Appendix 9: TASK 9- Evaluation of RFAAP Bioplant performance

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Executive Summary

A thorough analysis of the operation of the Bioplant at Radford Army Ammunitions Plant was performed. Along a sample round and the characterization of selected streams discharging into the Bioplant and Bioplant operational units, the assessment also included the review of:

- The Toxic Review Evaluation performed by BAE
- DBOC JV/Haskell's assessment of the wastewater systems at RFAAP
- Bioplant logsheets
- Historical information of flows and available parameters for UMS

The results from the study and recommendations are presented below.

- SVI values fall in the lower end of the recommended range
- Biomass appears to be viable
- Oxygen uptake much higher in the EQ basins than in the RBCs
- 83% BOD_5 removal and a BOD_5 effluent satisfying VPDES daily limits
- TCOD and SCOD data need further analysis
- Solvent evaporative processes at the EQ basis might be playing a significant role in SCOD removal
- Microtox toxicity found for a few streams (pH effects, NG and derivatives present in samples)
- Bioaugmentation products appear not to enhance NG removal (<10% degradation mostly to 1,3 DNG) although might enhance COD removal. This is most likely due to Bioplant operational conditions which are not adequate for promoting NG degradation
- High concentrations of graphite found in the EQ basins sludge
- DNT (mix of 2,4 and 2,6 DNT) only detected in UMS-6 sample; 96 ppb

Moreover, is recommended that the operator should assess the feasibility of increasing phosphorous amendment to the Bioplant as the values measured in the active treatment areas were below detection. The analysis of the flow through the Bioplant during 2023 shows a wide variation. The average and median flows were 0.92 and 0.9 mgd, respectively; however, the standard deviation was 0.15 mgd and the maximum and minimum values were 1.4 mgd and 0.53 mgd, respectively. It is possible that those swings also have an impact on the Bioplant level of treatment.

Regarding the recommendations made in the DBOC JV/Haskell, the tiered approach valued around $70M addressing the acid and industrial streams systems is sound and will help improve the current plant operation. As high NG concentrations that correlate well with microbial toxicity

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were found, it is recommended that the assessment of improvements in the NG interceptors as recommended in the report is prioritized. However, a technoeconomic analysis should be performed as the facility is aging and any major modification to the current plant will require Virginia DEQ approval and might face regulatory challenges. All equipment is subject to Virginia DEQ Sewage Collection and Treatment Regulations (SCAT), Section 9VAC25-790-450 "Treatment Works Design" Part D, "Flooding" which reads: "All mechanical and electrical equipment that could be damaged or inactivated by contact with or submergence in water (motors, control equipment, blowers, switch-gear, bearings, etc.) shall be physically located **above the 100-year level** or otherwise protected against the 100-year flood/wave action damage…"

A review of FEMA maps performed by Haskell shows that the Bioplant is in the Zone AE Floodway and the 100-year flood plain; thus, any major modification likely will require relocation of major process units at which time cost estimations will likely rise and open the possibility of considering the design of a new, state of the art, wastewater treatment plant able to process the industrial streams and the acid streams (upon neutralization) with the associated reduction of nutrient discharges to the New River.

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Introduction

There are three wastewater treatment systems in operation at RFAAP: the AB acid waste treatment plant (discharge: outfall 007), the municipal wastewater treatment plant (trickling filter, discharge outfall 026), and the Bioplant (RBC) treatment system (discharge through outfall 029). The focus of this task is the analysis of the Bioplant operation.

Wastewater generated in propellant manufacturing areas is treated at the Bioplant. Organics are removed using biological treatment. The wastewater is generally pH neutral. Three branches of the collection system combine at the Bioplant. Two branches are forced mains from pump stations. The first is from the 4th rolled powder area and mainly contributes wash-down water and water from sprinkler trips. Storm water from building downspouts is also a significant contribution from this area. The second branch carries mainly wastewater generated by New River Energetics (NRE) operations. A line also enters following the pump station from the DNT/TNT area. This area is not currently in use. If production were occurring in this area, the wastewater would be pretreated in activated carbon columns located in the TNT area. The activated carbon would remove nitro compounds. NRE wastewater collected in this branch has significant graphite content. The third branch carries most of the propellant production wastewater. This branch enters from the west and is a combination of 3 sub-branches. These originate from (1) the horseshoe area – rocket and NG2, (2) NRE and some RFAAP solvent recovery and water dry operations and (3) the remainder of the production area – solvent recovery, water dry, green lines, solvent stills, and NG1 (Figure 1).

In February 2019, SIT performed a comprehensive sampling event of all UMS (upstream monitoring stations) following by chemical and toxicological characterizations (using microalgae as target organism) of those wastewaters and found that some of them were toxic or partially toxic. Moreover, lately, the Bioplant discharge has not met operational requirements and an assessment of its operation is long overdue. This situation led to the issue of several Notes of Violation (NOVs) for TUa (acute toxicity units) and BOD (average and maximum) during the second half of 2021 and the first quarter of 2022. This investigation aims to evaluate the current operation of the Bioplant and perform a preliminary evaluation of the possibility of combining Bioplant biological with AB acid waste treatment with the aim to reduce BOD and TRI discharges into the New River.

Approach

Historical information was procured from RFAAP regarding the discharge flows at outfall 029 and flow readings from selected UMSs (Upstream Monitoring Stations 1, 3, 9, and 12). To close data gaps in the historical record of Bioplant operational parameters and the chemical/physical

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characterization of the streams that make up the Bioplant influent, a sampling program was developed as described in the scope of work (SOW) below.

