

# Analysis of Microbial Communities as Indicators of Microbially Induced Corrosion Potential in Stainless Steel Piping

September 2021

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Danielle Saunders

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Prepared for  
the U.S. Department of Energy  
under Contract DE-AC05-76RL01830

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## Summary

The 200 West Area Pump-and-Treat (200W P&T) facility is part of the final remedy decision for the 200-ZP-1 Operable Unit (OU) and is the interim remedial action for 200-UP-1 OU at the Hanford Site. The facility also treats water from other sources across the site, including 200-DV-1 OU perched water, groundwater from the 200-BP-5 OU, and leachate from the Environmental Restoration Disposal Facility. The facility originally operated a biological treatment process for nitrate that resulted in systemic biofouling and loss of injection well capacity, requiring frequent and costly well rehabilitation. Biofouling has also contributed to corrosion of welds and pitting of stainless-steel piping at the facility. The biological treatment for nitrate was suspended at the end of 2019 calendar year to eliminate negative impacts from biological treatments which improve facility operations and treatment of carbon tetrachloride.

Evaluation of the 200W P&T facility's response to operational improvements, specifically the suspension of the biological treatment, provides information on the potential for continued microbially induced corrosion (MIC). To this end, historical data sets (2015-2017) were compared with molecular biological analyses of 200W P&T water samples from 2018-2020 to quantify trends in total microbial biomass and specific microbial indicator species (iron, sulfate, nitrate, and manganese reducing bacteria) known to cause and enhance metal corrosion. Results showed unequivocal reductions (in excess of 90%) in all microbiological indicators measured following the suspension of the biological treatment.

Although abundance measurements for all corrosive MIC populations initially declined after removal of the treatment system, nitrate-reducing bacteria (*nirK*) and sulfate-reducing bacteria remain highly abundant in the system, an order of magnitude higher than the number of cells present prior to the removal of the biological treatment system [ $5.14 \times 10^8$  (1.4) cells/L, vs.  $1.14 \times 10^7$  (0.07) cells/L, reported as average ( $\pm$ SD) for  $n=3$ ]. In addition, there was an unexpected inflection point in 2019 where all microbial indicators increased unexpectedly in abundance in 2020 by as much as two orders of magnitude compared to 2019 samples.

Given the limited number of water samples available for this analysis, the cause of the increase in microbial indicators post-biological treatment has not yet been determined. Systematic sampling of the facility for continued monitoring is recommended so that the responses of MIC populations can be evaluated to ensure that further corrosion is avoided, allowing the 200W P&T facility to operate safely and efficiently at design capacity.

## **Acknowledgments**

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## Acronyms and Abbreviations

ITB	Interim Transfer Building
MIC	microbially induced corrosion
NQAP	Nuclear Quality Assurance Program
OU	operable unit
P&T	pump-and-treat
QPCR	quantitative polymerase chain reaction

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## 1.0 Introduction

Contaminated groundwater in the 200 Area of the Hanford Site is being treated ex situ by the 200 West Pump-and-Treat (200W P&T) facility. The 200W P&T facility removes several contaminants of concern from groundwater, including radionuclides and volatile organics, though biological treatment of nitrate was discontinued in 2020 as part of the 200W P&T Optimization Plan (DOE/RL-2019-038, Rev. 0). The biological treatment process was responsible for several operational issues stemming from pervasive biofouling, including biofouling of injection wells (Figure 1.1A), microbially induced corrosion (MIC) of the stainless steel piping weldments (Figure 1.1B), and electrochemical corrosion by Mn-oxide particles coating the inside stainless steel piping surfaces (Figure 1.1C).

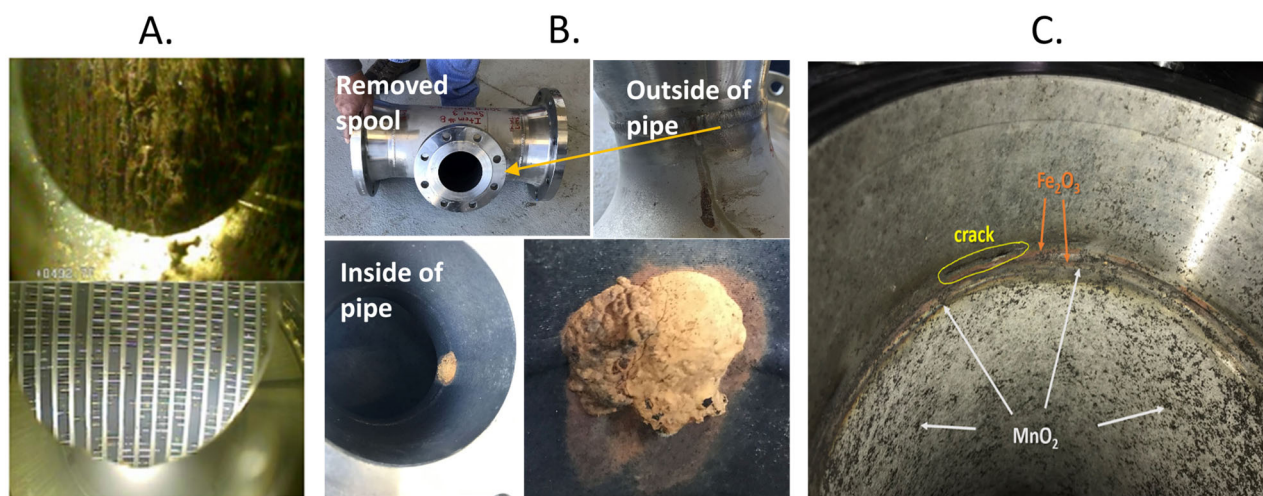


Figure 1.1. Various types of fouling observed at 200W P&T: (A) injection well screen fouled and cleaned in 2013 (SGW-61398, Rev. 0); (B) photographs of removed spool attacked by MIC with unidentified accretion (PNNL-30421); (C) removed spool showing internal crack with deposited Mn(III/IV)-oxides/hydroxides on internal surfaces.

During the biological treatment of nitrate at the 200W P&T facility, manganese [Mn(II)], along with eight additional trace metal nutrients, was consistently added to the fluidized bed reactors to help maintain a high rate of biological activity (denitrification). Excess manganese and other trace metals would exit the fluidized bed reactors as water was exchanged between treatment cycles and proceed through the system. Many aerobic microorganisms readily oxidize dissolved Mn(II) to form highly insoluble Mn(III/IV)-oxide and Mn(III/IV)-hydroxide particles (Ghiorse 1984). Reduced, inorganic manganese, Mn(II), will also rapidly be oxidized (abiotically) in well-aerated groundwater to produce these characteristically black particles. Evidence for Mn(III/IV)-oxide and Mn(III/IV)-hydroxide particle formation and accumulation has been well documented, from water samples collected at the 200W P&T (Figure 1.2) and visual inspection of replaced piping (Figure 1.1C). Biological and electrochemical deposition of Mn(III/IV)-oxide / hydroxide particles onto metal surfaces leads to ennoblement and the initiation of corrosion. Direct physical contact allows biotic and abiotic pathways to cause pitting and crevice corrosion on stainless steel surfaces.

