The Importance of Micronutrients to Biological Treatment, 200 West Pump and Treat, Hanford Site, Richland, WA – 14021

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ABSTRACT

The Department of Energy's 200 West Pump and Treat is designed to capture and treat contaminated groundwater to reduce the mass of carbon tetrachloride, trichloroethene, nitrate, chromium (Cr III and Cr VI), technetium-99, and iodine-129. The media (granular activated carbon) in the fluidized bed biological reactors was inoculated with denitrifying microorganisms. An organic carbon substrate, phosphoric acid nutrient, and a micronutrient blend promote microbial growth. Initial analytical results indicated the microorganisms effectively reduced many of the contaminants to less than cleanup levels. Shortly after start-up, however, operational challenges including carbon carry-over conditions, presence of a slimy biomass, the presence of free-floating white gelatinous masses, and "septic" odor associated with hydrogen sulfide impacted contaminant removal efficiency. The extraneous biomass was determined to be extracellular polymeric substance (EPS) generated as a result of a nutrient deficiency. A new micronutrient mixture and an increase in feed rate resulted in generation of less EPS, but carbon media was still being carried out of the bioreactors. Biofilm analyses for nutrient and micronutrient content indicated sulfide formed in the biofilm posed a demand for iron and copper many times the demand posed by biological needs. This competing sink between the chemical and the biological aspects of the system for micronutrients was satisfied by reducing the substrate feed to minimize the production of sulfide and increasing the iron and copper content in the micronutrient feed. This paper shares lessons learned and demonstrates that balancing nutrient and micronutrient content for both chemical and biological demand is critical to operational efficiency of biological treatment systems.

INTRODUCTION

In June 2012 CH2M HILL Plateau Remediation Company began operation of the 200 West pump and treat (P&T), a new biological groundwater treatment system, on the Hanford Site in Washington State. The system is built over the 200-ZP-1 Groundwater Operable Unit (OU) to remove technetium-99 and iodine-129 by ion exchange; nitrate, carbon tetrachloride, trichloroethene, and chromium using anoxic and aerobic bioreactors; and air stripping as a polishing step for volatile organic compound removal. The startup of this P&T system is important since it will ensure that 200-ZP-1 contaminants never reach the Columbia River. Table I presents the final cleanup levels and illustrates the ability of the system to remove contaminants to below cleanup levels. Note that the regulations express the activity levels in picocurie per liter (1 pCi – 0.037 Bq.).

TABLE I. 200 West P&T September 2013 influent and effluent concentrations and final cleanup levels for the 200-ZP-1 groundwater Operable Unit

Contaminant of Concern	Units	Influent Concentration	Effluent Concentration	Final Cleanup Level	Cleanup Level Basis
Carbon tetrachloride	µg/L	790	<1	3.4	MTCA, Method B
Chromium (total)	µg/L	28	4.43	100	Federal/State MCL
Hexavalent chromium	µg/L	24.3	3.7	48	MTCA, Method B
Iodine-129	pCi/L	<0.213	<0.223	1	Federal MCL
Nitrate as Nitrogen	mg/L	26.2	8.53	10	Federal/State MCL
Technetium-99	pCi/L	1,600	70	900	Federal/State MCL
Trichloroethene	µg/L	3.8	<0.5	1	MTCA, Method B
Tritium	pCi/L	3,200	3,200	20,000	Federal MCL

The treatment approach being used to remove the contaminants of concern is summarized in Table II. Note there currently is no cost-effective technology to remove tritium from groundwater.

Figure 1 illustrates the flow stream through the FBRs. Groundwater from wells containing radioactive contaminants is pumped through the ion exchange system, which removes technetium-99 and iodine-129 using a Purolite A530E[1] resin, and then on to the equalization tank where it is blended with groundwater from wells without radioactive contaminants. The water passes through the recycle tank and up through the FBRs. MicroCg™ (i.e., organic carbon substrate used as the electron donor in biological denitrification), phosphoric acid, and a micronutrient solution are fed immediately upstream of the FBRs to facilitate biological denitrification. These three chemicals provide food and nutrients for a denitrifying biofilm that is grown on granular activated carbon in the FBRs. The treated FBR effluent is discharged into carbon separators before the denitrified groundwater flows into a splitter structure to divide the stream amongst four MBRs for removal of solids by membrane filtration, residual carbon substrate by aerobic biodegradation, and carbon tetrachloride, chloroform, and trichloroethene by air stripping. Vapor emissions are collected for treatment with granular activated carbon. The final treated water is then transferred to the injection wells. Injection wells are installed both upgradient (to direct the contaminant flow toward the extraction wells) and downgradient (to slow contaminant flow toward the Columbia River). This paper focuses on the performance of the FBR system.