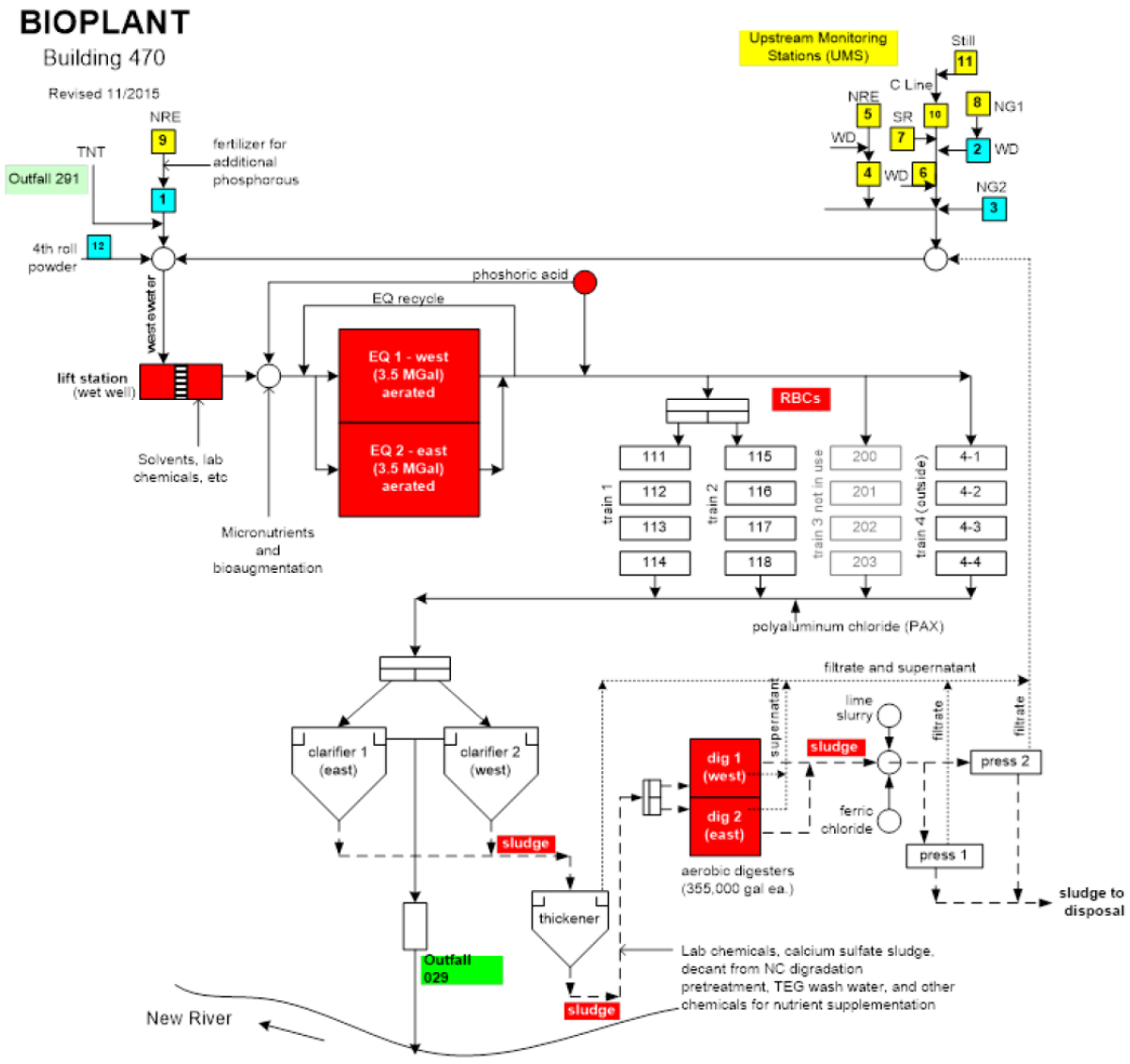

Figure 1. Bioplant schematics.

Scope of Work

The work was performed as per following subtasks:

Subtask 9.1: Procure and analyze historical data from RFAAP on discharges from outfall 029 and flows in UMSs 1, 3, 9, and 12.

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Subtask 9.2: Arrange with RFAAP a sampling round to be performed in the second quarter of the project. Three gallons samples were collected following the procedures used in February 2019. Beyond the 10 UMS samples taken in 2019, samples were collected from the lift station, equalization basins EQ1 and EQ2, the RBC's biomass from operating trains.

Subtask 9.3: Chemical and toxicological characterizations of the obtained samples as follows:

- pH
- TOC, COD, BOD
- Total phosphorous, NH4+, Nitrate, nitrite, sulfate
- NG, NC
- Conductivity and TSS
- Metals: Al, Ca, Cr, Cu, Fe, K, Na, Zn, Pb, As, Mn, Ni, and Cd
- Toxicity studies (Scenedesmus obliquus and Microtox)

Deliverables

Deliverables for this task include:

- Full characterization of the streams discharging into the Bioplant
- An assessment of the Bioplant operation. Identification of wastewater streams that might impair the Bioplant operation.

Work performed

Several telecoms were held with BAE/RFAAP and DEVCOM-AC to discuss practical aspects of the work to be performed and to present the results to the stakeholders. The first priority was the signing of the NDA between BAE/RFAAP and SIT. The NDA agreement was fully executed on 8/10/2022 covering information sharing and sampling events. Arrangements were made to transfer relevant Bioplant operational data, data from UMSs 1, 3, 9, 5, and 7B (the last one suggested by BAE, currently known as UMS10), NOVs (notices of violation), and historical chemical characterization of the streams fed into the Bioplant from RFAAP to SIT via DEVCOM-AC. Although NOVs occurred during 2021 and first quarter of 2022, changes in the performance of the Bioplant were detected at the beginning of 2019; thus, data from 2018 were requested in order to develop an operational baseline for the Bioplant's performance. Moreover, originally, we requested flow information for UMS12; however, we were informed that flow data from that UMS was not available.

During the period of performance of this study, we continuously monitored RFAAP Bioplant operation based on the information supplied by RFAAP. Moreover, we also reviewed the full DBOC JV/Haskell report (2023) along the Toxic Reduction Evaluation performed by BAE Systems Inc, (TRE, 2022). The schematic of the Bioplant is presented in Figures 1 and 2 along with a description of the upstream monitoring stations (UMSs), and a schematic of the Bioplant collection system (Figure 3). Data (flow, pH, and TC) from several UMS were also furnished by

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RFAAP. Samples were collected on February 15, 2023, and brought back to Stevens for characterization. The yellow highlighted samples in Table 1 were delivered to Stevens on 3/16/23. This report presents the results of the full characterization.

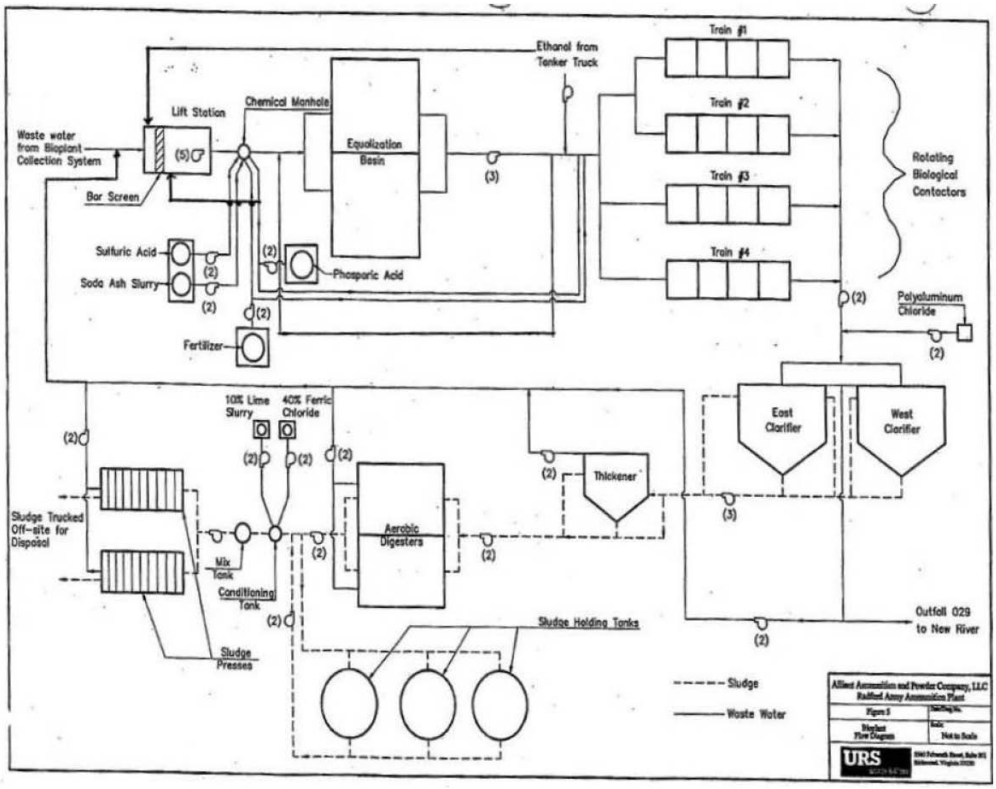

Figure 2. Bioplant schematic. Observe the discharge lines from the clarifiers, thickener, aerobic digesters, and sludge press to the lift station, EQ basins and RBCs.