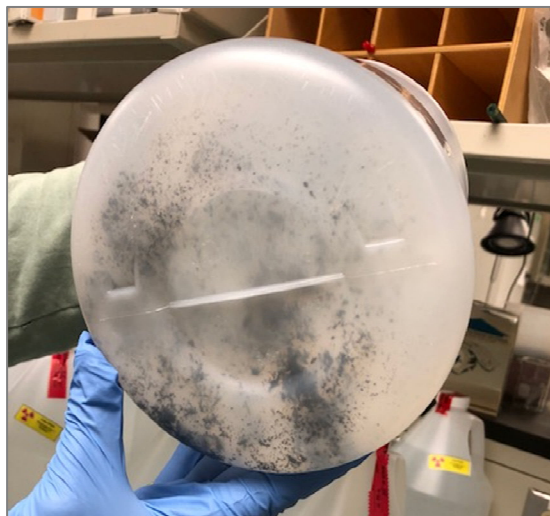


Figure 1.2. Black Mn-oxide / hydroxide particulates in a 200W P&T water sample.

Elimination of the biological treatment of nitrate has permitted the facility to operate at design capacity of 9500 L/min (3750 gal/min) given that biofouling has been significantly reduced and injection wells no longer require routine rehabilitation. Moreover, hypochlorite is being used to control microbial growth and aid in the oxidative removal of residual buildup on the inside of pipe surfaces. While these improvements have had a significant impact, the 200W P&T facility has not fully stabilized following these optimization actions, and residual microbial contamination and Mn(III/IV)-oxide / hydroxide coatings persist in the system. Consequently, the potential for corrosion (biotic and abiotic) or conditions that could exacerbate existing corrosion within the system remains a significant concern.

As part of a system-wide monitoring plan, a baseline assessment of MIC was recommended to quantify water quality with specific and routine monitoring of MIC potential within the facility. Specific bacterial groups that have been shown to contribute to the corrosion of stainless steel surfaces are categorically defined as bacteria capable of respiring  $\text{NO}_3$ ,  $\text{SO}_4$ , Fe(III), Mn(IV), and biofilm-forming and acid-producing bacteria (Lekbach et al. 2021). Each of these biological pathways is a known cause of ennoblement in stainless steel, a phenomenon that increases corrosion potential, specifically pitting corrosion (Linhardt 2004; Little et al. 1991).

The objective of this task was to demonstrate the value of MIC testing for corrosion monitoring by first establishing a temporal record and current baseline for total microbial biomass and MIC populations ( $\text{NO}_3$ ,  $\text{SO}_4$ , Fe(III), Mn(IV), reducing bacteria) that could be contributing to MIC in the 200W P&T facility. Historical data sets from 2015-2017 were analyzed for MIC, capturing a window of time when the biological treatment process was active. Additionally, stored groundwater from 2018-2020 at the 200W P&T facility was processed and analyzed for MIC by real-time quantitative polymerase chain reaction (QPCR).

This document reports on the results of these analyses, describing the methods in Section 2.0, followed by a discussion of the results in Section 3.0. Section 4.0 summarizes the preliminary conclusions of these analyses, followed by a technically defensive recommendation for continued monitoring of the system in Section 5.0. Finally, a description of the quality program under which this work was performed is provided in Section 6.0.

## 2.0 Approach

### Quantitative Microbial Analysis of 200W P&T Water

Table 2.1 summarizes the existing source data from 2015-2017 and descriptions of archived 200W P&T water samples spanning 2018-2020 used in this study. A corresponding facility map shows the sampled locations (Figure 2.1).

Table 2.1. Summary of source data and water samples.

Map ID	Year	Sampled Location	Description
1	2015-2017	Equalization tank	Previously collected data by Lee et al. 2017
2	2015-2017	Effluent tank	Previously collected data by Lee et al. 2017
3	2018	ITB 1, ITB 2	Non-chlorinated, available water from both transfer buildings combined for analysis
4	2019	ITB 2	Chlorinated
5	2019	Influent tank	Non-chlorinated
6	2020	ITB 1	Chlorinated

ITB = Interim Transfer Building.

The chlorinated water designation refers to the application of hypochlorite in the transfer buildings at the 200W P&T facility.