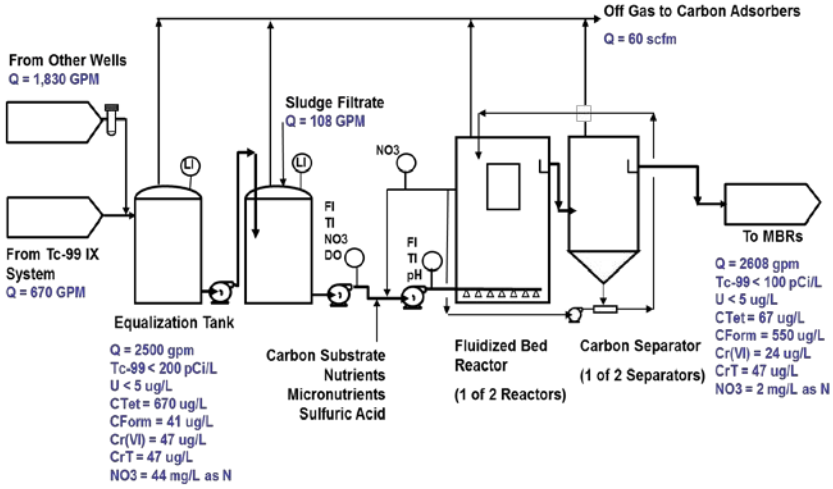

Fig. 1. Anoxic fluidized bed biological reactor schematic.

[1] Purolite is a registered trademark of BROTECH CORP., Bala Cynwyd, Pennsylvania.

TABLE II. 200 West P&T unit process descriptions

Unit Process	Process Benefit	Targeted Parameter
Ion exchange	Removal of technetium-99 and iodine-129	Technetium-99 and iodine-129
Anoxic biodegradation (FBR)[a]	Removal of nitrate, degradation of carbon tetrachloride and trichloroethene, and conversion of hexavalent chromium to trivalent form	Nitrate, carbon tetrachloride, trichloroethene, total and hexavalent chromium
Aerobic biodegradation (MBR)	Degradation/removal of residual organic carbon substrate	Biochemical oxygen demand
Membrane filtration (MBR)	Removal of particles, biomass, and precipitated trivalent chromium	Trivalent chromium, turbidity, and biochemical oxygen demand
Air stripping	Removal of volatile organic compounds (carbon tetrachloride and trichloroethene)	Carbon tetrachloride and trichloroethene
Sludge thickening	Thickens biological solids for dewatering process	Solids content
Sludge dewatering	Reduces water content to allow for landfill disposal	Water content
Lime sludge conditioning	Dry biomass and odor control	Water content
Treated water chemistry adjustment	Provides treated water stability	pH and alkalinity

a. FBR = fluidized bed biological reactor
b. MBR = membrane biological reactor

BACKGROUND OF FLUIDIZED BED BIOLOLOGICAL REACTOR PROBLEMS

The FBRs have been subject to operational challenges associated with the growth of a slimy biomass that fouled a straining apparatus (Fig. 2). In May of 2012, the slimy biomass increased to such a degree that the buoyancy of the carbon was reduced, resulting in a loss of carbon from the system. The carbon flowed into the downstream aerated solids tank and MBR, undesirable because the sharp edges of the carbon damages the MBR membranes.

The microorganisms in flocculated biological systems such as biofilms reside in a complex matrix of proteins, lipids, polysaccharides, nucleic acids, and humic substances called extracellular polymeric substance (EPS). Stresses, such as nutrient limitations, can cause an overproduction of EPS. The gelatinous masses observed on the biofilm media (Fig. 3) during the FBR upsets are believed to have been caused by EPS overproduction. Thus, the key question of this work is what caused the observed EPS overproduction?

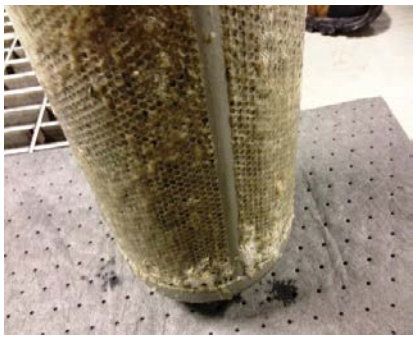

Fig. 2. Biofilm fouling of a straining apparatus.

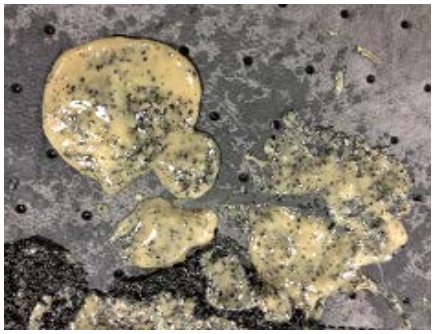

Fig. 3. Gelatinous biomass observed on the biofilm media

Many micronutrients were identified during the FBR upset as being fed at rates insufficient to sustain necessary biochemical reactions. In response, a new micronutrient mixture was created (approximately 10 times the strength of the original solution) and fed at an increased rate to meet micronutrient targets indicated in Table III. The micronutrient formulation in Table III was based on a previous denitrifying biofilm system design.

Table III. Denitrification organism macronutrient and micronutrient requirements

Nutrient and Micronutrient Element	Cell Requirements (% Dry Mass)
Phosphorus	2.40
Potassium	1.50
Sodium	1.30
Calcium	1.40
Magnesium	0.70
Chloride	0.50
Sulfur	1.00
Manganese	0.20
Iron	0.20
Cobalt	0.05
Molybdenum	0.05
Zinc	0.05
Copper	0.02
Nickel	0.01
Boron	0.01
Selenium	Not Established
Aluminum	Not Established

Source: Castle Peak Station Technical Memorandum (Nutrient Addition to Existing Biological Treatment System), CH2M HILL, 2012.