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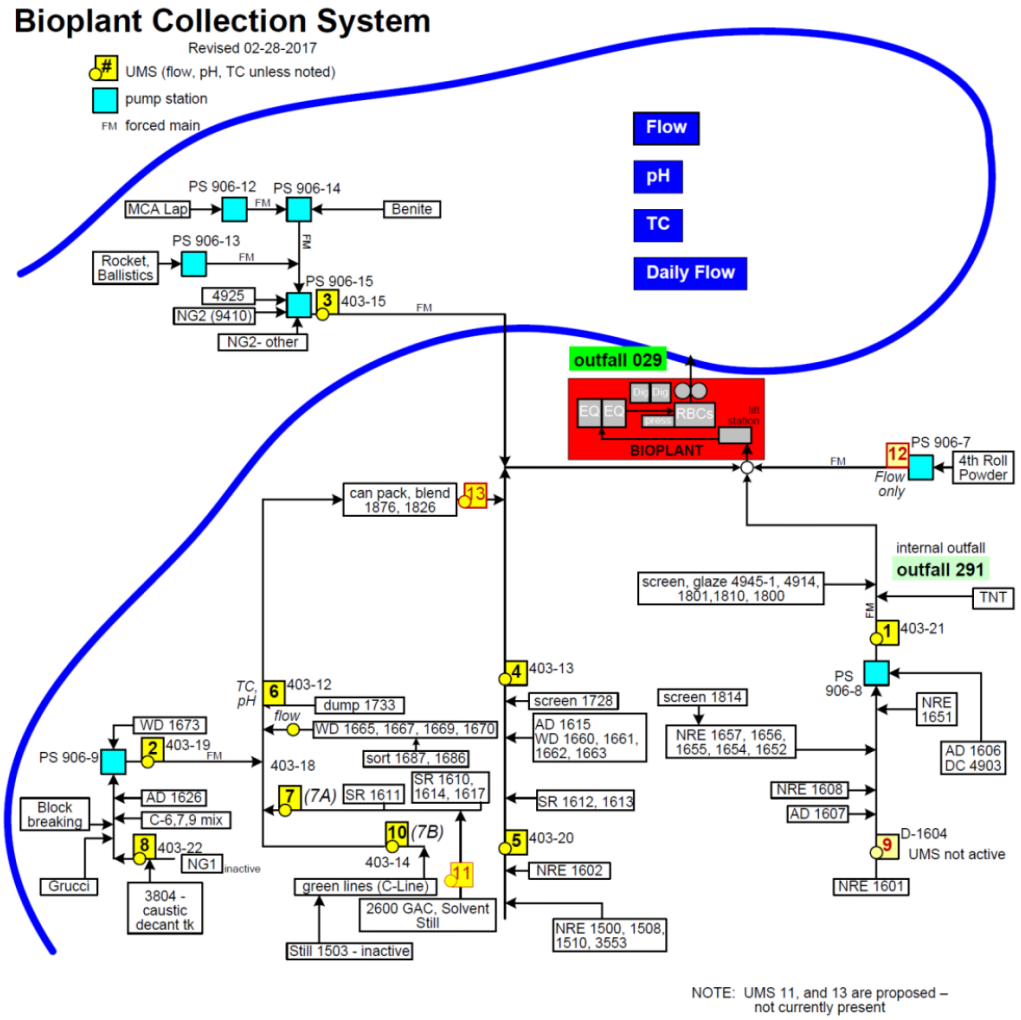

Figure 3. Bioplant collection system

UMS description (information furnished by BAE in a previous project)

Figure 3 presents a more detailed view of the Bioplant collection system. Below is the BAE provided description of the locations and monitoring performed at the different UMSs.

UMS 1: This station is located following pump station 906-8. Wastewater is mainly collected from NRE operations. This station includes monitoring for flow, pH, and TC. The flow meter is of the magnetic in-line type and the sample pump is pneumatic. UMS 9 is upstream of this station. UMS 1 is located 100 yards east of Bldg. 1658.

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UMS 2: This station is located following pump station 906-9. This pump station receives wastewater generated in the NG2 area and from some water dry buildings. This station includes monitoring for flow, pH, and TC. The flow meter is of the magnetic in-line type and the sample pump is pneumatic. UMS 8 is upstream of this station. UMS 2 is located 100 yards northwest of Bldg. 420-2.

UMS 3: This station is located following pump station 906-15. This station receives all wastewater generated in the horseshoe area of the plant. This includes wastewater from the Rocket area and NG2. This station includes monitoring for flow, pH, and TC. The flow meter is of the magnetic in-line type and the sample pump is pneumatic. UMS 3 is located 100 yards northwest of Bldg. 9410.

UMS 4: This station monitors wastewater generated in the center portion of the plant. This stream is comprised mainly of NRE wastewater and wastewater from RFAAP water dry and solvent recovery buildings. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. UMS 5 is located upstream of this station. The UMS 4 monitoring building is located 150 yards southwest of Bldg. 1728.

UMS 5: This station monitors wastewater generated in the NRE operations. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. The UMS 5 monitoring building is located 200 feet south of Bldg. 1612.

UMS 6: This station monitors wastewater from a variety of operations. This station is unique in that the flow monitoring and TC and pH monitoring are of separate streams. Flow is measured in a stream comprised of wastewater from a series of water dry buildings. The flow meter is of the capacitance probe type and the sample pump is pneumatic. The sample collected for pH and TC analysis at UMS 6 is taken from the main flow which combines wastewater from several operations (i.e., solvent recovery, green lines, solvent stills, water dry, and NG1). The flow for this stream can be calculated by adding the flow from UMSs 2, 6, 7, and 10. UMSs 2, 8, 7, 10 and 11 are upstream of this station (when considering pH and TC). The UMS 6 monitoring building is located 100 yards west of Bldg. 1672.

UMS 7 (Formerly 7A): This station monitors wastewater from a series of solvent recovery buildings. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. The UMS 7 monitoring building is located 150 yards west of Bldg. 1610.

UMS 8: This station monitors wastewater from the NG1 area. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. The UMS 8 monitoring building is located 150 feet south of Bldg. 3622.

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UMS 9: This station monitors wastewater from NRE operations in building 1601. This operation produces wastewater high in graphite. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. The graphite present in the wastewater frequently interferes with monitoring for pH and TC due to fouling and blocking of the sampling line. The UMS 9 monitoring building is located 100 feet east of Bldg. 1604.

UMS 10 (Formerly 7B): This station monitors wastewater form the green lines and solvent stills. This station includes monitoring for flow, pH, and TC. The flow meter is of the capacitance probe type and the sample pump is pneumatic. UMS 11 is located upstream of this station. The UMS 10 monitoring building is located 30 feet northeast of Bldg. 1999.

UMS 11: This station monitors the bottoms from solvent still building 1503. This station includes monitoring for pH and TC. Flow is not measured. The monitoring equipment is located on the 1st floor of building 1503.

UMS 12: This station monitors the wastewater from pump station 906-7. The wastewater is from operations in the 4th Roll Powder area. This station monitors flow only. The magnetic in-line flow meter is located in the pump station discharge line. There is no monitoring building, but the monitoring location is 200 yards north of Bldg. 9334-7.

Analysis of the information provided by RFAAP

Two lines of investigation were followed in the TRE (Toxic Release Evaluation); Bioplant operation and influent source identification/reduction. The conclusions of the study along with the corrected actions taken during the first quarter of 2022 are summarized as follows:

- No single event or toxicant responsible for failed toxicity tests
- Key reported points and observations
  - NOVs provided show COD_{Max}, BOD_{5Max}, BOD_{5ave}, and TUa violations for outfall 007 (last quarter 2021)
  - TUa exceedances in outfall 029 and 007 in Q3, 2021 and Q1, 2022 (colder months)
  - Insufficient amount of phosphates (corrected)
  - No NG adsorption within the Bioplant treatment train
  - Solid accumulation in the EQ basins (where most of the biological treatment occurs) (corrected)
  - Seeding products: Microsolve 118, Microclear M100, Bioremove COD (oil industry), MB2 (for NG), MB MN.
  - Inflow and Infiltration resulting in hydraulic overloading (corrected)

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- Solvent peaks (ethanol) (corrected)
- Monitor discharges from Northrup Grumman Defense Systems (solvent still recovery process)
- No direct correlation between pulses of NG and failed toxicity tests
- Currently NG is not being removed withing the Bioplant system. More NG detected in the clarifier than entering the system. Place of adsorption unknown.
- Clean up of the EQ tanks located in the pretreatment system for NG-2 did not decrease NG loading to the Bioplant
- Evidence for toxicity test strongly suggest that NG is not the only toxicant responsible for failed toxicity tests
- Graphite coatings can inhibit biofilm attachment and growth (RBCs) and movement of oxygen and nutrients through the biofilm
- Methyl centralite (1,3-Dimethyl-1,3-diphenylurea from coating operations) role in NOVs to be assessed
- More stringent upstream controls of solvents and graphite entering the Bioplant implemented early 2022 along with the cleanup of the equalization basins expected to result in a more balanced microbial community and improved treatment. Toxicity evaluation results from the second and third quarter of 2022 show toxicity values below 1.0 TUa within facility's VPDES (Virginia Pollution Discharge Elimination System permit) which seems to support expectations. The results, although encouraging, need to be taken cautiously as the monitoring period also coincided with higher ambient temperatures and enhanced microbial activity.
- NOVs coincide with periods of higher continuous TC measured at UMS6 although flow through was relatively small
- Discuss solids recycling within the Bioplant and effect of supernatants from clarifier, thickener and filter press to the EQ and RBCs (chemical amendments). As shown in Figure 1 and more clearly in Figure 2 above, no biomass recycling lines are shown in the Bioplant schematics. During conversations with RFAAP personnel it was stated the biomass is recycled to the RBCs and the EQ basins, however, detailed information regarding schedule and amounts of biosolids recycling could not be obtained.