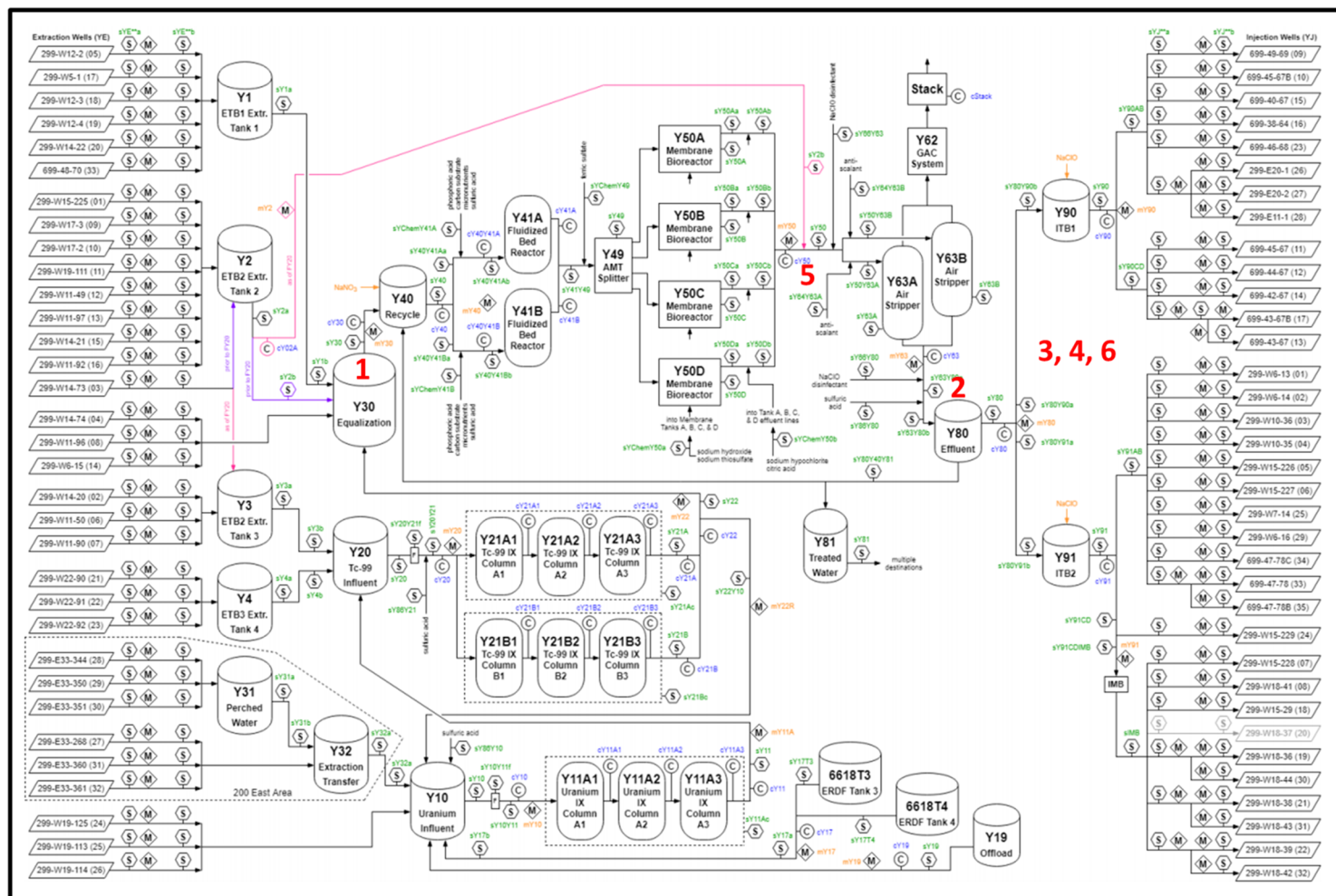


Figure 2.1. Schematic of 200W P&T facility showing locations of water collection corresponding to Table 2.1.

200W P&T facility water samples from 2018-2020 were held in cold storage (4°C) prior to analysis. Water (8L/location) was filtered (0.2-µm pore size) to recover microorganisms and genomic DNA extracted using the DNeasy PowerWater Sterivex Kit following chemical and ballistic disruption of filtered microbial cells. Purified genomic DNA was quantified using a NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE).

MIC targeted microbial populations (and genetic markers) for QPCR included Fe(III)/Mn(IV)-reducing bacteria (16S rRNA, *Geobacter spp*; Cummings et al. 2003), sulfate-reducing bacteria (*dsrA*; Spence et al. 2008), and denitrifying bacteria (*nirS/nirK*; Braker et al. 1998). Additionally, total bacteria (16S rRNA) were enumerated using established primers targeting conserved regions of the ribosomal RNA operon (Bagwell et al. 2019). QPCR assays were performed in triplicate on a Bio-Rad CFX96 Real-Time PCR Detection System using the SsoAdvanced™ Universal SYBR® Green Supermix. Thermal cycling conditions consisted of an initial denaturation step at 98°C for 3 min, followed by 35 cycles of denaturation (98°C for 20 sec), primer annealing (48°C for 15 sec), and extension (60°C for 20 sec). Fluorescence measurements were collected at the end of each cycle. Amplification specificity was assessed by melt curve analysis. Cell equivalents were calculated from calibration curves using commercially purchased DNA preparations (ATCC) from reference strains.



## 3.0 Results and Discussion

### 3.1 Sample Preparation

The historical data and 200W P&T facility water samples analyzed provide a snapshot of temporal patterns in microbial community and MIC populations responding to operational improvements at the 200W P&T facility – specifically, bypass of the biological nitrate treatment and disinfection of the transfer buildings (ITB 1 and 2) with hypochlorite. Comparison of water samples taken from the ITB buildings during hypochlorite treatment permits evaluation of disinfection efficacy. This study was supported by on-hand water samples and describes a limited data set. Single-point sampling does not provide an accurate characterization of water quality throughout the facility for any given year. A more systematic sampling approach is recommended for improved reliability and sensitivity (Section 5.0).

A 0.2- $\mu\text{m}$  pore size filter was used to concentrate microorganisms from 200W P&T facility water samples. A representative image of particulates accumulated from 4 L of filtered 200W P&T water is shown in Figure 3.1. All water samples analyzed had varying amounts of black particulates [Figure 1.2; previously identified as Mn(III/IV)-oxides and Mn(III/IV)-hydroxides], as well as aggregated material like the gray mass shown in Figure 2.1. The source and fate of this gray material is unknown; no characterization was performed under this investigation.



Figure 3.1. Filtrate from 200W P&T. Filter pore size is 0.2  $\mu\text{m}$ .

## 3.2 QPCR Results

Compiled QPCR results for total bacterial biomass and corrosive MIC populations are provided in Table 3.1. Total microbial and MIC population abundance measurements are reported (as averages  $\pm$  SD,  $n = 3$ ) in units of gene copies/L, which can be interpreted as cell equivalents (biomass)/L. Results are described in detail in the following sections.

Table 3.1. Summary of QPCR results of MIC indicators from 200W P&T effluent.

Sample Type	Total Bacterial Biomass (16S rRNA copies/L)	SO <sub>4</sub> Reducing Bacteria ( <i>dsrAB</i> copies/L)	Fe Reducing Bacteria (16S rRNA copies/L)	NO <sub>3</sub> Reducing Bacteria ( <i>nirS</i> copies/L)	NO <sub>3</sub> Reducing Bacteria ( <i>nirK</i> copies/L)
Equalization tank 2015-2017 (Lee et al. 2017)	2.82e <sup>8</sup> (0.7)	-	8.25e <sup>7</sup> (0.5)	2.1e <sup>7</sup> (0.3)	1.88e <sup>6</sup> (0.9)
Effluent tank 2015-2017 (Lee et al. 2017)	2.32e <sup>10</sup> (0.5)	-	4.78e <sup>9</sup> (0.2)	2.22e <sup>9</sup> (1.1)	1.88e <sup>8</sup> (0.2)
ITB1 & 2 mixed sample, non-chlorinated, 2018	2.93e <sup>8</sup> (0.5)	1.42e <sup>8</sup> (0.4)	3.13e <sup>4</sup> (0.03)	1.58e <sup>7</sup> (0.5)	2.3e <sup>10</sup> (2.36)
ITB2 chlorinated, 2019	3.1e <sup>6</sup> (0.8)	7.38e <sup>5</sup> (2.5)	1.47e <sup>2</sup> (0.09)	2.56e <sup>5</sup> (1.2)	1.22e <sup>7</sup> (0.1)
Influent tank non-chlorinated, 2019	5.32e <sup>5</sup> (0.8)	1.9e <sup>4</sup> (0.1)	1.39 (0.6)	7.84e <sup>4</sup> (0.0)	5.28e <sup>5</sup> (6.8)
ITB1 chlorinated, 2020	2.38e <sup>7</sup> (0.4)	1.14e <sup>7</sup> (0.07)	4.46e <sup>3</sup> (0.1)	2.36e <sup>6</sup> (0.1)	5.14e <sup>8</sup> (1.4)

Replicated QPCR assays are reported as averaged abundances ( $\pm$  standard deviation). ‘-’ for sulfate-reducing bacteria indicates no prior data was available for these specific corrosive microorganisms in 2015-2017.

