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Letter Report: Analysis of Injection Well Samples for Biofouling Constituents

November 2017

BD Lee
JW Morad
DL Saunders
KC Johnson

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Pacific Northwest National Laboratory
Richland, Washington 99352

Summary

The 200 West Area Pump-and-Treat (P&T) at the Hanford Site is a combined treatment train consisting of unit processes to remove uranium (U) and technetium-99 (Tc-99) and other inorganic (nitrate and hexavalent chromium) and organic (carbon tetrachloride and trichloroethene) contaminants. The ion exchange building of the P&T removes Tc-99 and U using ion exchange chromatography, while the other inorganic and organic contaminants are removed in the facility's BioPlant portion. Radioiodine (I-129) in Hanford groundwater is currently controlled using hydraulic containment. Treated groundwater is then reinjected into the subsurface using a network of injection wells.

Groundwater injectivity for most of the injection wells has diminished with time due to microbial growth and chemical precipitation in the screened interval of the wells. This biofouling, or bioclogging, requires rehabilitation to be performed on the injection wells on a regular basis to maintain adequate injectivity, so that the P&T can run at a sustained nominal capacity. A number of the injection wells have become biofouled to the extent that rehabilitation does not provide adequate recovery of injectivity, so installation of new injection wells is required to maintain adequate flow from the P&T.

Sediment samples from three new injection wells (YJ29, YJ30, YJ32) that were being installed in the 200-ZP-1 Operable Unit were analyzed for biofouling constituents, which included microbiological as well as chemical analyses. Six sediment samples (three above the water table and three below the water table), were analyzed for each well. Results from the analyses indicate that slime forming bacteria, capable of well fouling, were present in all samples analyzed. In two of the wells, the number of slime forming bacteria and the activity shown indicate the probability of aggressive slime formation, which could lead to relatively quick well fouling and loss of injection capacity. In a third well (YJ29), the risk of slime forming bacteria was low to moderate, indicating that the bacteria were present but slime formation would not be immediate.

Results from this study showed that the potential for slime forming bacteria varies depending on the location of the well in the injection well network. This finding is valuable because the results support the tendency of some wells to become fouled faster than others when they are put into service and/or not respond as favorably to well rehabilitation efforts. Once pumping of treated water has been initiated, the rate of fouling for YJ29 which showed low to moderate slime forming bacteria could be compared to fouling rates for YJ30 and YJ32 which showed high potential for slime forming bacteria. Since analyses were performed on sediments from fresh core samples, results also show that fouling is expected to start or is enhanced by the microbes present in the subsurface sediment formation and then propagates into the gravel pack and onto well screen. Comparisons between the injectivity loss within and between these wells over time would verify that biological growth is a primary issue with fouling, but more importantly that subsurface bacteria are driving fouling, not solely the 'quality' of water leaving the P&T.

Acknowledgments

This work was funded by the CH2M Hill Plateau Remediation Company as part of the 200-ZP-1 Operable Unit activities at the Hanford Site. The Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the DOE under Contract DE-AC05-76RL01830.

Acronyms and Abbreviations

BART	Biological Activity Reaction Tests
BDL	below detectable limits
bgs	below ground surface
MBR	membrane bioreactor
NO_3/NO_2-N	nitrate/nitrite nitrogen
OU	operable unit
P&T	pump-and-treat
SRB	sulfate reducing bacteria
TKN	total Kjeldahl nitrogen
TOC	total organic carbon

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1.0 Introduction

1.1 Background

In June of 2012, CH2M Hill Plateau Remediation Company completed construction of a 2500 gpm (maximum capacity) pump-and-treat (P&T) system designed to remove technetium-99 (Tc-99), uranium, carbon tetrachloride, trichloroethene, nitrate, and total and hexavalent chromium from incoming groundwater. The 200 West Area P&T, at the Hanford Site, consists of two buildings: an ion exchange building and a biological treatment building (Biological Plant, BP). Groundwater in which Tc-99 and uranium concentrations are above drinking water standards is pumped at a maximum flow rate of 600 gpm into the RAD building, where ion exchange is used to remove these contaminants. Treated groundwater is then mixed in an equalization tank with groundwater pumped from wells where radioactive constituents are below drinking water standards. Mixed groundwater is then pumped into two parallel, fluidized bed reactors containing microbes that remove nitrate, hexavalent chromium, and chlorinated solvents through reductive processes. Water from the fluidized bed reactors is then pumped through a carbon separation tank, and then into four membrane bioreactors (MBRs), where total suspended solids are removed and residual carbon is degraded under aerobic conditions. Water from the MBRs then goes to an air stripper, where remaining volatile compounds are removed, 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 31 injection wells for reintroduction into the aquifer.