Based on the information available, which includes discussions with DBOC JV (company which made an operational assessment of the Bioplant operation), an updated sampling plan which includes DBOC and SIT recommendations was compiled and shown in Table 1.

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Table 1. Sample recommendations by DBOC JV (Haskell, rows 1-5). Stevens' recommendations for additional sampling (rows 6-10)

Table 1: Sampling Recommendations by Haskell				
No.	Process	Locations	Parameters [1]	Notes
1	Pump Station No. 906-17 (Raw Influent)	Wet Well Liquid and Bottom Sludge	• Graphite concentration • Graphite particle size analysis • NG, NC • TSS, COD, pH, Temp, TP, TKN, Sulfate, Conductivity, ORP	Total 2 sample locations
2	EQ Tank, East and West	1) Mixed area, 2) Unmixed area in corner, & 3) Sludge at bottom	• Micro-organism identification [2] • Floc identification [2]	Total 6 sample locations
		Mixed area	• TSS, COD, pH, Temp, Conductivity, ORP • Dissolved Oxygen	
3	RBC Trains No. 1, 2 & 4 (No. 3 is offline)	Influent End & Effluent End (3 different units)	• Graphite • NG, NC • TSS, COD, pH, Temp, TP, TKN, Conductivity, ORP • Dissolved Oxygen	Total 6 sample locations
		Basin Middle & RBC Media Scrape (3 different units)	• Micro-organism identification [2] • floc identification [2]	Total 6 sample locations
4	Clarifiers, East and West	Influent (MLSS)	• Graphite • NG, NC • Sludge volume index (30 minutes) • TSS, COD, pH, Temp	
		Sludge	• Batch settleability curve test (see notes) • TSS	Measure sludge depth every minute for 40 minutes and plot.
5	Final Effluent	Near Flume	• Graphite • NG, NC • TSS, COD, BOD, pH, Temp, TP, TKN, Sulfate	
6	Monitoring Stations	UMSs 1,3,9,5, 10 (7B), and 11	• Graphite, • Solvents • NC, NG (and daughter products) • COD, pH, TSS	7 samples total
7	Thickener	Effluent wastewater (EQB and/or RBC return lines)	• Graphite • NG, NC • TSS, COD, pH • Al, Cl	1 sample

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8	Aerobic digesters	Effluent wastewater (EQB and/or RBC return lines)	• Graphite, • Solvents • NC, NG (and daughter products) • COD, pH, TSS	2 samples
9	Conditioner tankers/sludge press	Effluent wastewater from sludge press (EQB and/or RBC return lines)	• Fe, Cl, Al • TSS, pH, TSS, COD	1 sample
10	Clarifiers (East and West)	Effluent wastewater (EQB and/or RBC return lines)	• Graphite • NG, NC • TSS, COD, pH • Al, Cl	2 samples total. How often recycle occurs? This is the water discharged into the New River

1) All grab samples
2) Recommend an experienced biomass analysis expert to perform a microscopic review and consider the unique arrangement of this WWTP and provide conclusions and recommendations for operations adjustments and improvements. For example, Environmental Leverage Inc. It would be beneficial to repeat this analysis in January when the wastewater is coldest.

As several processes were not operational at the time of the sampling, the highlighted samples, rows 7 to 10, were collected by BAE/RFAAP personnel on March 15, 2023, once the sludge processing processes were operational, and delivery to SIT by 3/16/23. Currently, 100% of the sludge collected in the clarifiers is recycled either to the EQ basins or the head of the RBC trains. The visual inspection of the three operational RBCs trains shows a relatively small amount of biomass attachment. Scrap samples were taken from each of the 4 stages of the trains 1 and 2 and from stages 2-4 from train 4 and the rotators in stage 1 were not turning at the time of sampling. Moreover, samples of the bioaugmentation products used by BAE/RFAAP were also provided by the facility personnel for a preliminary assessment. These products are:

- MB-2: a highly specialized blend of bacteria which will remove BOD, COD, TOC, phenol, chlorinated hydrocarbons, aromatic and aliphatic hydrocarbons meta and ortho chlorinated phenols, propylene dichloride, styrene, formaldehyde, and alcohols.
- MB MN micronutrients: balanced blend of micronutrients specially formulated for industrial and municipal wastewater treatment (to be shipped at a later time).
- BioRemove™ COD (MB-8) is Novozymes' most versatile product for industrial wastewater and is effective for highly complex waste streams. It has a diverse set of microorganisms that target a wide range of organics found in a variety of industries. BioRemove™ COD has the broadest range of biological activity and helps keep plants in compliance and in control.
- MicroSolv™ 118: a high potency, bacteria-laden, powdered formulation, for use in degrading many types of waste. MicroSolv™ 118 contains a specially formulated blend of microorganisms, micro/macronutrients, fungi, and surface tension

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suppressants/penetrants, developed for use in bioaugmentation. Because of the diversity of microorganisms this bioaugmentation product is excellent for pulp & paper wastewater applications where there are heavy influxes of cellulosic fibrous organic solids. The enzyme activity enhances cellulosic reduction by converting the fibrous solids into carbon dioxide and water.

- Microclean^{R} 100: a proprietary formulation of micronutrients designed to enhance the performance of biological systems. It enhances biological growth, shortens lag time, increases biological activity, and provides the critical building blocks necessary to maintain a healthy floc-forming population. The formulation consists of micronutrients, trace minerals, amino acids, and vitamins. There are sixteen trace minerals, including calcium, iron, magnesium, manganese, and zinc. The formulation also includes twenty amino acids, such as glycine, and thirteen vitamins, including folic acid.

Table 2. Sample IDs and sampling locations from sampling event at RFAAP. S=Sludge, L=liquid.

Sample ID	Sample Location	Matrix
906-17 #1	pump station No.906-17	S
906-17 #2	pump station No.906-17	L
EQ E_MA	EQ tank, east, mixed area	L
EQ W_MA	EQ tank, west, mixed area	L
EQ E_UA	EQ tank, east, unmixed area	L
EQ W_UA	EQ tank, west, unmixed area	L
EQ E_S	EQ tank, east, sludge	S
EQ W_S	EQ tank, west, sludge	S
RBC1_Inf	RBC Train1, Influent	L
RBC1_Eff	RBC Train1, Effluent	L
RBC1_BM	RBC Train1, Basin media	S
RBC1_MS	RBC Train1, Scrape media	S
RBC2_Inf	RBC Train2, Influent	L
RBC2_Eff	RBC Train2, Effluent	L
RBC2_BM	RBC Train2, Basin media	S
RBC2_MS	RBC Train2, Scrape media	S
RBC4_Inf	RBC Train4, Influent	L
RBC4_Eff	RBC Train4, Effluent	L
RBC4_MS	RBC Train4, Scrape media	S
C-East	Clarifier East	L
C-West	Clarifier West	L
Effluent	Final effluent near flume	L
UMS-1	monitoring station 1	L
UMS-3	monitoring station 3	L
UMS-5	monitoring station 5	L

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UMS-6	monitoring station 6	L
UMS-9	monitoring station 9	L
UMS-7B	monitoring station 7B	L

The parameters selected for the initial characterization are as follow:

- pH
- Biosolids concentration (TSS, VSS)
- Biological Oxygen Demand at 5 days (BOD_5)
- Total organic carbon (TOC)
- Chemical Oxygen Demand (COD)
- Sludge volume Index (SVI)
- Inorganic nitrogen concentrations (nitrate, nitrite, ammonia)
- Selected energetic compounds (NG, DNT)
- Graphite
- Total Phosphorous
- Selected heavy metals

It should be noted that not all parameters were measured for all samples, but targeted analyses were applied to selected samples in accordance with significance and pre-established characterizations objectives. Characterization is ongoing at the time of releasing this report; however, preliminary results of analysis are included in the Results section.