The new micronutrient feed resulted in effluent concentrations of nitrate and chemical oxygen demand (COD) less than 4 milligrams per liter (mg/L) nitrate-nitrogen and 20 mg/L COD respectively. The old micronutrient solution routinely resulted in an effluent COD concentration greater than 100 mg/L. After an extended period of improved performance, the problematic gelatinous biomass and granular carbon carry-over reoccurred from September 2012 through early March 2013. This reoccurrence was thought to have been brought on by the use of diluted micronutrient solution that resulted from precipitation of some micronutrient constituents in the micronutrient solution reservoir.

A sampling campaign was initiated in March and April 2013 to identify potentially limiting macronutrients and micronutrients and to examine biofilm characteristics to determine other potential causes of EPS overproduction. This information was used to develop a new micronutrient formulation to satisfy the specific needs of this FBR system and to develop monitoring strategies to detect (and ideally avoid) an upset condition before it becomes severe.

HYPOTHESIZED MECHANISM OF UPSET

Bacteria complete metabolic processes with essential substrates that include an electron donor, electron acceptor, macronutrients (N and P), and micronutrients (e.g., Zn, Mn, Mo, Se, Co, Cu, Ni, S, K, Mg, Ca, Fe, Na, and Cl). Macronutrients are required in greater quantity than micronutrients, which are required only in trace amounts. The amount of the feed constituents (e.g., electron donor, electron acceptor, macronutrients, and micronutrients) needed for biomass growth is known as the stoichiometric requirements. There is general consensus concerning the stoichiometric requirements for electron donor, electron acceptor, and macronutrients (phosphorus and nitrogen). In contrast, there is less consensus concerning the requirements for micronutrients because different bacteria have different requirements and because it is difficult to separate the biological requirement from physical/chemical interactions.

In biofilm reactors, fluid containing the electron donor, electron acceptor, macronutrients, and micronutrients passes over the surface of a biofilm-covered substratum. Macronutrients and micronutrients must be provided in proportion to the mass of carbon source fed to the systems.

If macronutrients and micronutrients are not available, either because they are not fed in sufficient quantity or because they are diffusion limited, they will limit biochemical transformations, causing undesirable results such as EPS overproduction. Bacterial cells produce EPS to facilitate biofilm formation. EPS provide further functions, such as serving as an emergency food source and protecting bacterial cells from harmful toxic materials by providing a buffer that the toxic material must diffuse through before reaching the cell wall. The absence of sufficient macronutrients and micronutrients can place biofilm-entrained bacteria under distress. Similarly, exposure to sufficient quantities of toxic materials (e.g., metals and organic chemicals) places biofilm-entrained bacteria under distress. The symptoms of a distressed biofilm system include retarded biochemical conversion (i.e., conversion rates less than optimal) and the increased production of EPS. Fang et al. [1] reported that the production of EPS increased up to 100 percent in marine biofilms exposed to seawater containing toxic metals and chemicals such as Cr(III). Chénier et al. [2] reported increased EPS in biofilms whose growth was limited by the availability of a macronutrient (phosphorus or nitrogen) when compared with biofilms limited by a terminal electron donor/acceptor.

Potential Mechanism for Fluidized Bed Reactor Upset

Sulfate (SO_4^{2-}) can serve as an electron acceptor for the growth of sulfate-reducing bacteria (SRB) when readily biodegradable organic matter (e.g., MicroCg™) is present and more readily utilizable electron acceptors such as dissolved oxygen and nitrate nitrogen are absent. The growth of SRB will create an additional macronutrient/micronutrient sink in the FBR because macronutrients and micronutrients are also needed for SRB growth. While nitrate-nitrogen may be present in the bulk liquid, it can become depleted inside a denitrifying biofilm due to diffusional resistance if organic carbon is fed in excess of the stoichiometric requirement for denitrification. The reduction of sulfate yields hydrogen sulfide (H_2S). Thus, sulfide can be produced in a biofilm reactor even when nitrate is present in the bulk liquid and the process effluent.

Hydrogen sulfide is a weak acid and exists predominantly in two ionic forms (H_2S or HS^-; Equations 1–3) over the pH range of 6 to 8 in the FBR system. Although hydrogen sulfide is produced when nitrate is absent, it may also be used as an electron donor for denitrification by autotrophic bacteria (Equations 4–6). Furthermore, S^{2-} and HS^- precipitate with metals, including metal micronutrients, to form complexes (Equations 7–9). Metal precipitation in the biofilm would render micronutrients unavailable for biological assimilation and generate additional micronutrient demand.

Some reactions relevant to the reduction of sulfate by SRB include:

$$SO_4^{2-} + \text{organic matter} \rightarrow S^{2-} + H_2O + CO_2 \quad \text{(Eq. 1)}$$

$$S^{2-} + H^+ \leftrightarrow HS^- \quad \text{(Eq. 2)}$$

$$HS^- + H^+ \leftrightarrow H_2S \quad \text{(Eq. 3)}$$

Autotrophic denitrification with hydrogen sulfide:

$$5\ H_2S + 8\ NO_3^- \rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O + 2\ H^+ \quad \text{(Eq.4)}$$

$$10\ H_2S + 8\ NO_3^- \rightarrow 5\ S_2O_3^{2-} + 4\ N_2 + 9\ H_2O + 2\ H^+ \quad \text{(Eq. 5)}$$

$$5\ H_2S + 6\ NO_3^- \rightarrow 5\ SO_3^{2-} + 3\ N_2 + 3\ H_2O + 4\ H^+ \quad \text{(Eq. 6)}$$

Precipitation of bisulfide (HS^-) and metal:

$$HS^- \leftrightarrow S^{2-} + H^+ \quad \text{(Eq. 7)}$$

$$M^{2+} + S^{2-} \leftrightarrow MS_{(s)} \quad \text{(Eq. 8)}$$

$$M^{2+} + HS^- \leftrightarrow MS_{(s)} + H^+ \quad \text{(Eq. 9)}$$

The presence of a strong hydrogen sulfide odor, a probable byproduct of sulfate reduction, is an indicator that anaerobic biochemical activity (by SRB) is occurring in the FBRs. Their presence is consistent with the symptoms reported for the 200 West P&T FBRs (i.e., slime, white billowing foam).

