having less biomass than FBBR B was still noted.

Microbial Diversity – 16S rRNA Metagenomes

Phylogenetic analysis of different components of the BP was performed using next generation sequencing in an effort to understand stability of the FBBR microbial community, as well as the unit processes surrounding the FBBR, such as feed tanks, and the membrane bioreactor. In addition, the microbial community in the plant effluent was also sequenced to determine diversity.

Three depths were sampled in both FBBR A and FBBR B, and phylogenetic analysis showed a dominance of the phyla *Proteobacteria* and *Bacteroidetes*. *Proteobacteria* accounted for ~50 to 70% of the community, depending on the sample location (Fig. 2). Dominant genera in the FBBR biofilm included *Flectobacillus* species accounted

for nearly 20% of bacteria present in FBBR B, while species in this genus were less than 10% of the community in FBBR A (Fig. 3). Percentage of *Flavobacteria* species sequences was more even when comparing the two FBBR

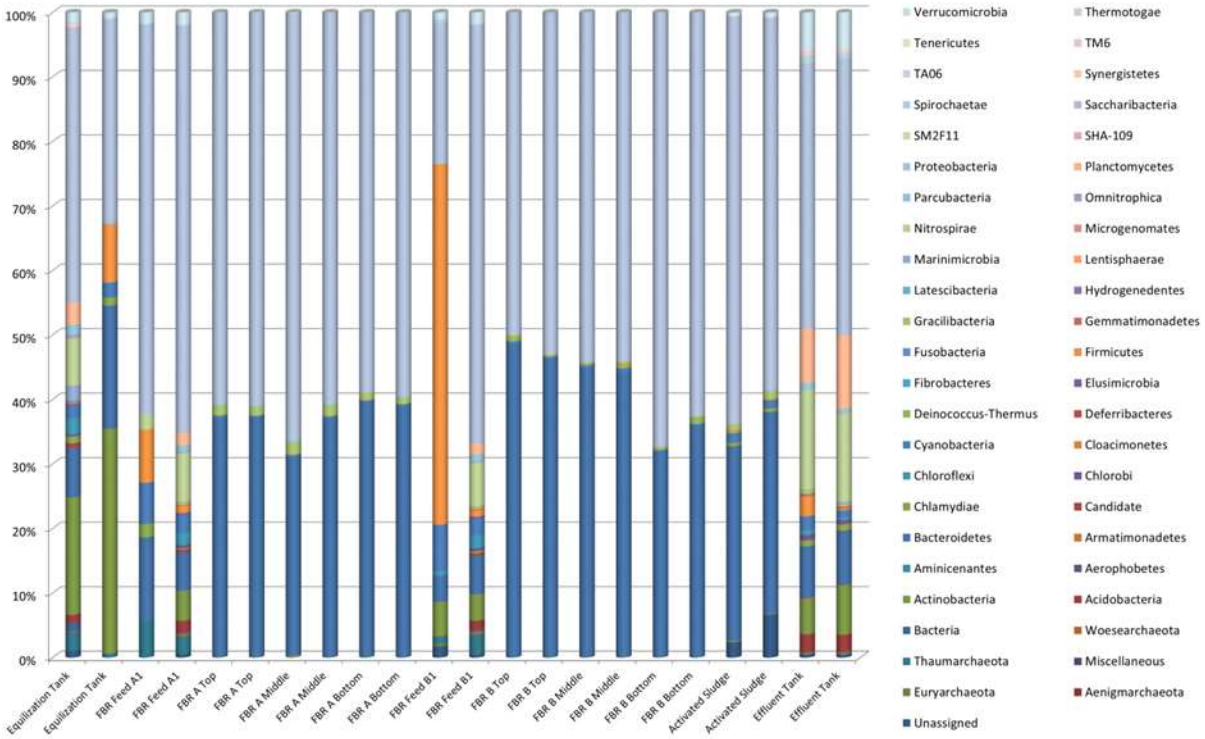

Fig. 2. Bacterial phyla present in samples taken at different locations throughout the biological plant at the 200 West Pump and Treat Facility.

microbial communities, accounting for ~20% of the bacteria present. Other dominant species in the bed include *Aromonas* and genera in the *Comomonadaceae* and *Rhodocyclaceae* Families. Bacteria in the genera *Hydrotalea*, *Rhodobacter*, *Simplicispira* and species in the *Enterobacteriaceae* Family rounded out the community.