Analytical Methods

A brief description of analytical methodologies utilized during the sample's characterization process is presented in this section. Other analytical procedures will be made available in future reports as additional parameters are characterized.

- Total suspended solids (TSS) and volatile suspended solids (VSS) analysis are conducted according to procedure described in the standard methods 2540D and 2540E. The solids procedure involves a measured volume of samples (i.e., 5-20 mL) passed through a prepared, pre-weighed ceramic crucibles provided with dedicated glass fibers filters (0.45µm, 934-AH, Whatman, Fisher scientific). After filtration, the filter and any collected particles are dried in an oven at 104 ± 1°C for two hours. After drying, the filter is reweighed and the TSS is calculated by the difference in weight respect to the sample volume filtered. Following determination of the TSS, the same samples are then placed in a furnace at 550 °C for at least 30 min, for VSS determination. A modification of this process, as per BAE information, will be utilized to determine graphite content of selected samples as described in Table 26.

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- The pH measurements are carried out by using glass probe with standard BNC connection and bench pH meter Thermo Orion Stara1110 Star A111 (Thermo Scientific) calibrated with standard buffer solutions of 4.0, 7.0 and 10.0 value.
- Chemical Oxygen Demand (COD), total and soluble. Total Chemical Oxygen Demand (COD) is measured using HACH COD TNT plus vial kits (Kit number 822, testing range 20-1500 mg/L). The kits procedure follows the reference method 8000 which is USEPA approved for wastewater analysis under 40 CFR 136.3 and based on standard method 5220D. The total COD (TCOD) is measured using sampled mixed liquor while the soluble COD (SCOD) refers to the soluble portion found in the collected effluent after separation of any suspended solids through sample filtration using disposable syringe filters (pore size 0.45 μm).
- Sludge volume Index (SVI) is determined according to the Standard Method 2710D. Briefly, the SVI is the volume in ml occupied by 1 g of a sludge suspension after 30 minutes settling time; it is typically used to monitor the settling characteristics of activated sludge. In general, a good quality sludge has an SVI in the range of 50-150 mL/g.
- Nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_4^+). Nitrite and nitrate concentrations are analyzed by using IC (Dionex Asap Series) with a AS18 column (2 mm×250 mm, Dionex) as stationary phase and DI water with 26 mM KOH as mobile phase. The flow rate was fixed to 0.25 ml/min. For the conditions described the nitrite and nitrate had a retention time of 5.58 and 8.50 min, respectively. Ammonia concentrations were measured using IC with IonPac ®AS16 column (4 mm×250 mm, DIONEX), equipped with a guard column (IonPac®AG16 (4 mm×50 mm) DIONEX). The eluent was given by a solution of 26 mM of methanesulfonic acid (0.1 M MilliporeSigma, Supelco 555171L) while the flow rate was fixed to 1.0 ml/min. At such conditions, the retention time of ammonia was 9.1 min.
- Biodegradable Oxygen Demand at 5 days (BOD_5) analysis is conducted following the procedure described in the standard method 5210B. Briefly, the method consists of filling with diluted and seeded sample an airtight bottle with a ground-glass stopper and a flared mouth of specified size (300 mL) and incubating it at 20 °C for 5 days. Dissolved oxygen is measured at the beginning and at the end of the incubation period; the BOD is then computed from the difference between initial and final DO by considering sample and seed volume. BOD seed inoculum was added through use of Polyseed capsule (HACH); each capsule is dispersed directly into the dilution water and mixed for an hour to allow seed revival. Afterwards, the solution is settled for 15 minutes, and decanted supernatant is used as seed source; standard procedure requires the addition of 4 mL of seed to each BOD bottle. Each capsule supplied about 500 mL of seed solution; the solution was prepared fresh for each batch of BOD tests. For each selected sample, the BOD test is performed in duplicate and for each batch test, a glucose control standard (120 mg/L) is added as an additional sample in order to check the validity of the test.

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- The BOD_5 test procedure was utilized also as base to execute an oxygen uptake test on few sludge samples to test the viability of the biomass. In this instance, instead of using HACH Polyseed as source of seed, about 1 g of sludge sample was dispersed in 500 mL of BOD_5 dilution water and aerated following the same procedure required for Polyseed. The decanted supernatant was then used directly to fill the BOD bottles with the addition of 0.5 g of glucose as biodegradable organic. The bottles where incubated for a period of about 20 hours and the DO difference between the initial and final values was recorded as oxygen uptake.

Results

Table 3 shows the analytical results for operational parameters of the Bioplant (pH, BOD, SCOD, TCOD, SVI, TSS, VSS and oxygen uptake). Experimental values indicate that the SVI falls in the lower end of the recommended range and that the biomass appears to be viable as per the 20 hours oxygen uptake measured. However, oxygen uptake appears to be much higher in the EQ basins than in the RBCs, which aligns with previous assessments reporting that most of the biological treatment occurs in the EQ basins. BOD_5 was assessed at three locations (lift station, influent to RBC2, as a measure of EQ basins BOD reduction, and final effluent near flume) to get an idea of its fate across the treatment system. The results show an overall 83% BOD_5 removal and a BOD_5 effluent satisfying VPDES daily limits. TCOD and SCOD data need further analysis; however, the picture portraited by the Bioplant influent data, EQ data and UMS data, suggest that solvent evaporative processes at the EQ basis might be playing a significant role in SCOD removal.

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Table 3. Characterization of RFAAP samples collected on 2/15/2023 and 3/15/2023.

Sample ID	Matrix	pH	BOD5 (mg/L)	SCOD (mg/L)	TCOD (mg/L)	*SVI	TSS (g/L)	VSS (g/L)	^Oxygen Uptake (mg/L)
906-17 #1	S			40.0			0.421	0.281	
906-17 #2	L	7.01	335	300	2000		0.083	0.050	
EQ E_MA	L	7.04		57.6	2670	56.7	0.871	0.685	
EQ W_MA	L	6.69		59	2590	56.7	0.782	0.628	
EQ E_UA	L	7.07		37.3			0.830	0.626	
EQ W_UA	L	6.79		42.1			9.84	8.03	
EQ E_S	S						36.4	27.3	5.28
EQ W_S	S						47.3	38.4	3.51
RBC1_Inf	L	7.36		34.7	2910				
RBC1_Eff	L	7.42		40.0	3540				
RBC1_BM	S								
RBC1_MS	S						0.0507°	0.0403°	1.63
RBC2_Inf	L	7.52	214	40.3	3100				
RBC2_Eff	L	7.55		35.2	3190				
RBC2_BM	S	7.17		39.2					
RBC2_MS	S						0.0464°	0.0298°	1.81
RBC4_Inf	L	7.45		40.3	3040				
RBC4_Eff	L	7.33		30.5	3450				
RBC4_MS	S						0.0428°	0.0342°	1.87
C-East	L	7.88		52.4					
C-West	L	7.72		43.6					
Effluent	L	7.38	56	82.1					
UMS-1	L	6.17		1840					
UMS-3	L	7.56		170					
UMS-5	L	6.94		750					
UMS-6	L	7.03		241					
UMS-9	L	6.80		2300					
UMS-7B	L	7.14		260					
T_EW	L	7.39							
AD#1_EW	L	4.27							
AD#2_EW	L	4.11							
SP_EW	L	12.32							
C_East_EW	L	7.35							
C_West_EW	L	7.38							

*Measurement was performed from composite sample (EQ_E_MA and EQ_W_MA).