TABLE IV. Summary of sampling program

Information Collected	Purpose
Quantification of electron acceptors (nitrate and sulfate) and donors (organic carbon, sulfide) in the groundwater, FBR influent and FBR effluent	Evaluate and benchmark system performance
Quantification of other groundwater quality characteristics (alkalinity, ammonia, total Kjeldahl nitrogen, etc.), FBR influent and FBR effluent	Identify other contributing factors behind FBR upsets
Quantification of macronutrients and micronutrients in the groundwater, FBR influent, and FBR effluent	Identify potential limiting nutrients Benchmark values for future comparison
Quantification of macronutrients and micronutrients in the biofilm	Identify potential limiting nutrients Estimate the true micronutrient needs of the system
EPS extraction and characterization	Determine if EPS characteristics match typical observations of stressed systems Benchmark EPS quantity for future comparison
Quantitative Polymerase Chain Reactor (qPCR)	Determine if a significant fraction of the biofilm consisted of populations other than denitrifying bacteria (i.e., SRB)
Fluorescence In Situ Hybridization (FISH)	Examine the change in redox condition through the depth of the biofilm

There was evidence that the 200 W P&T FBR system may be subject to macronutrient and micronutrient limitations and the limitations may be a result of:

- Inadequate macronutrients and micronutrients in the feed,
- The system having an increased demand for macronutrients and micronutrients due to the co-existence of heterotrophic denitrifiers and SRB in mixed-culture biofilms.

APPROACH

A sampling program was developed based on the hypothesized mechanisms for the FBR upsets described above. The sampling program was designed to provide a system mass balance. In addition, information was collected to characterize biofilm nutrient content, biofilm structure, and bacteria types present in the biofilm. The following was collected:

- Reactor operational characteristics
- Influent stream characteristics (including phosphorus, micronutrients, and COD doses)
- Effluent stream characteristics
- Biofilm covered granular activated carbon characteristics (samples analyzed for solids, bacteria type and quantity, and nutrient content)

Table IV summarizes the characterization efforts and the purpose of each measurement.

Sampling

FBR A was used for all sampling efforts. At the time of the sampling, FBR A was operating better than FBR B and had more granular activated carbon media. Sampling was only performed while the FBR was in forward feed mode. Three sampling events were conducted, each about two weeks apart.

During each sampling event, both water and biofilm samples were collected. The samples were delivered to the laboratory on the day of sampling to ensure the samples met all criteria for temperature and holding times. The micronutrient feed solution was also analyzed to determine the micronutrients fed to the system.

Biofilm samples were collected using a peristaltic pump and a long sampling tube. A camera was lowered into the FBR to determine the fluidized bed height. Biofilm samples were collected from three bed depths: one foot below the fluidized bed surface, from the middle of the bed, and one foot off the bottom of the bed. All three samples were analyzed during the first sampling period. Only the middle sample was analyzed from the second and third sampling events.

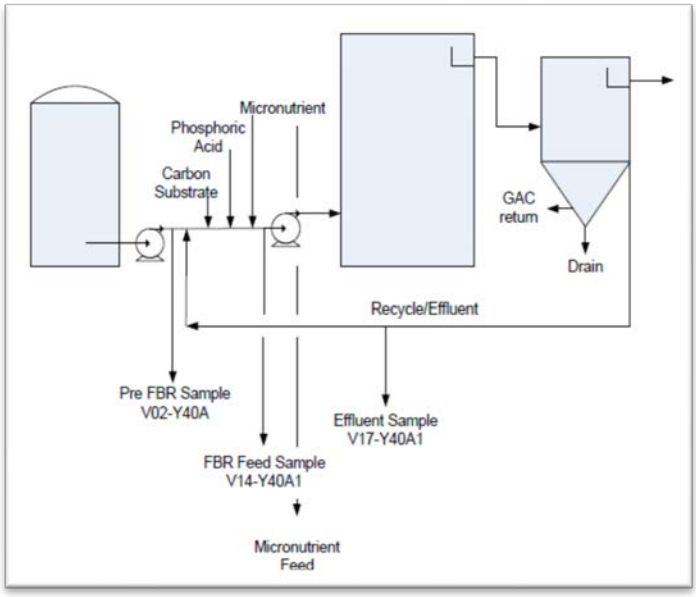

Fig. 4. Location of Samples in Process Stream (numbers indicate sample location/valve identifiers).

Water samples were collected from locations shown in Fig. 4. In each case, composite samples were collected over the course of several hours. The specific time interval varied with each sampling event to accommodate operational schedules. In addition to the water quality and biofilm samples, reactor operational characteristics were recorded.