Injection wells for the 200 West P&T were originally designed to receive 150 gpm per well (DOE-RL 2014). Over time, microbial communities and chemical precipitates have developed at the screened interval in the wells, causing clogging and an overall decrease in injection capacity. Injection capacity of most wells has decreased with time, requiring frequent cleaning and rehabilitation of the wells in an attempt to return the capacity to near the design requirements.

Analysis of samples from the injection wells showed the presence of microbial biofilms, as well as what was thought to be manganese oxide precipitates (Carlson et al. 2015). Molecular analysis of the injection wells indicated that the microbial community in the injection well was different than the microbial community in the effluent tank from the P&T.[1] While the 200 West P&T is meeting effluent requirements, for chemical oxygen demand (COD), nitrate, iron, and manganese, amounts reaching the injection wells are high enough to stimulate microbial activity and associated chemical reactions.

[1] Lee BD, SD Hoyle, E Cordova, MA Carlson, J Geiger, K Bral, M Niebauer, DL Saunders, SM Brooks, WL Garcia, BB Christiansen, and MH Lee. "Chemical and Microbial Sampling of the Hanford 200 West Pump-and-Treat Biological Plant and Injection Well System" (In preparation).

2.0 Objectives

The overall objective of the work performed was to test sediment samples taken from different depths during drilling of new injection wells to monitor for biofouling constituents. Sediment from three boreholes were analyzed, comparing biofouling constituents in three samples above the water table and three samples below the water table.

3.0 Methods

3.1 General

New injection wells are currently being drilled for the 200 West P&T. Split-spoon soil samples are being collected as drilling progresses and will be analyzed for chemical and microbiological signatures associated with biofouling/bioclogging. Samples will be taken from three separate wells in the vadose zone (80, 140, and 200 ft bgs) and from the saturated zone (240, 340, and 410 ft bgs). To ensure expedited analytical results, sample analysis is being performed in house using Hach kits for total iron, manganese, phosphorus, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), total heterotrophic bacteria plate counts, and Biological Activity Reaction Tests (BART) for slime forming, iron-related bacteria (iron reducing and iron oxidizing bacteria) or sulfate reducing bacteria (SRB).

TKN is a measure of organic nitrogen and ammonia in a system. Slime forming bacteria are bacteria that will produce an extracellular polymeric substance that allows biofilm formation as well as uptake and precipitation of minerals within the biofilm. Iron-related bacteria are defined as bacteria that are involved in redox cycling of iron in water systems. Bacteria that can oxidize iron and produce iron precipitates are of interest, as are bacteria that reduce iron, producing ferrous iron that can be precipitated with sulfide in the environment. SRB are bacteria that reduce sulfate to sulfide, producing metals sulfides.

3.2 Sample Processing

Samples from all three wells have been processed, as well as one well from the chromium plume in the 200-UP-1 Operable Unit (OU) that was used as a negative control for the BART test mentioned above. Sample information is shown in Table 1.

Sample (B3B124, C9635, 354.05 bgs) from near the chromium plume in the 200-UP-1 OU was processed as a negative control for the injection well samples since it lies outside the influence of the current injection well network. Upon sample receipt, cores were logged and the tops and bottoms of each core were photographed. Using aseptic technique, subsamples of the cores were then taken and refrigerated for analysis. Chemical and microbiological analyses were performed on the supernatant created after extracting potential biofilm from the sediment by using 0.9% sodium chloride at a sediment- solution ratio of 1:10. Supernatant was used directly for all analyses, with the exception of the total heterotrophic plate counts, where the supernatant was serially diluted, and a range of dilutions spread on nutrient agar for growth.

Table 1. Injection well samples analyzed.

Injection Well Designation	Borehole	Well ID	Sample Number	Depth (ft bgs)
YJ32	C9563	299-W18-42	B398T1	77.85
			B398T2	140
			B398T3	197.63
			B398T4	237.23
			B398T5	340.2
			B398T6	340.7
			B398T7	407.99
YJ29	C9561	299-W6-16	B398H3	78.09
			B398H4	132.6
			B398H5	196.72
			B398H6	235.6
			B398H8	335.15
			B398H7	335.15
			B398H9	405.78
YJ30	C9565	299-W18-44	B39927	78.79
			B39928	138.6
			B39929	198.02
			B39930	237.66
			B39931	338.13
			B39933	405.5
			B39932	406
	C9635	699-30-70	B3B124	354.05

Specific analyses performed on each of the samples are shown in Table 2.