^values were obtained as difference in DO over a period of 20 hours.

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°values are in unit of g/g.

Table 4 presents the results of macronutrients and TOC at different points throughout the Bioplant treatment system (including selected UMSs). Results in Table 4 suggests that the system could benefit from an increase in phosphorous dosage as total phosphorous concentration throughout most of the system were below the detection limit.

Table 4. Results of macronutrients and TOC assessment throughout the Bioplant and collection system.

No	Sample ID	Matrix	TP (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	TOC (mgC/L)
2	906-17 #2	L	ND	0.509	<1	ND	59.7
3	EQ E_MA	L	ND	0.426		0.3	4.8
4	EQ W_MA	L	ND	4.13		0.6	4.1
5	EQ E_UA	L	ND	0.879	<1	1.3	5.0
6	EQ W_UA	L	0.037	0.563	<1	19.5	6.8
9	RBC1_Inf	L	ND	0.678	<1	0.6	4.5
10	RBC1_Eff	L	ND	1.09	<1	0.7	4.5
13	RBC2_Inf	L	ND	0.736	<1	0.6	4.4
14	RBC2_Eff	L	ND	0.799	<1	0.7	4.1
17	RBC4_Inf	L	ND	0.524	<1	0.8	4.8
18	RBC4_Eff	L	0.004	0.689	<1	0.7	4.7
21	C-East	L	ND	4.001	<1	ND	4.5
22	C-West	L	0.002	3.94	<1	ND	4.7
23	Effluent	L	0.003	4.29	<1	ND	4.2
24	UMS-1	L	ND	13.12	9.352	ND	453.4
25	UMS-3	L	ND	68.69	24.36	ND	55.2
26	UMS-5	L	ND	5.54	1.236	ND	165.4
27	UMS-6	L	ND	1.75	1.342	ND	65.2
28	UMS-9	L	0.119	23.19	28.44	ND	386.2
31	UMS-7B	L	ND	0.9579	<1	ND	68.4
32	T_EW	L	<0.05	6.8	ND		2.8
33	AD#1_EW	L	0.198	194.7	ND		23.3
34	AD#2_EW	L	1.178	298.6	ND		68.6
35	SP_EW	L	0.323	155.71	ND		37.5
36	C_East_EW	L	0.05	5.58	ND		3.6
37	C_West_EW	L	0.052	5.46	ND		3.0

Table 5 presents the results for selected metals throughout the Bioplant treatment system. The results shown in Table 5 indicate that there is no evidence of degraded Bioplant performance due to metal contamination.

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Table 5. Selected metals concentration throughout the Bioplant and collection system.

No	Sample ID	Sample Location	Al (mg/L)	Ca (mg/L)	Cr (ug/L)	Cu (ug/L)	Fe (ug/L)	K (mg/L)	Na (mg/L)	Zn (mg/L)	Pb (ug/L)	Co (mg/L)	Mo (mg/L)	Mn (ug/L)	Ni (mg/L)	Cd (ug/L)
2	906-17 #2	pump station No.906-17	1.88	22.42	5.4	87.9	32.3	45.06	52.48	0.16	252.6	N.D	N.D	13.4	N.D	0.6
3	EQ E_MA	EQ tank, east, mixed area	0.36	30.40	2.6	24.7	246.4	56.88	137.7	0.3	67.9	0.02	0.04	3.6	0.02	0.7
4	EQ W_MA	EQ tank, west, mixed area	0.25	29.04	9.1	30.9	365.8	70.96	105.1	0.78	92.8	N.D	0.02	9.7	N.D	5.7
5	EQ E_UA	EQ tank, east, unmixed area	0.02	29.94	3.5	N.D	N.D	56.4	136.88	0.42	N.D	0.1	0.38	1.1	0.12	2.1
6	EQ W_UA	EQ tank, west, unmixed area	8.52	92.60	93.6	53.1	30.8	142.24	110.28	1.38	1094.1	N.D	0.1	2.1	0.04	62.5
9	RBC1_Inf	RBC Train1, Influent	17.49	30.32	46.9	152.0	196.1	57.82	137.78	0.3	2978.5	0.04	0.08	227.2	0.04	11.2
10	RBC1_Eff	RBC Train1, Effluent	2.09	29.18	13.2	97.6	565.6	56.96	138.52	0.22	269.1	N.D	0.02	24.2	N.D	4.6
13	RBC2_Inf	RBC Train2, Influent	0.75	30.32	3.4	49.8	614.0	60.16	143.28	0.22	135.2	N.D	0.02	7.6	N.D	1.3
14	RBC2_Eff	RBC Train2, Effluent	1.26	29.50	27.4	86.4	N.D	59.28	140.26	0.18	209.0	N.D	0.02	31.6	N.D	20.2
17	RBC4_Inf	RBC Train4, Influent	24.87	32.64	67.6	1018.5	11333.5	60.08	142.44	0.26	2519.7	N.D	0.02	152.8	N.D	29.8
18	RBC4_Eff	RBC Train4, Effluent	2.68	16.90	11.3	124.0	1309.5	44.34	134.04	N.D	318.6	N.D	N.D	20.7	N.D	2.4
21	C-East	Clarifier East	1.15	31.36	24.4	81.6	904.7	57.98	141.94	0.22	188.1	N.D	0.02	27.7	N.D	18.7
22	C-West	Clarifier West	0.17	16.94	2.4	3.3	52.7	44.8	133.76	0.02	23.2	N.D	0.02	2.4	N.D	1.7
23	Effluent	Final effluent near flume	0.79	17.26	11.6	32.2	388.7	44.6	135.34	N.D	73.3	N.D	N.D	12.6	N.D	7.0
24	UMS-1	monitoring station 1	7.84	28.02	41.0	88.5	527.4	109.38	13.44	0.02	882.1	N.D	N.D	15.6	N.D	24.1
25	UMS-3	monitoring station 3	0.01	14.98	1.4	N.D	N.D	2.98	485.04	0.04	3.6	N.D	N.D	0.2	N.D	0.6
26	UMS-5	monitoring station 5	N.D	25.76	0.3	N.D	N.D	39.62	11.4	0.04	11.1	N.D	0.02	0.0	N.D	0.5
27	UMS-6	monitoring station 6	1.09	31.00	17.2	71.0	829.7	3.92	21.46	0.1	166.8	N.D	N.D	21.8	N.D	12.8
28	UMS-9	monitoring station 9	0.64	14.86	15.6	56.3	N.D	196.26	8.74	0.02	108.2	N.D	0.02	18.3	N.D	8.0
31	UMS-7B	monitoring station 7B	2.92	16.94	39.0	157.0	164.4	3.06	14.14	N.D	365.9	N.D	N.D	4.7	N.D	25.9
32	T_EW	Thickener, Effluent wastewater (EQB and/or RBC return)	7.01	28.52	19.8	283.8	8303.1	66.92	109.12	0.08	884.4	N.D	N.D	39.5	N.D	2.6
33	AD#1_EW	Aerobic, Effluent wastewater (EQB and/or RBC return)	0.06	225.04	1.2	6.2	3.1	116.88	235.76	3.32	6.4	N.D	0.08	0.2	0.16	1.4
34	AD#2_EW	Aerobic, Effluent wastewater (EQB and/or RBC return)	0.03	296.28	5.9	N.D	N.D	193.64	243.24	5.92	2.7	N.D	0.16	0.5	0.28	0.4
35	SP_EW	Sludge press, Effluent wastewater (EQB and/or RBC return)	6.46	296.36	37.4	460.7	1760.0	111.12	190.6	0.08	841.7	N.D	0.04	37.6	N.D	3.2
36	C_East_EW	clarifier, Effluent wastewater (EQB and/or RBC return)	0.15	26.80	6.4	0.6	25.5	61.36	104.64	0.04	15.9	N.D	N.D	1.8	N.D	1.5
37	C_West_EW	clarifier, Effluent wastewater (EQB and/or RBC return)	0.07	27.84	7.6	2.9	59.1	63.68	110.6	0.08	15.6	N.D	N.D	2.5	N.D	2.5

Graphite levels were assessed throughout the system. For most of the samples, graphite was not detected; however elevated levels were found in the unmixed area of the west equalization basin and in the sludge of both EQs (1673, 8008, and 7095 mg/L in EQW_UA, EQE_S, and EQW_S samples, respectively). Graphite concentrations between 4 and 10 mg/L were found in the biomass scraped from RBCs 1, 2 , and 4.