A similar phylogenetic distribution was found when analyzing samples from the Activated Sludge return line, which is representative of bacteria that would be found in the MBBR. Approximately 35-40% of the community was represented by *Bacteroidetes* species, while 60-65% were represented by *Proteobacteria* species. In the Activated, sludge, *Flavobacterium* was the dominant *Bacteroidetes* genera, while the Families *Comamonadaceae* and *Rhodocyclaceae* were the dominant *Proteobacteria* genera. Species in the *Bdellovibrio* genus also became more

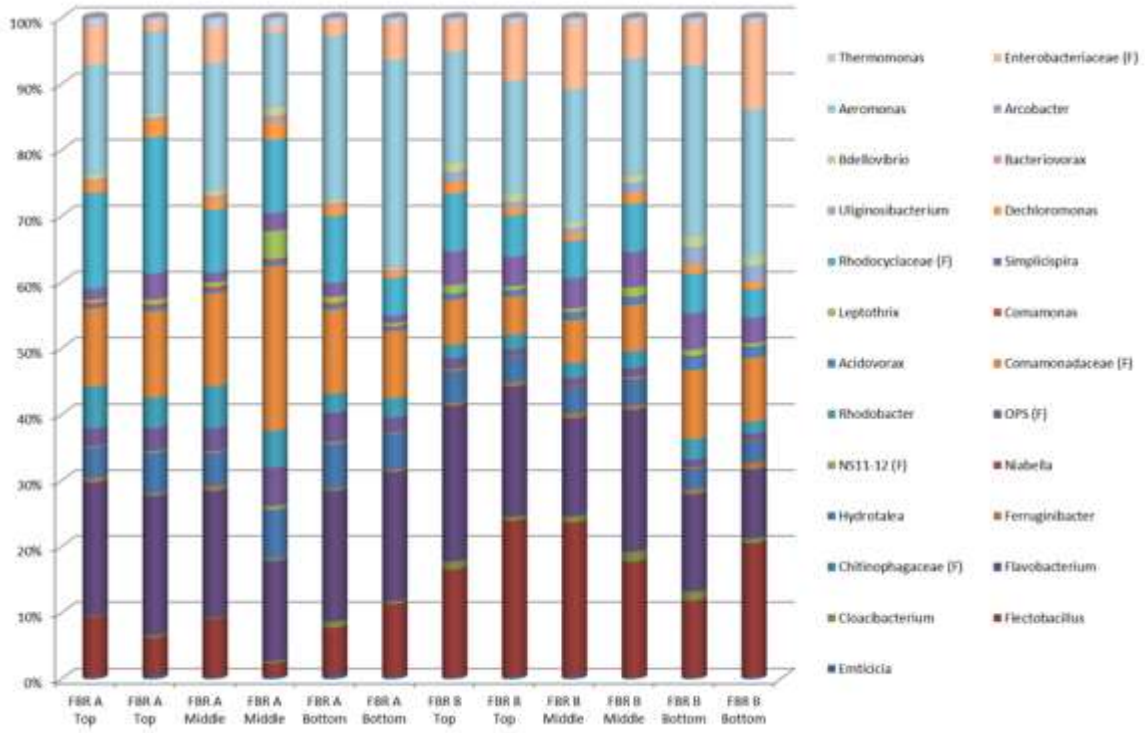

Fig. 3. Bacterial genera extracted from GAC biofilm in FBBR used for treating nitrate.

dominant in the Activated Sludge. *Flectobacillus* and *Aeromonas* species abundance dropped substantially compared to numbers found in the FBBRs.

Phylogenetic distribution in water samples from the Equilization Tank that feeds the FBBRs showed *Proteobacteria* abundances between 35 and 45%, but *Bacteroidetes* species were 15% or less. *Actinobacteria* were dominant in the water samples from the tank. In water samples from the FBR feed tanks, *Proteobacteria* accounted for approximately 70% of the phyla present. Phylogenetic distribution of the Centrate sample indicated a similar distribution of *Proteobacteria* and *Bacteroidetes* as was shown in the FBBR.

Sludge samples that were tested showed 20 to 30% *Flavobacteria*, species in the *Comamonadaceae* Family represented 5 to 17% of the community depending on the sample. As was demonstrated with the activated sludge, the number of *Aeromonas* species present dropped substantially compared to the FBBRs. The most substantial change in phylogenetic distribution occurred in the Stored Sludge. *Flavobacteria* abundance decreased to below 10% and a number of new or less abundance became more dominant.

Similarity of bacterial abundance between the FBBR samples indicates that the microbial community has stabilized with time. As with previous analyses, many of the bacterial genera found in the FBBR microbial community have been shown to contain species capable of denitrification, especially *Flavobacterium* species [9]. Interestingly, many of these species were found in acetate-utilizing aerobic granules used to treat nitrate in a municipal wastewater treatment plant [1].