Table 2. Analytical methods used to analyze ZP-1 injection well samples.

Analysis	Method
Total Heterotrophic Plate Count	APHA Method 9215A
BART	Hach BART Test Combo Package
TKN	Hach s-TKN Method (10242)
Orthophosphate	Hach USEPA PhosVer3 Method (8048)
Iron, Total	HACH USEPA FerroVer Method (8008)
Manganese	Hach Method 8149
Total Organic Carbon	APHA Method 5310B

4.0 Test Results

4.1 Injection Well YJ32

Analytical results for injection well YJ32 are shown in Table 3. In addition to TKN, the table also shows total nitrogen as well as nitrate/nitrate nitrogen (NO_3/NO_2-N).

Table 3. Analytical results from sediment samples from injection well YJ32 (C9563, 299-W18-42).

Sample	Depth (ft bgs)	Iron (mg/L)	Phosphorus (mg/L)	Total N (mg/L)	NO_3/NO_2-N (mg/L)	TKN (mg/L)	TOC (mg/L)	Manganese (mg/L)
B398T1	77.85	0	0.4	5.65	0.228	5.42	30.12	0.12
B398T2	140	0.9	6.9	1.7	1.39	0.31	12.69	0.58
B398T3	197.63	BDL	1.4	0.623	0.213	0.41	11.42	0.48
B398T4	237.23	0.3	1.1	0.376	0.419	0.043	13.68	1.93
B398T5	340.2	0.7	0.1	5.93	0.159	5.77	10.38	0.71
B398T6	340.7	0.5	0.2	3.26	0.176	3.08	9.33	0.96
B398T7	407.99	0.9	0.3	2.3	2.12	0.18	8.33	0.65

BDL – Below detection limits

Total iron was highest in cores from 140 and 407.99 ft bgs, and was below 1 mg/L in all core material analyzed. Manganese was highest in the sample taken from 237.23 ft bgs, and was more than triple the manganese concentrations found in the other cores. Phosphorus was highest in the sample from 140 ft bgs (6.9 mg/L), while phosphorus in all other samples was below 1.5 mg/L. The sample from 140 ft bgs was very tightly packed and dry. TKN was the highest in the sample from 77.85 ft bgs at a high of 5.42 mg/L, which indicates elevated organic nitrogen and ammonia in the samples. TKN is low in the other three samples, while nitrogen attributed to NO_3/NO_2-N was elevated in the samples taken from 140 ft bgs and 407.99 ft bgs. TKN also accounted for most of the nitrogen in samples analyzed from the 340.7 ft bgs interval. TOC was the highest in the sample taken from 77.85 ft bgs (30.12 mg/L), while concentrations in the other samples averaged approximately 10 mg/L.

Sediment samples from YJ32 were tested for the presence of slime forming, iron-reducing, and sulfate reducing bacteria. In general, the BART test can be considered to be qualitative to semi-quantitative depending on how quickly the bacteria in the sample grow. For most tests, when bacterial growth is noted with associated visual indicators within the first 3 days of incubation, a grade of "aggressive" is given to the growth pattern of the bacteria in the sample. Aggressive slime forming bacteria means that there are 6.7×10^4 to 1.75×10^6 colony forming units per milliliter in the sample. These bacterial densities are in line with values seen in previous analyses of Hanford groundwater and sediment. With the exception of the sample from 140 ft bgs, all samples were graded as "aggressive" related to slime forming bacteria, and moderate to aggressive for iron-related bacteria. Little or no growth was noted in the SRB incubations, except in the sample from 237.23 ft bgs. There was no growth noted in the BART tests for the sample from 140 ft bgs, which is expected when samples are fairly dry at the time they are collected.

Total heterotrophic bacteria plate counts support the data generated from the BART experiments. Samples from the 77.85 ft bgs interval showed higher bacterial numbers than samples from 197.63 and

237.23 ft bgs. Lower total counts were also noted for the samples from 407.99 ft bgs. As seen with the BART analyses, no growth was noted in the sample from 140 ft bgs.