Timing of Sampling and Micronutrient Dose Adjustments

The three sampling events described above were performed during a time when the micronutrient feed rate was being increased to help reduce EPS overproduction. A grab sample of the micronutrient feed was collected on March 5, 2013 and the analytical results indicated an increased micronutrient dose was required. Following the increased dosage, the first of three sampling events occurred from March 12 to March 14, 2013. The micronutrient feed was increased again on March 27, 2013 and the second (April 1 to 3) and third (April 16 to 17) micronutrient sampling events occurred to determine the appropriate micronutrient feed.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) provides a characterization of relative abundance of microbial communities chosen for analysis. For the current project, the following microbial communities were targeted for analysis: SRBs, methanogenic bacteria, iron-reducing bacteria, denitrifying bacteria, and anaerobic ammonia oxidizing bacteria. Their abundance is compared against the quantification of total Eubacteria and Archaea to estimate relative abundance.

RESULTS AND DISCUSSION

Denitrification Performance

In the absence of FBR upsets, the primary indication of system performance is based on the ability to meet the target nitrate removal. Carbon substrate in the form of MicroCg™ is added to the FBR to facilitate denitrification. Additional carbon is also added to remove dissolved oxygen, which is present in the influent, because the presence of this oxygen interferes with the denitrification process. The carbon dose is determined based on an equation provided by the system supplier (Envirogen). Equation 10 shows how the carbon dose is determined.

$$\text{Carbon Dose (lb COD)} = [\text{Influent DO (lb)} \times 2.33] + \{[\text{Influent } NO_3 - \text{Effluent } NO_3 \text{ Target}](\text{lb N}) \times 6.54\} \quad \text{(Eq. 10)}$$

The process factors that determine how much carbon to add (6.54 lb COD/lb NO_3-N for nitrate and 2.33 lb COD/lb O_2 for dissolved oxygen) are derived from vendor recommended parameters. The vendor recommendations are based on the mass of a MicroCg™ to add. MicroCg™ is a proprietary compound with 670,000 mg/L COD. The dosing rate for micronutrients and phosphoric acid is based on the carbon dose derived from Equation 10. The denitrification performance of the system is routinely monitored during normal system operation and was examined in detail during the sampling campaign. Table V summarizes the results of the three detailed sampling events.

TABLE V. Fluidized bed reactor performance results for nitrate and chemical oxygen demand

	Effluent Nitrate Concentration		Total Mass of Nitrate Removed		Effluent COD Concentration
	Target (mg/L N)	Measured (mg/L N)	Target (ppd)	Measured (ppd)	Measured (mg/L)
March 12	5	8.0	244	122	43
March 14	5	6.0	244	153	20
April 1	10	10.1	141	121	45
April 3	10	9.09	141	122	20
April 16	10	13.4	125	73	19
April 17	10	11.4	125	116	12

COD = chemical oxygen demand; mg/L = milligrams per liter; ppd = pounds per day.

The first sampling event (March 12 and March 14) occurred during a time when FBR performance was improving but the micronutrient dose was still insufficient. Comparing the target effluent nitrate concentration to the measured concentration shows that the system was not meeting the target of 5 mg/L N for effluent nitrate. More importantly, the MicroCg™ was being dosed to remove about 100 pounds per day more nitrate than was actually removed. This COD overdose was intentional driven, in part, by the reduced carbon bed media available (reduced reactor volume) requiring an excess of COD to drive the kinetics of nitrate reduction. This situation results in a significant excess of electron donor available to support microbial populations other than denitrifying bacteria, and these other populations would use micronutrients to sustain their biochemical conversions. Since the groundwater source has a significant influent sulfate loading, the excess carbon probably supports a population of SRB. In addition to creating a biochemical macronutrient and micronutrient demand, the production of sulfide by SRB also encourages metal precipitation, which exerts an additional micronutrient demand in the system.

The FBR system was able to meet effluent nitrate targets for the second sampling event (April 1 and April 3), which occurred just after the micronutrient dose was increased. While the mass of nitrate removed was much closer to the target value, there was still excess COD in the effluent (i.e., the effluent COD was greater than 10 mg/L). The effluent nitrate concentration target was not met during the third sampling event (April 16 and April 17) but there was less excess COD in effluent. This suggests that the process could have been operating in a COD limited mode, which is the preferred operational approach.

The ideal operational strategy for the FBR regarding carbon dosing is to meet the effluent nitrate targets for concentration and mass removed with little excess COD in the effluent (i.e., to operate in a carbon-limited mode, as described above). It is recommended to maintain the FBR effluent COD less than 20 mg/L to avoid overdosing of carbon. There are a number of detrimental side effects of overdosing COD. Some of these were discussed above, but a more complete list includes:

- Fouling of downstream pipes, tanks, and injection wells with unwanted biofilms
- Increased chemical costs associated with MicroCg™
- Additional microbial communities, including SRB, exerting a micronutrient demand
- Chemical precipitation of micronutrients caused by production of sulfide by SRB
- Hydrogen sulfide odor production caused by SRB

The FBR vendor provided carbon dosing targets (process factors for nitrite and oxygen) that can be adjusted over time to optimize process performance. These process factors will also need adjustment over time when/if the groundwater characteristics (i.e., nitrate concentration or sulfate concentration) change and the FBR reactor beds are restored to their full design volume.