Metagenome libraries showed the presence of bacterial genera capable of growth using oxygen, as well as alternate electron acceptors such as nitrate in biofilm material extracted from the FBBR support material. Similar functional groupings of bacteria have been found in wastewater and pilot-scale water treatment systems used for removing nitrate from the influent stream. These findings are relevant to the FBBR because oxygenated groundwater, near saturation (8 mg/L oxygen), is fed into the FBBR and is not completely removed in the FBBR effluent. The effluent from the FBBR typically contains between 1 and 2 mg/L oxygen. While anaerobic zones are expected in microenvironments within the sludge, microbial communities within the bed, especially at the surface of the biofilm are expected to be aerobic or facultative anaerobes. Functionally this means that residual oxygen in the groundwater being treated is removed by bacteria on the surface of the biofilm and denitrification in bacteria colonizing inner layers of the biofilm.

Functional Analysis Using Quantitative Polymerase Chain Reaction

While understanding phylogenetic diversity in the FBBR bed is important, knowing the functional capacity of bacteria in the bed will provide insight that will help optimize operation and contaminant removal. Following extraction of the biofilm from the GAC support from the different levels within the FBBR, DNA was extracted and assayed using qPCR primers that targeted specific nitrogen cycling genes or 16S rRNA primers to determine total numbers of Bacteria and Archaea. When comparing bacteria to archaea, copies of bacterial 16S rRNA genes dominated in samples taken from the FBBR bed (Fig. 4). Total numbers of bacteria were fairly stable regardless of which FBBR was sampled, or the location in the FBBR.

Quantitative PCR analysis of functional genes controlling reduction of nitrogen was also performed. Membrane bound nitrate reductase is the enzyme that catalyzes reduction of nitrate to nitrite, and is encoded by the *narG* gene. Copy numbers of this gene per gram of GAC sampled, was higher copy numbers for even bacterial 16S rRNA. These results can be explained by the fact that some bacteria can have more than one copy of some functional genes in the genome. Another nitrate reductase gene, *napA*, encodes a periplasmic nitrate reductase. This gene was present at copy number densities similar to the bacterial 16S rRNA densities. Two types of nitrite reductase genes, *nirK* and *nirS* were present at lower densities in the GAC than the nitrate reductase genes. The presence of these genes at fairly high copy number densities indicates that there was some denitrification to the extent of nitric oxide. The final gene analyzed was the nitric oxide reductase gene, *cnor*. Density of this gene within the GAC medium was even higher than the nitric oxide reductase genes, indicating that nitric oxide produced was likely converted to nitrous oxide.

Copies of all of the genes dropped substantially in the activated sludge, but were then again present in higher numbers in the concentrated and stored sludge. Low numbers of bacteria were found in water from the equalization tank and FBR feed samples. Bacterial gene copies in the effluent tank were in the range of 2.8×10^5 per ml of water. Denitrification genes were also present in the effluent tank samples indicating the potential for denitrification in these samples.