The presence of NG and its degradation products throughout the Bioplant system has been a concern for the facility operator. An assessment of NG in the system was performed and the results are presented in Table 6. As observed in the table, high NG concentration levels were measured in UMS 1, 3, and 9, while concentration levels between 7 and 20 mg/L of 1,3 DNG (dinitroglycerin) were measured in the supernatant return line from the clarifiers and aerobic digestors. UMS 3 showed a high concentration of 1,2 DNG at the time of the sampling. Moreover, another energetic of concern, DNT (assessed as a mixture of 2,4 and 2,6 DNT) was only found in the UMS 6 sample (96 μg/L).

Table 7 presents the results of the Microtox assessment (expressed as 15 minutes EC50% concentration). As the table shows, is a strong correlation between toxicity and the presence of high NG concentration in the sample. This might be one of the reasons of the Bioplant operational upsets, if the contribution of a high NG concentration stream to the total daily flow

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at a given time is significant , microbial activity might be affected and proper biological treatment diminished.

Table 6. NG and denitrated byproducts assessment (concentration expressed and mg/L).

No	Sample ID	1-MNG (ppm)	2-MNG (ppm)	1,2-DNG (ppm)	1,3-DNG (ppm)	NG (ppm)
2	906-17 #2	<0.5	0.70	<0.5	2.32	1.78
3	EQ E_MA	<0.5	<0.5	<0.5	1.58	0.95
4	EQ W_MA	<0.5	<0.5	<0.5	1.52	0.97
5	EQ E_UA	<0.5	<0.5	<0.5	2.32	1.07
6	EQ W_UA	<0.5	<0.5	<0.5	2.28	1.08
9	RBC1_Inf	<0.5	<0.5	<0.5	2.15	0.92
10	RBC1_Eff	<0.5	<0.5	<0.5	2.08	1.09
13	RBC2_Inf	<0.5	<0.5	<0.5	1.47	1.08
14	RBC2_Eff	<0.5	<0.5	<0.5	1.93	0.85
17	RBC4_Inf	<0.5	<0.5	<0.5	1.95	0.88
18	RBC4_Eff	<0.5	<0.5	<0.5	1.97	1.21
21	C-East	<0.5	<0.5	<0.5	1.99	0.97
22	C-West	<0.5	<0.5	<0.5	1.52	0.91
23	Effluent	<0.5	<0.5	<0.5	1.99	0.94
24	UMS-1	<0.5	0.42	1.20	3.50	89.68
25	UMS-3	<0.5	2.83	17.64	8.54	204.6
26	UMS-5	<0.5	0.00	0.54	2.65	10.66
27	UMS-6	1.83	2.67	<0.5	2.12	1.64
28	UMS-9	<0.5	<0.5	4.85	5.23	240.3
31	UMS-7B	<0.5	<0.5	<0.5	1.65	0.97
32	T_EW	<0.5	<0.5	<0.5	<0.5	1.03
33	AD#1_EW	<0.5	<0.5	<0.5	15.69	0.73
34	AD#2_EW	<0.5	0.00	<0.5	19.92	1.09
35	SP_EW	<0.5	3.25	<0.5	8.70	0.73
36	C_East_EW	<0.5	<0.5	<0.5	8.67	0.85
37	C_West_EW	<0.5	<0.5	<0.5	7.36	1.10

Evaluation of Bioaugmentation products (BGPs) for RFAAP Bioplant: oxygen uptake tests

Introduction

A set of commercial bioaugmentation products (BGPs) samples (3) were obtained from RFAAP during the sampling events in February and March 2023 and delivered at the Center for Environmental Systems (CES) (Stevens Institute of Technology). The samples were prepackaged dry powders of 1 lb. each in weight; each sample represents a commercial product for enhancement of COD removal from wastewater from different vendors. Specifications and information on each of the samples can be found in Table 8 below.

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Table 7. Microtox toxicity assessment.

MICROTOX TOXICITY		Expressed as 15 minutes EC50 % concentration			NT: non-toxic (or hormesis) NA: non-applicable		
No	**Sample ID**	**Sample Location**	**Matrix**	**Microtox Toxicity**	**NG**	**1,2 DNG**	**1,3 DNG**
1	906-17 #1	pump station No.906-17	S	NA			
2	906-17 #2	pump station No.906-17	L	NT	1.78	0	2.32
3	EQ E_MA	EQ tank, east, mixed area	L	NT	0.95	0	1.58
4	EQ W_MA	EQ tank, west, mixed area	L	NT	0.97	0	1.52
5	EQ E_UA	EQ tank, east, unmixed area	L	NT	1.07	0	2.32
6	EQ W_UA	EQ tank, west, unmixed area	L	NT	1.08	0	2.28
7	EQ E_S	EQ tank, east, sludge	S	NA			
8	EQ W_S	EQ tank, west, sludge	S	NA			
9	RBC1_Inf	RBC Train1, Influent	L	NT	0.92	0	2.15
10	RBC1_Eff	RBC Train1, Effluent	L	NT	1.09	0	2.08
11	RBC1_BM	RBC Train1, Basin media	S	NA			
12	RBC1_MS	RBC Train1, Scrape media	S	NA			
13	RBC2_Inf	RBC Train2, Influent	L	NT	1.08	0	1.47
14	RBC2_Eff	RBC Train2, Effluent	L	NT	0.85	0	1.93
15	RBC2_BM	RBC Train2, Basin media	S	NA			
16	RBC2_MS	RBC Train2, Scrape media	S	NA			
17	RBC4_Inf	RBC Train4, Influent	L	NT	0.88	0	1.95
18	RBC4_Eff	RBC Train4, Effluent	L	NT	1.21	0	1.97
20	RBC4_MS	RBC Train4, Scrape media	S	NA			
21	C-East	Clarifier East	L	NT	0.97	0.00	1.99
22	C-West	Clarifier West	L	NT	0.91	0.00	1.52
23	Effluent	Final effluent near flume	L	NT	0.94	0.00	1.99
24	UMS-1	monitoring station 1	L	48.51%	89.68	1.20	3.50
25	UMS-3	monitoring station 3	L	31.88%	204.57	17.64	8.54
26	UMS-5	monitoring station 5	L	NT	10.66	0.54	2.65
27	UMS-6	monitoring station 6	L	NT	1.64	0.00	2.12
28	UMS-9	monitoring station 9	L	25.75%	240.32	4.85	5.23
31	UMS-7B	monitoring station 7B	L	NT	1.88	0.00	1.65
32	T_EW	Tickener, Effluent wastewater	L	NT	1.94	0.00	0.00
33	AD#1_EW	Aerobic, Effluent wastewater	L	58.88%-63.9%*	2.00	0.00	15.69
34	AD#2_EW	Aerobic, Effluent wastewater	L	40.09%	2.06	0.00	19.92
35	SP_EW	Sludge press, Effluent	L	100% **	2.12	0.00	8.70
36	C_East_EW	clarifier, Effluent wastewater	L	NT	2.18	0.00	8.67
37	C_West_EW	clarifier, Effluent wastewater	L	NT	2.24	0.00	7.36

*pH related, extrapolate data, test repeated

** without pH adjustment very toxic at all levels.

The listed BGPs are currently used at the RFAAP Bioplant to improve and support the efficacy of plant's biological processes (i.e., RBC) in degradation of COD loads. As such, the products are also thought to support degradation of residual NG and derivative compounds (i.e., DNGs, MNGs) concentrations in the plant influent. In order to evaluate the extent of such performance and verify the claim, the team at Stevens designed and performed a series of qualitative oxygen (O_2) uptake tests based on the established methodology for BOD_5 assessment. Samples and blanks were prepared using the received BGPs (individual and mixed) and a synthetic solution containing

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fixed concentrations of NG and byproducts; the testing program was carried out by monitoring two main parameters:

1. O_2 uptake
2. NG and byproducts concentrations

Table 8. Bioaugmentation products description.