Sulfur Monitoring

A strong hydrogen sulfide odor from the FBR system was detected and the production of hydrogen sulfide is a strong indication of SRB presence. Sulfur was present in the biofilm at concentrations between 3.6 and 9.2 mg/L depending on the sampling date. However, the detailed sampling of the liquid process streams showed no sulfate removal (Table VI). This result suggests that sulfate reduction is not a significant source of carbon utilization in the FBR but does not rule out the possibility of SRB activity deep in the biofilm. Even small amounts of sulfate reduction would be sufficient to create a precipitation condition and the detection of sulfur in the biofilm provides evidence the sulfur precipitation was occurring. Additionally, the sulfide could be driven into the gaseous phase as hydrogen sulfide, which was not included in the analyses. The presence of hydrogen sulfide in larger quantities could be detected by its odor but incorporation of hydrogen sulfide sampling as part of routine reactor monitoring could be used to optimize the carbon dose and reduced if hydrogen sulfide is detected in the gaseous stream.

Phosphate Monitoring

Phosphate is an essential macronutrient for biological conversions and is dosed as a ratio of the amount of COD fed to the system to maintain a residual of at least 0.1 mg/L (as P). FBR effluent sampling confirmed adequate phosphorus was being added.

Micronutrient Analysis

The micronutrient concentrations in the groundwater, FBR influent, and FBR effluent were analyzed to determine if any micronutrients were identified as clearly limiting. The results from the groundwater and FBR effluent are summarized in Table VII.

TABLE VI. Fluidized bed reactor performance results for sulfur compounds (mg/L)

Sample Date	Sulfate Concentration		Dissolved Sulfide Concentration		Total Sulfide Concentration	
	Groundwater (pre-FBR)	FBR Effluent	Groundwater (pre-FBR)	FBR Effluent	Groundwater (pre-FBR)	FBR Effluent
March 12	46.4	47.8	ND	ND	ND	ND
March 14	47.7	48.0	ND	ND	0.60	ND
April 1	47.8	47.3	ND	ND	ND	ND
April 3	45.9	47.9	0.63	ND	0.63	ND
April 16	45.7	46.7	ND	ND	ND	ND
April 17	45.4	47.7	ND	ND	ND	ND

ND – Not Detected

The micronutrient concentrations shown in Table VII demonstrate that, as expected, many micronutrients were not detected in the groundwater feeding the FBR system; hence the need to add micronutrients to the FBR feed. Sampling of micronutrients in the effluent shows that aluminum was not detected during the first sampling event (March 14), which occurred before the micronutrient dose was increased. Aluminum was found in excess during the second (April 3) and third (April 17) sampling events following the increase in micronutrient dose. The other micronutrients do not appear to show a significant increase after the dose was increased, though copper appears to have a wider variability in effluent concentration than the other metals examined. This variability would be consistent with variations in hydrogen sulfide production and copper precipitation variations between sampling periods. This variability could also be related to variability in the groundwater copper concentration.

TABLE VII. Micronutrient concentrations in the groundwater and fluidized bed reactor effluent (µg/L)

Micronutrient	Groundwater (Pre FBR Sample)			Effluent (Post FBR Sample)		
	March 14	April 3	April 17	March 14	April 3	April 17
Iron	ND	ND	ND	0.064	0.600	0.436
Aluminum	ND	ND	0.0228	ND	0.018	0.043
Manganese	ND	0.000424	ND	0.183	0.194	0.148
Nickel	0.000313	0.000352	0.000296	0.011	0.022	0.015
Cobalt	ND	ND	ND	0.049	0.057	0.042
Copper	0.00328	0.00292	0.000984	0.006	0.058	0.013
Zinc	ND	0.00614	ND	0.044	0.049	0.030
Molybdenum	0.00426	0.00354	0.00344	0.057	0.056	0.044
Selenium	0.00306	0.00261	0.00292	0.003	0.003	0.003
Boron	0.0551	0.053	0.0497	0.070	0.060	0.060

ND – Not Detected

Sampling for micronutrients in the effluent of the system is not ideal to determine its specific micronutrient needs. The primary purpose of effluent sampling is to screen for micronutrient concentrations that may be low (like aluminum in this case). However, even an undetected result for the micronutrient in the effluent does not necessarily mean the biofilm is limited. If the dosage and utilization rates are well balanced, the biomass will get the necessary micronutrient but leave no excess nutrient in the effluent. Additionally, increased micronutrient dosage could simply encourage further precipitation reactions that would not be captured by sampling the liquid streams around the FBR system.

The micronutrient concentration in the biofilm is more indicative of the system's true micronutrient demands as this characterization captures micronutrient demands associated with both biological activity and chemical precipitation. Table VIII summarizes the biofilm micronutrient concentrations measured during each sampling event. Concentrations less than the micronutrient recommendations from previous experience (Table III) are highlighted in yellow in Table VIII. In order to characterize the micronutrient content in the biofilm, the biomass was removed from the granular activated carbon growth medium and digested to solubilize the micronutrients. The total Kjeldahl nitrogen content of the biomass was also measured and used to estimate the total biomass removed from the biofilm based on the assumption that biomass contains ten percent nitrogen on a dry weight basis.