### 3.2.1 Total Bacterial Biomass

Quantification of total bacterial biomass targeted the 16S rRNA gene, a universal genetic marker for bacterial detection and enumeration. The results are plotted in Figure 3.2; averaged ( $\pm$  SD) abundance values are reported in log scale along the y-axis and sampled locations are ordered chronologically along the x-axis. ITB water samples from 2019-2020 are noted as receiving hypochlorite treatment (designated CL for chlorinated) or not (designated Non-CL for non-chlorinated).

High bacterial concentrations were reported in treated water exiting the 200W P&T facility between 2015 and 2017 (Lee et al. 2017), when the biological treatment system was operational. These values are consistent with the many reports citing biofouling issues and loss of injection well capacity at the facility during that time (Thomle et al. 2017). Suspension of the biological treatment process, as part of operational optimization plan, has effectively reduced total bacterial biomass by 99%. Comparison of results from the 2019-2020 ITB samples based on hypochlorite treatment suggests that disinfection had a limited role in controlling total bacterial biomass, particularly since total biomass increased significantly for the chlorinated sample in 2020. It is important to note that the analysis performed does not differentiate active (viable) bacterial cells from bacterial cells that are inactive or dead. In Section 5.0, a modified procedure is recommended to specifically eliminate dead cells to permit measurement of live bacteria only from 200W P&T water samples.

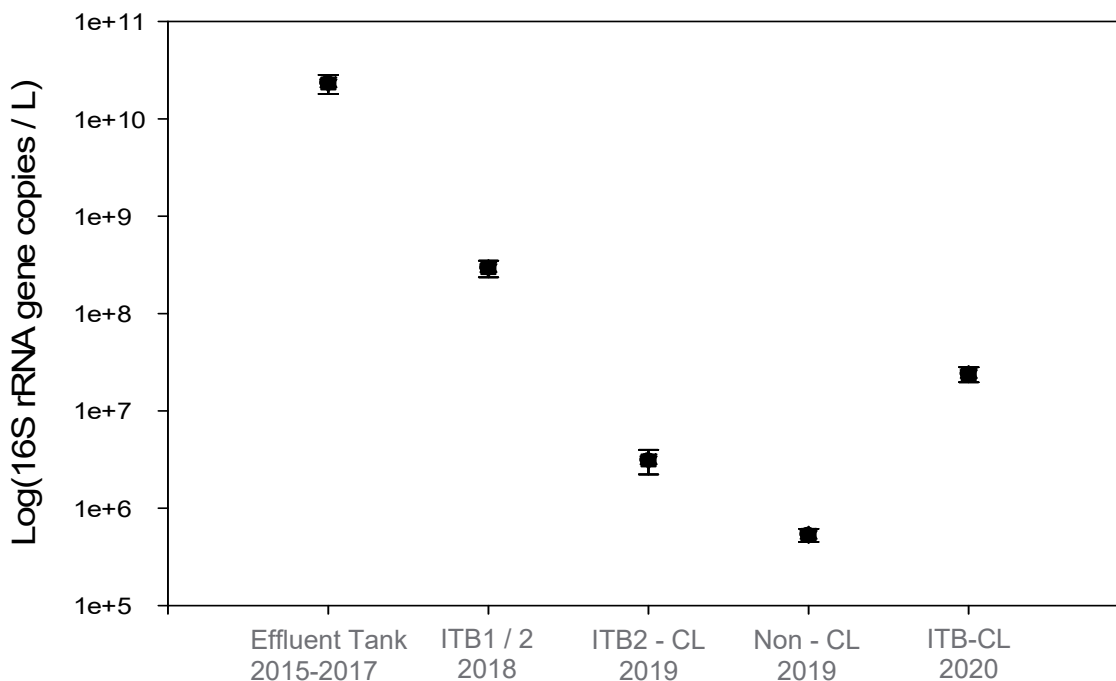


Figure 3.2. Quantification of total microbial biomass (16S rRNA gene) at the 200W P&T facility. QPCR assays were conducted in triplicate; averaged results (standard deviation) are plotted.

### 3.2.2 Sulfate-Reducing Bacteria

Sulfate-reducing bacteria have been extensively characterized for direct (by metabolic coupling) and indirect (sulfide production) contributions to metals corrosion (Enning and Garrelfs 2014; Lekbach et al. 2021), and testing of stainless steel alloys has shown that H<sub>2</sub>S can initiate pitting corrosion at lower potentials than chloride (Ringas and Robinson 1988; Sun et al. 2011). No data was collected for sulfate-reducing bacteria at the 200W P&T facility during 2015-2017 (Lee et al. 2017). A plot of QPCR results for sulfate-reducing bacteria (*dsr*) in 200W P&T water is provided in Figure 3.3. The averaged ( $\pm$  SD) abundance values are reported in log scale along the y-axis and sampled locations are ordered chronologically along the x-axis. ITB water samples from 2019-2020 are noted as receiving hypochlorite treatment (designed CL for chlorinated) or not (designated Non-CL for non-chlorinated).