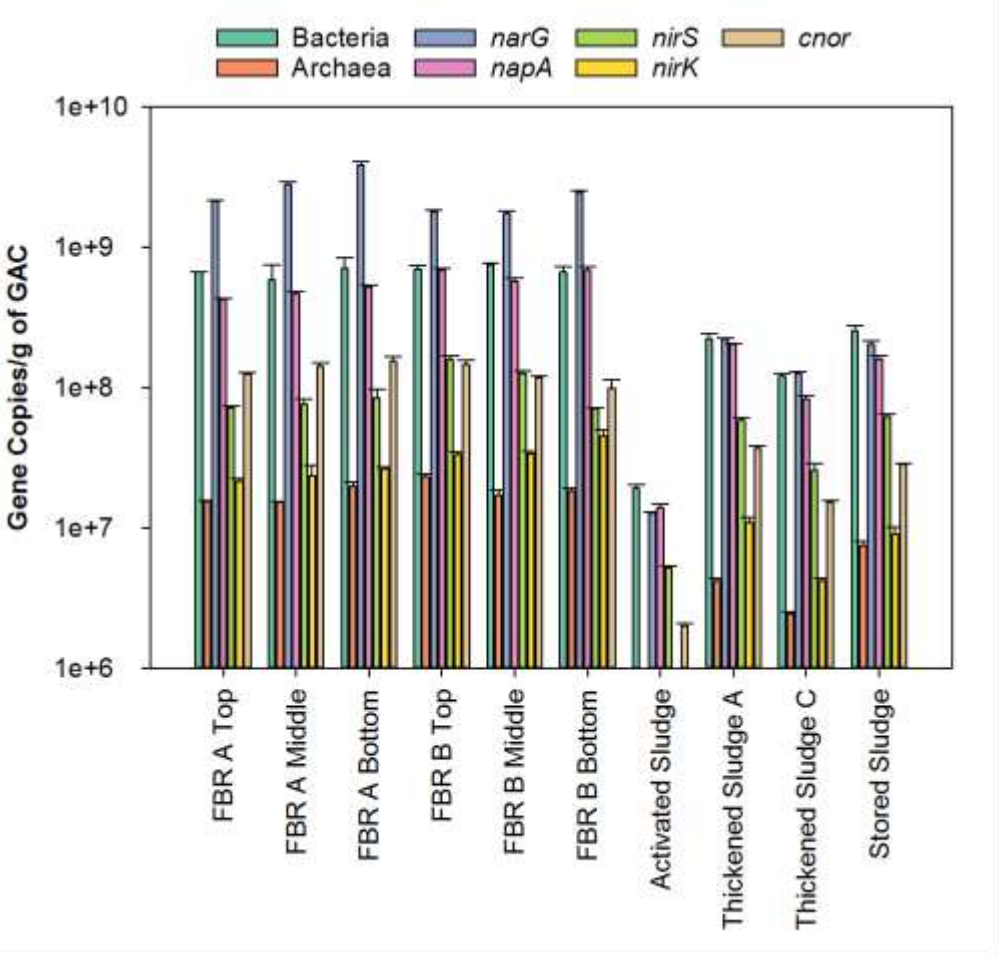

Fig. 4. Quantitative PCR results showing distribution of bacteria, archaea, and different genes associated with denitrification. Key: *narG* – membrane bound nitrate reductase; *napA* – periplasmic nitrate reductase; *nirK* – Cu-containing nitrite reductase; *nirS* – cytochrome cd1 nitrite reductase; and *cnor* – nitric oxide reductase.

Additional targets for analysis that will be performed at a later date, include genes for an additional nitric oxide reductase, nitrous oxide reductase, genes related to sulfur metabolism and 16S rRNA genes targeting iron reducing bacteria. In addition, since ammonia is high in the GAC medium, the gene responsible for dissimilatory nitrite reductase to ammonia will also be assayed. Quantitative PCR has not yet been performed on samples beyond the effluent tank. These samples will be analyzed in the near future.

CONCLUSIONS

Numerous water and sludge samples were taken within the biological plant at the 200 West Pump and Treat Facility in an effort to better understand operation and potential for optimization of parameters within the plant. Chemical and microbiological/molecular analyses were performed on the samples to determine performance at that specific time. Chemical analyses showed removal of nitrate in the bed and associated buildup of nitrite and ammonia, indicating conversion of nitrate through dissimilatory processes ending in either nitrogen gas or ammonia.

Metals analysis indicated that many metals were concentrating in the biomass attached to the GAC. Copper and iron were the two metals that were concentrated to the highest concentrations in the GAC support. Since the sludge from the BP is separated from the water, the plant continues to achieve established discharge goals for contaminants, as well as organic added to the FBBRs to drive the reductive processes. Orthophosphate was the most plentiful constituent in the GAC material and was present in g/kg quantities. Chemical analysis of the effluent tank, the injection well distribution tank and the injection well, indicated that concentrations of most metals and other measured parameters were stable across the system, including bailed water from the injection well. Following remediation of the injection well, values of many constituents in the system increased substantially, in addition to the pH of the water becoming acidic. Levels of TOC, nitrate, phosphate, sulfate and other constituents would provide chemicals for biofouling, as well as corrosion to occur in the well casing.

Microbiological and molecular analysis of the GAC material indicated that a stable community made up of primarily bacteria in the *Proteobacteria* and *Bacteriodetes* families. These families represent groups of common facultative anaerobes that can survive in environments with oxygen, as well as other alternate electron acceptors such as nitrate and oxidized metals and radionuclides. At a genus level, *Flavobacteria*, members of the *Comamonadaceae* family, and *Aeromonas* appear to be dominant in the GAC biomass material. Bacteria present in the equalization tank are at low numbers and the phyla present are somewhat different than what is in the FBBRs. Likewise, while there are bacteria in the plant effluent tank, the community present is quite different than the community in the FBBR, so carryover from the FBBR is likely not the source of bacterial in the injection wells; however, this won't be known for sure until molecular analyses are completed on the injection well and injection well distribution tank samples.

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