TABLE VIII. Biofilm micronutrient concentrations expressed as percentage of biomass (dry weight basis)

Micronutrient	Table III Recommended Micronutrient Concentrations	March 14	April 2	April 17	Average April 2 and April 17
Iron	0.20	0.311	8.3	6.8	7.525
Aluminum	Not established	ND	0.081	0.013	0.047
Manganese	0.20	0.029	0.125	0.039	0.082
Nickel	0.01	0.001	0.013	0.005	0.009
Cobalt	0.05	0.004	0.028	0.004	0.016
Copper	0.02	0.007	0.092	0.068	0.080
Zinc	0.05	0.002	0.046	0.014	0.030
Molybdenum	0.05	0.006	0.028	0.006	0.017
Selenium	Not Established	0.001	0.016	0.001	0.009
Boron	0.01	0.012	0.027	0.017	0.022

Note: Concentrations less than the target values are highlighted in yellow. ND – Not Detected.

The micronutrient concentrations of many metals in the biofilm on the first sampling event (March 14) were lower than the micronutrient needs from the reference plant. The aluminum concentration was below the detection limit for the first sampling event, consistent with the results from the effluent aluminum measurements. The low overall micronutrient concentrations and the undetected aluminum measurement give a strong indication that micronutrient limitation was still occurring during the first sampling event.

The micronutrient concentration in the biofilm increased significantly for the second sampling event (April 2), and the system was not in an upset condition at that time. However, though the FBR system was performing well during the third sampling event (April 17), the micronutrient concentrations decreased during the third sampling event, resulting in micronutrient concentrations that were lower than the reference plant. Since the FBR system was operating well during both the second and third sampling events, the micronutrient results from those events were used to create an updated micronutrient formulation for all metals except iron. The iron concentration measured in the biofilm is very high in all cases, even during the first sampling event. High iron concentrations suggest iron adsorption or precipitation or both were likely occurring in the system.

TABLE IX. Extracellular polymeric substance micronutrient concentration (µg/L)

Micronutrient	March 14 Sample	April 2 Sample	April 16 Sample
Iron	0.15	0.297	0.130
Aluminum	ND	ND	ND
Manganese	ND	ND	ND
Nickel	ND	ND	ND
Cobalt	ND	ND	ND
Copper	ND	0.025	ND
Zinc	ND	ND	ND
Molybdenum	ND	ND	ND
Selenium	ND	ND	ND
Boron	0.185	0.172	0.141

ND – Not Detected

The potential for metal precipitation was evaluated further by measuring the micronutrient concentration in the EPS (Table IX). EPS is different from the total biofilm result (Table VIII). The biofilm result (Table VIII) represents micronutrients present both inside the bacteria themselves and present external to the bacteria in the EPS matrix. The results in Table XII are the micronutrient concentrations measured only external to the bacteria and, because of how the EPS sample was processed, the results in Table VII provide only an indication of which micronutrients might be precipitating. To obtain the results in Table VII, the soluble fraction was washed from the solid biofilm material and the biofilm solids were resuspended in a phosphate-buffered saline solution. No other chemical or physical manipulations were made to the sample. Removing the soluble fraction and the micronutrients it contained would disrupt the equilibrium of precipitated metals in the EPS matrix and encourage dissolution to reach a new equilibrium condition. Therefore, measurement of micronutrients in the EPS control sample provides only an indication of which micronutrients may be precipitating. The results in Table IX support the observation that iron is precipitating in the biofilm matrix. Iron is present in relatively high concentration relative to the other micronutrients in the feed stock. Thus, a high degree of iron precipitation would be expected. The results in Table IX also suggest boron and copper precipitation may be occurring. These metals and others are present in much lower concentrations compared to iron, so a small amount of precipitation could result in micronutrient limitation. Because precipitation occurs faster than biological assimilation, the precipitation reaction can make the nutrient unavailable as bacteria require dissolved nutrients to be present.

Table X summarizes the recommended micronutrient formulation for the system. Because of the strong evidence of iron precipitation, the updated micronutrient formulation used the iron dose provided during periods of good operation rather than the concentration in the biofilm.

The presence of iron precipitation in the biofilm would also support the possibility of a population of iron-reducing bacteria in the biofilm as these organisms are uniquely capable of deriving energy from an iron-sulfate precipitate. The potential for iron-reducing bacteria to thrive under the conditions present in the FBR system creates another biological micronutrient demand in the system. Therefore, iron-reducing bacteria are one of the microbial communities targeted by the qPCR efforts.

TABLE X. Recommended micronutrient formulation for biological treatment systems

Micronutrient	Percent of Dry Mass	Source
Iron	1.24	Plant Experience
Zinc	0.030	Biofilm
Manganese	0.082	Biofilm
Copper	0.080	Biofilm
Molybdenum	0.017	Biofilm
Cobalt	0.016	Biofilm
Nickel	0.009	Biofilm
Selenium	0.0085	Biofilm
Boron	0.022	Biofilm
Aluminum	0.047	Biofilm

Quantitative Polymerase Chain Reaction Results

Before performing the qPCR measurements, the Pacific Northwest National Laboratory performed a preliminary qualitative screening to determine the presence or absence of genetic material associated with each microbial community targeted. Table XI summarizes the qualitative PCR results. As expected, denitrifying bacteria were present in all samples. SRB and iron-reducing bacteria were also detected in all three samples. This result supports the hypothesized mechanism for FBR upsets whereby microbial communities other than the denitrifiers specifically fostered in the FBR system create an additional micronutrient demand (chemically and/or biologically), reducing the micronutrient availability and leading to micronutrient limitation.