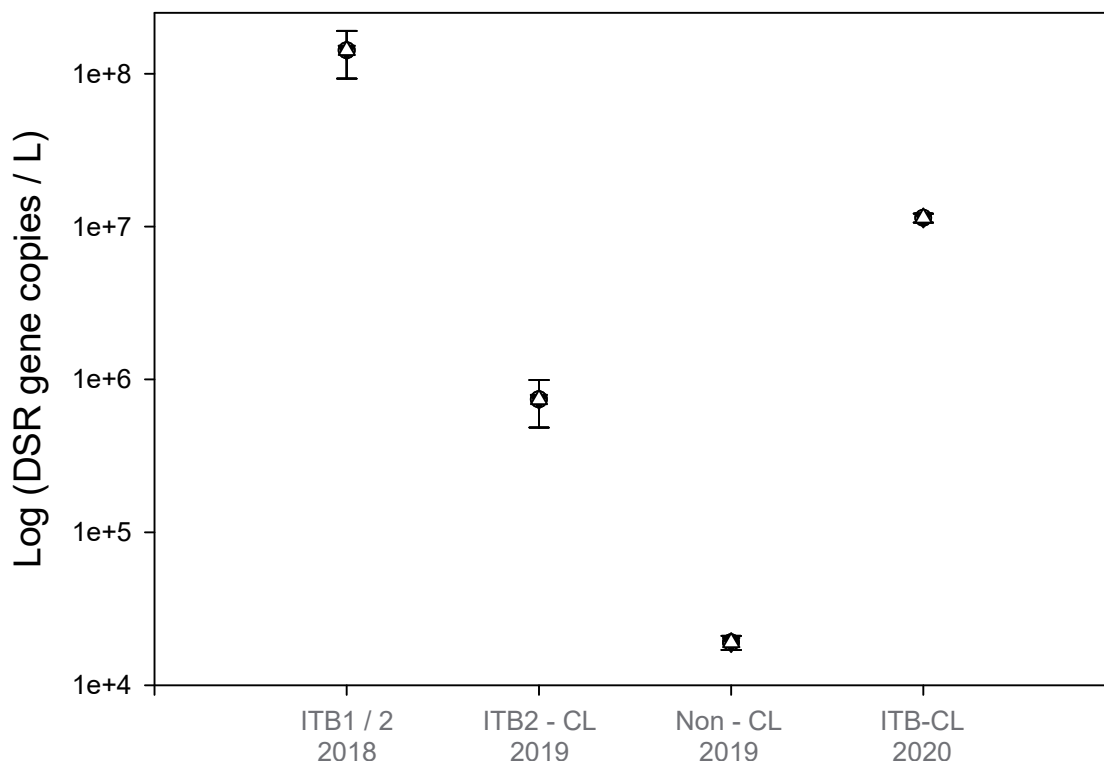


Figure 3.3. Quantification of sulfate-reducing bacteria (*dsr*) at the 200W P&T facility. QPCR assays were conducted in triplicate; averaged results (standard deviation) are plotted.

Sulfate-reducing bacterial abundances were exceptionally high in 2018 but decreased significantly in 2019, most likely due to the elimination of the biological nitrate treatment, which provided carbon/energy for bacterial proliferation throughout the 200W P&T facility. As measured for the total microbial community (Figure 3.2), this trend was unexpectedly reversed in 2020 with a notable increase in sulfate-reducing bacteria measured. Chlorinated ITB water samples maintained higher levels of sulfate-reducing bacteria than the non-chlorinated water sample.

### 3.2.3 Iron-Reducing Bacteria

Iron-reducing bacteria have been studied extensively for reductive pathways that contribute to the damage and removal of Fe(III) passive films on iron alloy surfaces (Herrera and Videla 2009; Lekbach et al. 2021). Iron-reducing bacterial biofilms also create electrical-chemical cells that promote and potentially accelerate corrosive reactions.

The iron-reducing bacterial primers used in this investigation were devised specifically for *Geobacter spp.* and closely related genera, keystone Fe(III) reducers that are also capable of Mn(III/IV) reduction. The phylogenetic and taxonomic breadth of detection for these primers is narrower than the other functional primer pairs used in this study (*nirS*, *nirK*, *dsr*). The gene sequence databases used to develop comprehensive QPCR primers is smaller for Fe/Mn reducers because these reactions are not catalyzed by highly specific genes or pathways that can be targeted by molecular assays as is the case for NO<sub>3</sub> and SO<sub>4</sub> reduction.



A plot of QPCR results for iron-reducing bacteria is provided in Figure 3.4. The averaged ( $\pm$  SD) abundance values are reported in log scale along the y-axis, and sampled locations are ordered chronologically along the x-axis. ITB water samples from 2019-2020 are noted as receiving hypochlorite treatment (designated CL for chlorinated) or not (designated Non-CL for non-chlorinated).

Iron-reducing bacterial estimates were remarkably high between 2015 and 2017 (Lee et al. 2017), though the abundance of these taxa has significantly diminished and has remained very low following implementation of the optimization plan. Again, hypochlorite treated water did not correspond with lower estimations for Fe/Mn-reducing bacteria relative to the non-chlorinated water.

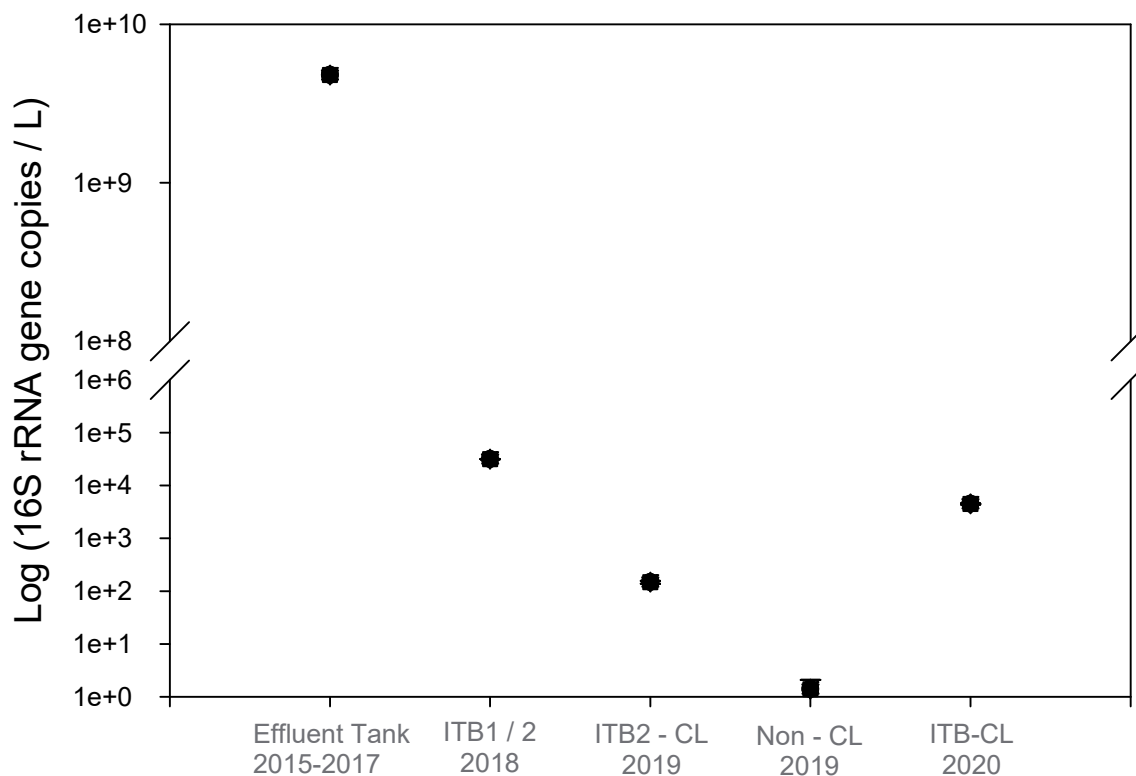


Figure 3.4. Quantification of iron-reducing bacteria (16S rRNA gene for *Geobacter* spp. and closely related genera) at the 200W P&T facility. QPCR assays were conducted in triplicate; averaged results (standard deviation) are plotted.