4.2 Injection Well YJ29

Analytical results from injection well YJ29 are shown in Table 4. Total iron in samples from this injection well ranged from 2.1 to 13.9 mg/L. The highest iron values were found in samples from 132.6 ft bgs in the vadose zone and from 335.15 ft bgs in the saturated zone. Phosphorus concentrations were higher in the samples from below the water table, with the exception of the deepest sample, which was below 1 mg/L. Total nitrogen was highest in samples from the vadose zone, and the majority of the nitrogen was present as TKN. Elevated TKN is important because this indicates the presence of organic nitrogen and ammonia, which is evidence of conversion of nitrate, which is expected in the samples, to other nitrogen species, which are less likely to occur in the subsurface. The initial sample from below the water table also showed elevated total nitrogen, but the speciation was evenly distributed between NO_3/NO_2-N and TKN. Samples from 335.15 and 405.78 ft bgs showed primarily NO_3/NO_2-N. TOC was elevated in all of the samples tested. Manganese concentrations were highest in the sample from 405.78 ft bgs.

Table 4. Analytical results from sediment samples from injection well YJ29 (C9561, 299-W6-16).

Sample	Depth (ft bgs)	Iron (mg/L)	Phosphorus (mg/L)	Total N (mg/L)	NO_3/NO_2-N (mg/L)	TKN (mg/L)	TOC (mg/L)	Manganese (mg/L)
B398H3	78.09	4	0.1	4.57	0.802	3.77	21.53	0.71
B398H4	132.6	13.9	0	9.76	0.25	9.51	11.35	0.64
B398H5	196.72	3	0.4	6.98	1.23	5.74	11.82	1.18
B398H6	235.6	2.1	4.8	4.07	1.17	2.9	15.15	1.07
B398H8	335.15	13.8	8	1.68	1.08	0.6	9	1.07
B398H9	405.78	2.7	0.8	2.39	2.42	-0.04	10.41	4.88

BART analysis of samples from YJ29 showed that slime forming bacterial activity did not appear until after 5 days of incubation, indicating low numbers and moderate activity for these types of bacteria in this well. Activity of iron bacteria and sulfur reducing bacteria were also moderate in all of the samples.

4.3 Injection Well YJ30

Results from analysis of samples from injection well YJ30 are shown in Table 5. Total iron in the three vadose zone samples and the shallowest aquifer sample was above 4 mg/L, while the samples from 338.13 and 405.5 ft bgs showed total iron concentrations below 0.5 mg/L. Phosphorus concentrations in the vadose zone samples from 78.79 and 138.6 ft bgs were an order of magnitude higher than the concentrations in the deepest vadose zone sample and the three samples below the water table. Total nitrogen in the samples was between 1.37 and 5.28 mg/L, with the highest concentration seen in the sample from 138.6 ft bgs. Nearly all of the nitrogen in samples from YJ30 was in the form of NO_3/NO_2-N. These results indicate that nitrate typically found in Hanford groundwater has not been converted to organic nitrogen compounds or ammonia. TOC values were also elevated in the samples, ranging from 10.4 to 17.33 mg/L. Manganese was highest in the vadose zone sample taken from 198.02 ft bgs, compared to other vadose zone and saturated zone samples.

Table 5. Analytical results from sediment samples from injection well YJ30 (C9565, 299-W18-44).

Sample	Depth (ft bgs)	Iron (mg/L)	Phosphorus (mg/L)	Total N (mg/L)	NO_3/NO_2-N (mg/L)	TKN (mg/L)	TOC (mg/L)	Manganese (mg/L)
B39927	78.79	15.1	4.6	1.57	1.48	0.09	11.38	1.51
B39928	138.6	34.8	8.4	5.28	5.25	0.02	11.96	0.48
B39929	198.02	4.3	0.6	1.76	1.79	-0.02	17.33	3
B39930	237.66	13.9	0.4	1.37	1.28	0.08	15.88	0.84
B39931	338.13	0.3	0.9	3.65	3.6	0.06	16.92	1.33
B39933	405.5	0.4	1.8	2.24	1.97	0.27	10.4	1.37

BART analysis of sediment samples from YJ30 showed bacterial growth after 3 days of incubation, which is considered aggressive. The sample from 198.02 ft bgs was the only sample that showed slime forming activity associated with the growth. Exceptions were the samples from 78.79 ft bgs and 405.5 ft bgs, in which slime forming bacteria activity was moderate or low, and the sample from 138.6 ft bgs, which showed growth after 1 day of incubation.