Table XI. Qualitative polymerase chain reaction results
("X" indicates the targeted community was detected in the sample)

Targeted Community	March 14	April 4	April 16
Total Bacteria	X	X	X
Total Archaea	-	-	-
Sulfate-Reducing Bacteria	X	X	X
Methanogens	-	-	-
Iron-Reducing Bacteria	X	X	X
Denitrifying Bacteria	X	X	X
Anaerobic Ammonia Oxidizing Bacteria	-	-	-

The qPCR results for denitrifying bacteria are presented in Table XII. The total bacterial abundance is measured based on the number of copies of the eubacterial 16s rRNA gene. Denitrifier abundance is measured based on the copies of two genes known to encode nitrite reductase in bacteria (nirS and nirK). Typically, bacteria will contain either nirS or nirK but not both, so the relative abundance of denitrifiers is determined through the addition of nirS and nirK results [3]. The relative denitrifier abundance during the first sampling event (March 14) shows that only 10 percent of the total bacteria present are denitrifying bacteria, which was surprising since the FBR system is operated for the primary purpose of denitrification. The denitrifier relative abundance increased to 51 percent for the second sampling event (April 4) but decreased to 30 percent relative abundance for the third sampling event (April 16).

TABLE XII. Quantitative polymerase chain reaction results for denitrifying bacteria

2013 Sample Date	Eubacterial 16s rRNA (copies/gram sample)	nirS Gene (copies/gram sample)	nirK Gene (copies/gram sample)	Relative Denitrifier Abundance (% of total bacteria)
March 14	5.43E+06	3.84E+05	1.70E+05	10.20%
April 4	4.18E+06	8.55E+05	1.28E+06	51.08%
April 16	2.36E+06	4.45E+05	2.76E+05	30.55%

Table XIII presents the results for relative abundance of sulfate-reducing and iron-reducing bacteria. The total bacterial abundance is measured based on the number of copies of the eubacterial 16s rRNA gene (result presented in Table X). The abundance of SRB is measured based on the number of copies of a gene encoding for adenosine-5'-phosphosulfate (apsA). The abundance of iron-reducing bacteria is based on the number of copies of a region in the 16s rRNA gene specific to iron-reducers (geobacter). Table XIII results show that sulfate-reducing and iron-reducing bacteria are present, but only at low levels. This would be consistent with the small amount of sulfate being used as an electron acceptor when excess carbon is present in the system. The fact that sulfate reducers are present supports the notion that sulfate reducers can be facilitating micronutrient precipitation, and this conclusion is supported by the presence of iron-reducing bacteria.

TABLE XIII. Quantitative polymerase chain reaction results for sulfate-reducing and iron-reducing bacteria

2013 Sample Date	apsA Gene (copies/gram sample)	Relative Sulfate-Reducer Abundance (% of total bacteria)	Geobacter (copies/gram sample)	Relative Iron-Reducer Abundance (% of total bacteria)
March 14	2.27E+04	0.4%	3.11E+03	0.1%
April 4	3.85E+04	0.9%	6.26E+03	0.1%
April 16	2.04E+04	0.9%	1.08E+03	0.5%

The qPCR results presented in Table X and Table XI were evaluated in the context of FBR denitrification performance and micronutrient concentration results. During the first sampling event, excess carbon was present in the system, but the micronutrient concentrations were very low. Given that only 10 percent of the total bacterial population consisted of denitrifiers at this time, it is clear that another group of bacteria (not sulfate-reducing or iron-reducing bacteria) represented the dominant population at that time (Table XI). It is likely that aerobic heterotrophic bacteria are the dominant population. Since oxygen needs to be depleted at the surface of the biofilm before denitrification can occur, the available micronutrients may have become depleted, making one or more micronutrients the limiting substrate for denitrification.

Reduced carbon dosing coupled with increased micronutrient dosing increased the relative abundance of denitrifiers to 50 percent during the second sampling event. The decrease in the relative abundance of denitrifiers in the third sampling event could be explained by operation of the system in carbon-limited mode combined with reduced micronutrient concentrations. The

qPCR results, particularly the results from the first sampling campaign, demonstrate the need for tight supervision of FBR operation and performance. Shifts in carbon or micronutrient dosing create shifts in the community structure of the biofilm system and encourage the growth of other bacterial communities that compete with denitrifiers for micronutrients. The presence of sulfate-reducing and iron-reducing communities suggests precipitation was also occurring.

CONCLUSIONS

The results of the sampling campaign provide strong evidence that micronutrient limitation was the cause of EPS overproduction that led to FBR upsets. The qPCR results show that the relative abundance of denitrifiers in the FBR system is correlated to the micronutrient concentration. This may be due to micronutrient competition from aerobic microorganisms resulting in one or more micronutrients becoming the limiting substrate for denitrification. The relative abundance of denitrifiers also appears to be a function of carbon dosing. Routine monitoring of the nitrate removal performance relative to the target is recommended. Avoiding excess carbon dosing by maintaining the effluent COD concentration less than 20 mg/L can help reduce micronutrient demands exerted by bacterial populations other than denitrifiers that metabolize carbon.

Strong evidence of iron precipitation was also discovered. Iron precipitation encourages the growth of iron-reducing bacteria and demonstrates that precipitation is also reducing the availability of micronutrients in the FBR system. A new micronutrient formulation was developed based on the results from biofilm micronutrient characterization. The new formulation should satisfy the unique requirements of the 200 West P&T FBR system.

The microbial community is dynamic and changes as the nature of the influent changes. A change in the microbial community may result in changes in the micronutrient demand.

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