### 3.2.4 Nitrate-Reducing Bacteria

Unlike Fe(III) and SO<sub>4</sub> reducing bacteria, which have been studied extensively for MIC, corrosion by nitrate-reducing bacteria has received less attention but remains an important pathway for the 200W P&T facility. Numerous investigations have shown that nitrate-reducing bacterial species can form surface biofilms under anaerobic, NO<sub>3</sub>-containing conditions that cause severe pitting of stainless steel that is comparable to pitting by sulfate-reducing bacteria (Jia et al. 2017; Xu et al. 2013). More recently, Yu et al. (2020) demonstrated that *Bacillus cereus* biofilms can rapidly eliminate the protective passive film on 304 stainless steel through the anodic dissolution reaction under aerobic conditions.

The *nirS* and *nirK* genes signify distinct genetic populations of nitrate-reducing bacteria. Both populations are functionally equivalent in carrying out denitrification but are composed of structurally and ecologically distinct bacterial species. It is well established that both genetic populations coexist in natural and engineered environments (Lu et al. 2014; Tiedje et al. 1983), including the 200W P&T biological treatment system for NO<sub>3</sub> reduction to nitrous oxide gaseous products (Lee et al. 2014). *nirS* and *nirK* populations are distinct in the ecological niches where they proliferate, which is primarily controlled by organic substrate availability (quality and quantity).

Plots for nitrate-reducing bacteria (*nirS* and *nirK*, respectively) are shown in Figure 3.5 and Figure 3.6. The averaged ( $\pm$  SD) abundance values are reported in log scale along the y-axis and sampled locations are ordered chronologically along the x-axis. ITB water samples from 2019-2020 are noted as receiving hypochlorite treatment (designed CL for chlorinated) or not (designated Non-CL for non-chlorinated).

Trend comparison between nitrate-reducing bacterial genetic populations (*nirS* and *nirK*) reflects changes in water quality at the 200W P&T facility. It is well established that *nirS* populations categorically represent fast-growing, opportunistic nitrate-reducing populations that take advantage of carbon and energy replete conditions. Previous studies, including the 2015-2017 results presented here, have documented that the *nirS* populations of nitrate-reducing bacteria were more abundant during the operational period of the biological treatment system when carbon and energy supplies were plentiful (Lee et al. 2014). Conversely, the *nirK* populations are considered slow-growing, fastidious species that will be more persistent under low carbon and energy conditions. Consistent with this characterization, suspension of the biological treatment system at the 200W P&T facility has had a more significant impact on the *nirS* populations (Figure 3.5), while *nirK* populations remain abundant in the system despite all exogenous supplies of organic carbon or energy being cut off (Figure 3.6). The significance or potential impact of persistent nitrate-reducing bacterial populations (*nirK*) in the 200W P&T facility is uncertain but should be monitored because these corrosive MIC populations are persistent and remain highly abundant in the 200W P&T facility.

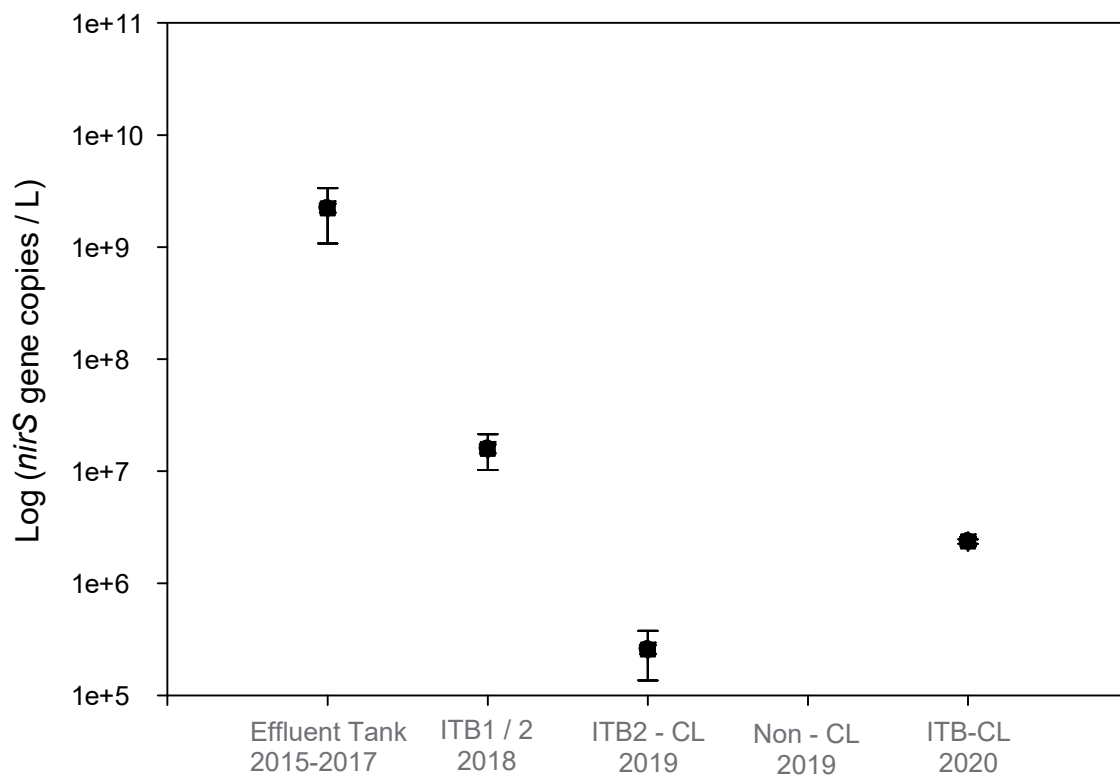


Figure 3.5. Quantification of nitrate-reducing bacteria (*nirS*) from the 200W P&T facility. QPCR assays were conducted in triplicate; averaged results (standard deviation) are plotted.