4.4 Negative Control Well

Since the BART tests have not been previously used to track microbial communities and associated activities on Hanford sediments, a split-spoon sample from borehole C9635 (699-30-70, 354.05 ft bgs) was processed for analysis. This location was selected because it is in the chromium plume in the 200-UP-1 OU and should be well outside the influence of existing injection wells for the P&T. Total iron in the sample was 6.7 mg/L, while phosphorus was below 1 mg/L. Total nitrogen was 3.41 mg/L and was present primarily as NO_3N/NO_2-N. TOC level in the sample was 10.9 mg/L, which was similar to other samples tested. Manganese in the samples was 3.43 mg/L, which is high compared to most of the samples tested.

BART analysis of the microbial community extracted from this sample showed aggressive growth and activity associated with slime forming bacteria. Iron bacteria were also ranked as aggressive in the samples.

5.0 Discussion

Parameters tested that seem to vary the most among the different samples and the control from the 200-UP-1 OU are the BART results for slime forming bacteria and the distribution of nitrogen species in the samples. In general, BART analyses showed the presence and activity of slime forming bacteria in wells YJ30 and YJ32. These results indicated aggressive activity by these bacterial types. Injection well YJ-29, on the other hand, did not show the presence or activity of slime forming bacteria, and results indicated moderate to low activity in the samples tested. The presence and activity of slime forming bacteria was also shown for the 200-UP-1 sample chosen as a potential negative control. In general, these results would indicate that there are slime forming bacteria present in all of the Hanford sediments tested using the BART analyses, but that slime formation may occur sooner in wells showing aggressive slime forming bacteria compared to wells with low to moderate slime forming bacterial activity. A schematic of the extraction/injection well network for the 200 West P&T is shown in Figure 1.

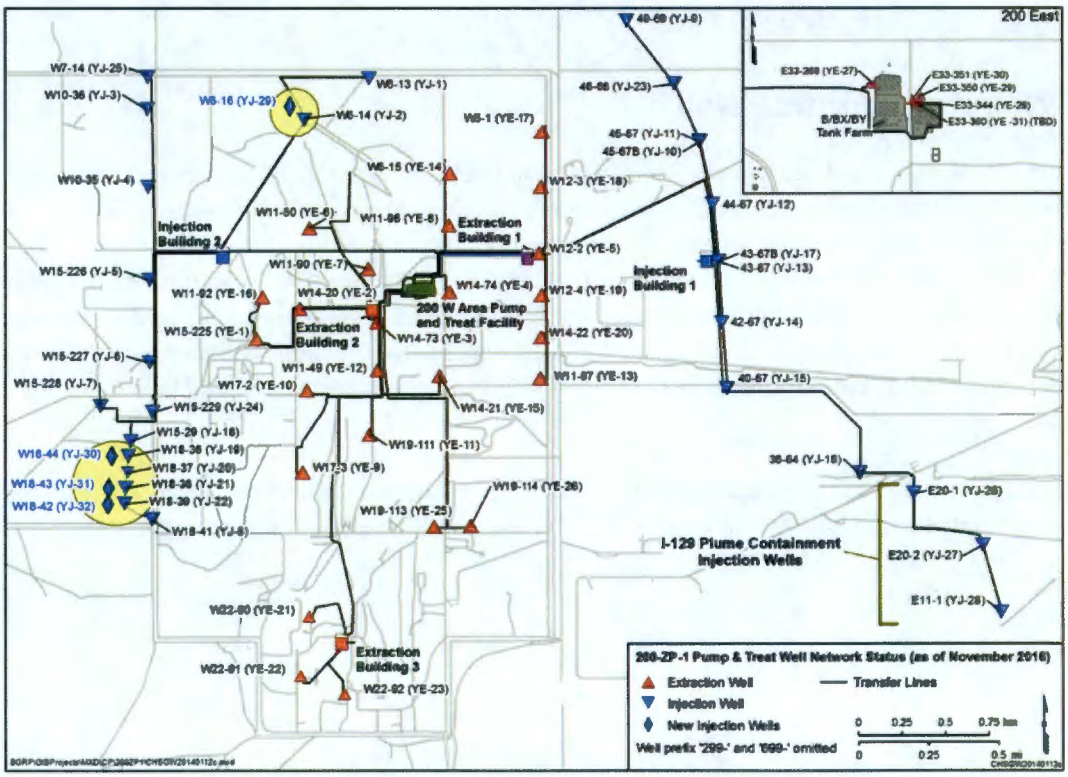

Figure 1. Extraction and injection well network associated with the 200 West Area P&T at Hanford.