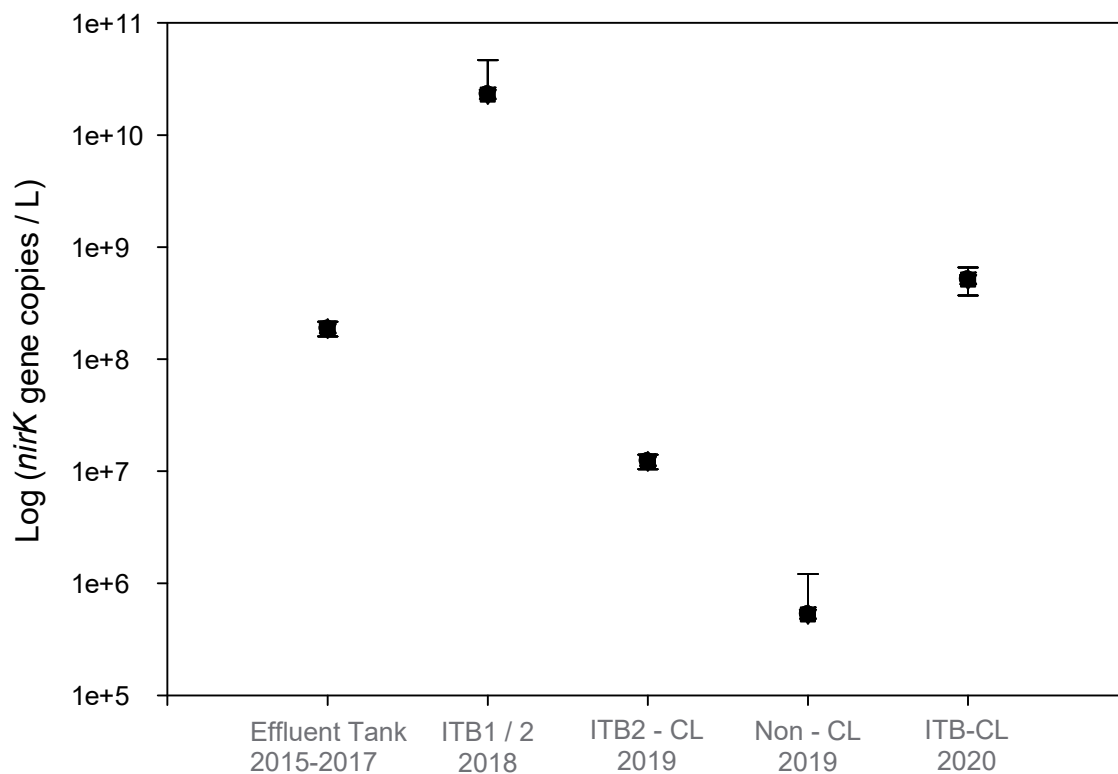


Figure 3.6. Quantification of nitrate-reducing bacteria (*nirK*) from the 200W P&T facility. QPCR assays were conducted in triplicate; averaged results (standard deviation) are plotted.

## 4.0 Conclusions

Microbial cell attachment and biofilm formation enables direct access for metabolic, chemical, or electrical attack of metal surfaces that results in pitting and mass loss over time. Surface-attached microbes are embedded in an extracellular polymeric film that affords protection and enables persistence. MIC is a serious and costly problem in many engineered systems, and stainless steel is not immune from MIC pitting and corrosive attacks. Type 304 stainless steel, which is used at the 200W P&T facility, has no antibacterial properties and therefore surface-attached biofilms cause corrosion when the surface passivation film is damaged. Nondestructive imaging techniques can be used to monitor and measure pipe wall thickness at locations where corrosion is known to be occurring or suspected. The application of molecular biological tools (like QPCR) to monitor MIC by targeting the microbes responsible provides a rapid, inexpensive, and unbiased evaluation of bacterial communities that can be used alongside traditional water quality measures to evaluate corrosion in the system.

The results presented in this report demonstrate that the 200W P&T Optimization Plan (DOE/RL-2019-038, Rev. 0), specifically the elimination of the biological treatment of NO<sub>3</sub>, has successfully reduced microbial biomass, including specific corrosive bacteria, and biofouling in the 200W P&T facility. It is worth noting that the microbial contamination that has built up in the system over many years persists and will be eliminated slowly over time. Discontinuation of the biological treatment process effectively cuts off all supplies of bioavailable carbon and energy to the system, which should prevent further microbial growth and proliferation. When the facility is operating at or near design capacity, shear force, as well as hypochlorite treatments in the conveyance network, will help to slough off microbial biofilm from the inside of pipe surfaces. As microbial biomass is eliminated from the 200W P&T system, though, it will be transported to and injected down-well throughout the injection well network, leaving open the possibly for residual biofouling potential or physical clogging of well screens.

Key findings from this investigation are highlighted below.

- Total microbial biomass and targeted MIC populations decreased in abundance by 99% following discontinuation of the biological treatment system at the 200W P&T facility. An apparent inflection point was noted in 2019, resulting in a rebound in measured microbial indicators in 2020. Due to limited sample availability, the cause and the potential to impact corrosion or well fouling are not known.
- The most abundant MIC populations remaining in the system were nitrate-reducing bacteria (*nirK*) measured at  $5.14e^8$  (1.4) cells/L and sulfate-reducing bacteria at  $1.14e^7$  (0.07) cells/L.
- Total microbial community and corrosive MIC population estimations, as performed, do not differentiate between live and dead bacterial cells. This could have an important impact on this evaluation, particularly if residual biofouling and surface-attached biofilms are being consistently eliminated from the system in response to facility optimization. A modified approach is recommended in Section 5.0 for continued monitoring by specifically removing inactive and dead bacterial cells before measurement to permit quantification of only active bacterial cells in 200W P&T water.

A recommended strategy for systematic monitoring of the 200W P&T facility for MIC is provided in Section 5.0. The purpose of continued monitoring is to evaluate facility equilibration and stabilization in response to P&T optimization.

## 5.0 Recommendations

The results reported here demonstrate that 200W P&T facility optimization to bypass the biological treatment of  $\text{NO}_3$  has had a significant impact by improving overall water quality, decreasing total microbial counts, and decreasing MIC counts. Continued operations, the physical action of water flow and chlorination will continue to help clean away the chemical and biological residues that have built up inside and on pipe surfaces over time. Eventually, these residues exit the system and will be injected into the subsurface. Continued testing is recommended to monitor the stabilization of water quality in response to further improvements in the facility, as well as at the well head to help understand if reinjected residues could potentially impact well capacity and performance.

**Purpose:** Recommend continued testing of 200W P&T facility effluents for microbial indicators of corrosion and water quality measures that directly impact well head capacity and performance.

- Water sampled from specific locations within the 200W P&T facility will quantify total live microbial counts and specific microbial groups that contribute to or exacerbate pitting and crevice corrosion.
- Sampled locations within the 200W P&T facility will quantify the impact of chlorination as a measure of total live microbial counts and microbial indicators of corrosion, as well as the extent and duration that residues (solids) are being removed from pipe surfaces within the facility.
- Water sampled from specific locations along the conveyance system will quantify total microbial counts (live and dead) and residues (solids) being injected back into the subsurface and potentially affecting well head capacity and performance.

**Approach:** A schematic of the 200W P&T facility with proposed sampling locations is provided in Figure 5.1. Monthly water testing (4 L/location) is proposed from the following facility locations.

- Equalization tank as groundwater enters the facility (Figure 5.1, A)
- An intermediate location where water has been chlorinated (Figure 5.1, B)
- Effluent tank as treated water exits the facility (Figure 5.1, C)
- ITB 1 and 2 (Figure 5.1, D)
- Near or from injection well heads (Figure 5.1, E)

P&T effluent samples will be filtered to collect microbial biomass, residues, and solids. Total live microbial biomass and MIC targets (i.e.,  $\text{NO}_3$ ,  $\text{SO}_4$ , Fe, and Mn respiring bacteria) will be quantified by QPCR and reported as a concentration, cells/L. New data will be plotted and tracked relative to values reported here (2015-2020) to monitor patterns and trends in water quality over time. Once microbial measurements stabilize, the sampling frequency may be reduced to quarterly or biannually to save on cost.

A single-step modification will be made to the microbial extraction protocol to allow for the quantification of live bacteria as a percentage of total microbial counts. This modification will provide additional information about microbial growth in the system and the potential for active microbial corrosion and will discern whether biological debris passing through the system consists of live microbes or dead biomass coming from pipe surfaces.

Concentrated solids, residues, and debris (like those shown in Figure 1.1, Figure 1.2, and Figure 3.1) may be characterized for chemical composition by scanning electron microscopy – energy dispersive X-ray spectroscopy, X-ray diffraction, or elemental analysis if the frequency or abundance (mass) of collection warrants investigation. This may become particularly important as accumulated materials may have negative impacts on injection well capacity and performance over time.

**Expected Outcomes:**

- Comparative analyses (total live microbial and MIC, residues) from water sampled over time at locations A, B, and C in Figure 5.1 will permit assessment of system stabilization as a function of water quality measurements in response to operational optimization. Specifically, changes in water quality as water passes through the facility will indicate the extent of biological and chemical residue accumulation on pipe surfaces, as well as potential impacts of chlorination to reduce biofouling, and will help to eliminate buildup within the facility. Removal of surface residues and debris is important as these materials will eventually be injected down well and may have negative impacts on well capacity and performance.
- Similarly, comparative analysis of water sampled from locations C, D, and E in Figure 5.1 will provide information about the extent of chemical and biological residues within the conveyance system, mass estimates, and transport of these residues to the injection well head.
- Water quality assessments within the facility and through the conveyance system will provide specific information about the potential for MIC, as well as microbial and chemical residues being eliminated from the system, which could potentially impact injection well performance and capacity. This evaluation will identify the primary source of residues and debris within the treated water, leading to specific recommendations or strategies to mitigate future impacts to injection well performance.

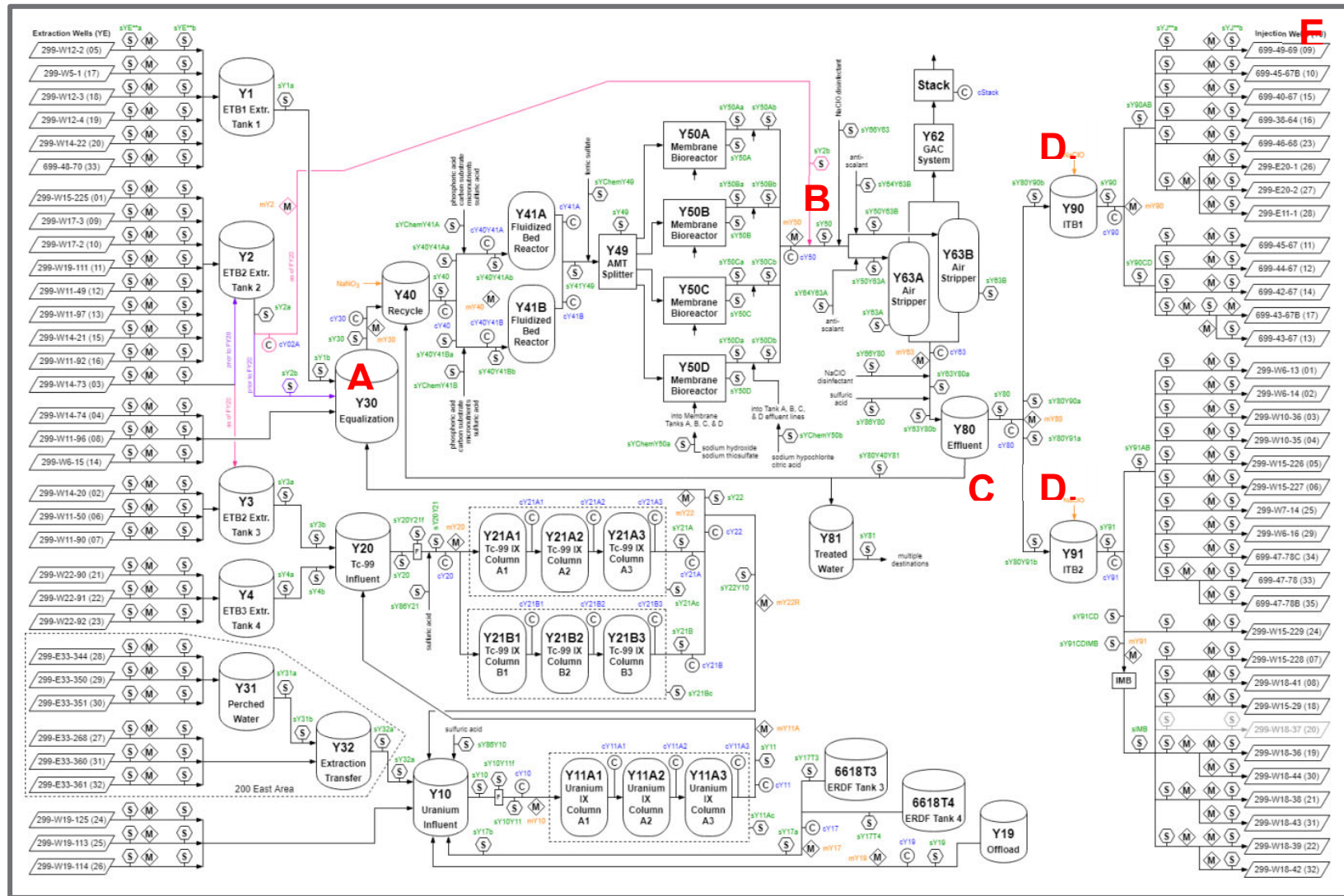


Figure 5.1. Schematic of 200W P&T facility. Proposed sampled locations for microbial monitoring are as follows: (A) equalization tank as groundwater enters the facility; (B) water sampled after chlorination, specific location TBD; (C) effluent tank as treated water exiting the facility; (D) ITB buildings; and (E) at or near injection well head, specific location TBD.



## 6.0 Quality Assurance

This work was performed in accordance with the Pacific Northwest National Laboratory Nuclear Quality Assurance Program (NQAP). The NQAP complies with DOE Energy Order 414.1D, *Quality Assurance*. The NQAP uses NQA-1-2012, *Quality Assurance Requirements for Nuclear Facility Application*, as its consensus standard and NQA-1-2012 Subpart 4.2.1 as the basis for its graded approach to quality.

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