When considering the wells selected for analysis, YJ30 and YJ32 are located in an area that has a high density of existing injection wells, compared to YJ29, which is somewhat isolated from other existing injection wells. Injection well performance data for the wells YJ18, YJ19, YJ20, YJ21, and YJ22 show substantial degradation of capacity, or wells have been taken out of service. In contrast, YJ2, which is a well associated with YJ-29, shows less degradation of well capacity.

From the data generated, it is not known whether the slime forming bacteria are present in the formation and can be stimulated when challenged with appropriate nutrients, or whether the microbial community in

the aquifer has already been stimulated by nutrients added through injection of treated water from the P&T. Regardless, the data do show that even if these areas have not been impacted by injected water from the P&T, when adequate nutrients are provided, slime forming bacteria that could cause clogging are able to grow. This hypothesis supports previous analysis of injection well microbial communities showing that the P&T was not adding bacteria to the subsurface, but instead was providing bacteria in the groundwater with nutrients to grow.

Nitrogen species also differed in samples from the different wells. In well YJ29, TKN was elevated in numerous samples compared to NO_3/NO_2-N, indicating the presence of organic nitrate and ammonia. Samples from injection well YJ32 varied in species depending on the sample. In contrast, NO_3/NO_2-N accounted for most of the nitrogen found in samples from YJ30. While nitrogen species were different among samples from the different wells, no direct correlation to bacterial growth could be made.

6.0 Conclusions

Results from the analyses performed indicate that slime forming bacteria, capable of well fouling, were present in the samples analyzed. In two of the wells, the number of slime forming bacteria and the activity shown indicate the probability of aggressive slime formation, which could lead to relatively quick well fouling and loss of injection capacity. In a third well (YJ29), the risk of slime forming bacteria was low to moderate, indicating that the bacteria were present but slime formation would not be immediate.

The study was valuable in showing that the potential for slime forming bacteria varies depending on the location of the well in the injection well network. Results support the tendency of some wells to become fouled faster than others when they are put into service and/or not respond as favorably to well rehabilitation efforts. Once pumping of treated water has been initiated, the rate of fouling for YJ29 which showed low to moderate slime forming bacteria could be compared to fouling rates for YJ30 and YJ32 which showed high potential for slime forming bacteria. Since analyses were performed on fresh core samples, results also show that fouling likely starts or is enhanced by the microbes present in the sediment formation and then grows into gravel pack and onto well screen. Comparisons between the injectivity loss within and between these wells over time would verify that biological growth is a primary issue with fouling, but more importantly that subsurface bacteria are driving fouling, not solely the 'quality' of water leaving the facility.

These results also support other analyses performed that showed that the fouling was caused by macronutrients (i.e., carbon, nitrogen and phosphorus) coming from the P&T that were stimulating the bacteria in the formation and the wells. The higher the concentration of nutrients added, the more growth that will be demonstrated. Results indicate that TOC, nitrate/ammonia and phosphate coming from the P&T should be further decreased in an effort to slow biofouling. Likewise, adding antimicrobial compounds or disinfectants as part of the overall treatment or injection system may also help mitigate or reduce fouling. Depending on the capacity for growth of slime forming bacteria, the amount of antimicrobial could be varied.

While chemical analyses, especially of nitrogen species, showed differences between the wells and samples, no correlation to well fouling could be made using the data.

Of the analyses performed during this study, BART analysis of slime forming bacteria appeared to be the most useful. BART tests for slime forming bacteria are straight-forward and fairly easy to complete and could be applied to sediment samples when wells are drilled. Doing these analyses simultaneously as the well characterization would allow for the development of a graded approach for up front mitigation strategies, prior to placing the well in use and/or fouling issues arise. BART could provide a relative estimate of the level of slime forming bacteria; these data could be used to determine if mitigation is necessary OR if the well field will produce without major incidence of fouling. For example, if the mitigation strategy was determined to be chemical addition to oxidize microbes present, then BART analyses during characterization would inform the amount of oxidant that needed to be added for mitigation (e.g. higher the number of slime forming bacteria determined using BART would require a higher amount of oxidant). This approach does not require extensive sampling and analyses; testing of one or two samples in the interval where the well screen is installed should be sufficient to determine the amount of slime forming bacteria in the local formation. The BART analyses are common for wastewater treatment and could be easily run in the lab on site.

7.0 References

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