Sample ID	Vendor	Description	Operational pH
MB2	Novozymes Biologicals Inc	blend of bacteria for degradation of petrochemical, chemical , pharmaceutical, tar distillation and phenolic species	6.5-9.0
MB8	Novozymes Biologicals Inc	microorganisms that target a wide range of organics found in a variety of industries	6.5-9.0
MicroSolv 118	Environmental Leverage Inc	bacteria-laden, powdered formulation containing a blend of microorganisms, micro/macro nutrients, fungi, and surface tension suppressants/penetrants. This product is for pulp & paper wastewater applications	5.2-9.0

Methodology

A series of samples and blanks were prepared following standards procedures listed for BOD_5 assessment (standard method 5210B). Briefly, the method consists of filling with diluted and seeded sample an airtight bottle with a ground-glass stopper and a flared mouth of specified size (300 mL) and incubating it in a dark environment at 20°C for 5 days; in this case, the incubation period was fixed to 48 hours. Dissolved oxygen concentrations were measured at the beginning and at the end of the incubation period with the difference representing oxygen uptake which related to organic carbon sources consumption. A total of four (4) samples were prepared to test each individual BGPs plus a mixed sample with all BGPs to evaluate any synergistic effects. The concentration of each BGPs was fixed by simulating a final bacterial count (CFU/g) comparable to that of standard BOD test inoculum (i.e., Polyseed, HACH) (Table 9). Each BGPs was activated following standards procedure outlined for activation of the Polyseed (i.e., mix fixed amount of product in 1 L of oxygen saturated dilution water for 1 hour, settle 30 minutes, decant; use 4 mL

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of supernatant x test bottle). The pH of all samples and control blanks was kept in a range compatible with the operational range indicated by the BGPs specs (pH range 6.70-7.20).

For preparation of the NG and derivatives synthetic solution, a concentrated NG stock solution in methanol (5% w:w) was used as source. In order to reduce interference from solvent, the NG solutions was prepared by evaporating 20 mL of NG methanol stock for 24 hours and then diluting up to 1 L with DI water. The solution was then mixed for 48 hours or until complete dissolution of NG. The solution was then further diluted to obtain levels of NG and derivatives compounds comparable to those found in the plant influent (Table 10).

Table 9. Concentration and bacterial count for each BGPs used in oxygen uptake tests.

BGPs	Bacteria count (CFU/g) [SDS]	Operational pH	BGPs amount x DW (g/L)	Bacterial count x DW(CFU/g)^	Volume of BGPs stock x test (ml)
MB2	$5*10^{\wedge 9}$	6.5-9.0	0.36	$1.8*10^{\wedge 9}$	4
MB8	$5*10^{\wedge 9}$	6.5-9.0	0.36	$1.8*10^{\wedge 9}$	4
M118	$1*10^{\wedge 9}$	5.2-9.5	1.5*	$1.5*10^{\wedge 9}$	4
Mix**	/	/	/	/	12

* Dosage adjusted based on the consideration of additional elements (i.e., nutrients) per weight of product and lower CFU/g
^ Calculated
** MB2+MB8+M118 in equal amounts

Table 10. Composition of prepared NG stock solution in DI water used for the tests. *NG derivatives originally present in stock solution.

	Avg compounds concentration (mg/L)
NG	21.7 ±0.5
1,2 DNG	1.95±0.08
1,3 DNG	3.15±0.05

Along with the test bottles, two (2) sets of controls and a set of blanks were prepared for each BGPs and their mix in order to assess the viability of the bacteria population as well as offset for any contribution of trace solvents residuals to the oxygen uptake. The negative controls consisted of each BGPs without any external carbon source while the positive control consisted of BGPs plus glucose. The glucose control was prepared following a theoretical oxygen demand of 120 mg/L as recommended for BOD tests. For each sample, the test was performed in duplicate and for each batch of BOD tests, a glucose-glutamic standard was added as an additional sample in order to check the validity of the test.

Before and after the end of the incubation period, NG and deriving organic by products such as 1,2 dinitro glycerin (1,2-DNG) and 1,3 dinitro glycerin (1,3-DNG) were quantified using high-performance liquid chromatography (HPLC) (Agilent, Infinity Series, 1260). A Hypersil ODS

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column reverse phase (RP) (Thermo Scientific) was used for the separation of NG and byproducts using a gradient mobile phase consisting of deionized water and acetonitrile at a flow rate of 1.0 mL/min and detector wavelength at 210 nm. Certified reference standards (1000 mg/L) in acetonitrile (Sigma Aldrich) were utilized for calibration of the analytical method for NG and respective byproducts.

Results

Oxygen uptake in relation to organic load consumption was evaluated by measuring the dissolved oxygen (DO) concentrations in all samples, blanks, and controls before and after the established incubation period (i.e., 48 hours). Difference between the initial and final DO concentrations are reported in Figure 4; as expected, the negative controls showed minimal difference in the initial and final DO concentrations resulting in a non-significant oxygen uptake (i.e., $\leq$ 6% uptake). On the other hand, positive controls showed significant oxygen uptake (i.e., average 9.0 mg/L) which was consistent with viable bacteria population for each of the tested BGPs.

In addition to the positive controls, significant uptake was also observed in the solvent (methanol) blanks followed by an average uptake of 3.0 mg/L in the NG-containing samples; the higher uptake in the solvent blanks indicates that any solvent residue (although most likely insignificant) in the synthetic NG solution would not affect the activity of the BGPs.

Overall, the uptake in the NG containing samples could not compare with either blanks or positive control although a slight increase in uptake was observed for the mix matrix (MB2+MB8+M118) sample.

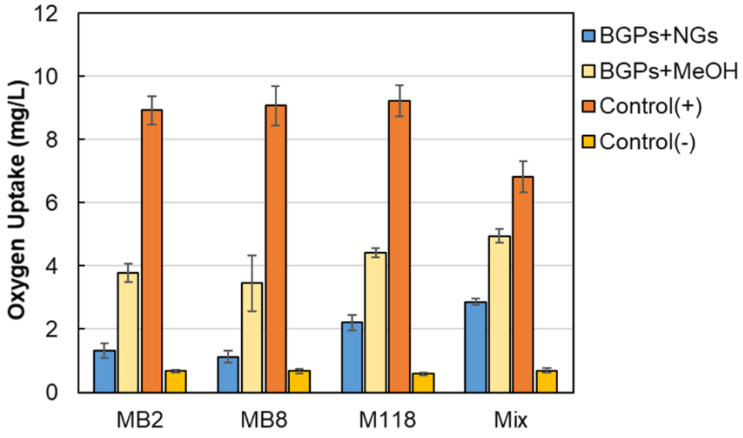

Figure 4. Oxygen uptake by BGPs (MB2, MB8, M118) in presence of NG and derivative compounds, methanol blank and controls; Uptake time period: 48 hours; Mix= MB2+MB8+M118. Error bars represent standard deviations from two replicates.

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The low oxygen uptake in the BGPs-NG samples was consistent with the minimal NG removal observed at the end of the incubation period (Figure 5a). In fact, across all samples tested, the NG removal averaged only to about 10%; in addition, the removal could not be attributed to degradation or decomposition of the main glycerin chain but most likely to denitration mechanisms resulting in transformation of the NG to DNGs compounds. Such hypothesis can be further supported by increases in the DNGs concentrations observed in some BGPs samples at the end of the incubation period (Figure 5c).

The low NG removal and oxygen uptake in the tested BGPs samples overall suggests that such bacteria consortiums will not play key role in degradation of NG and parent compounds in the Bioplant influent; however, efficacy of the same in degradation of other COD contributing sources (i.e., solvents) shall not be considered equally insignificant based on the results of these tests.

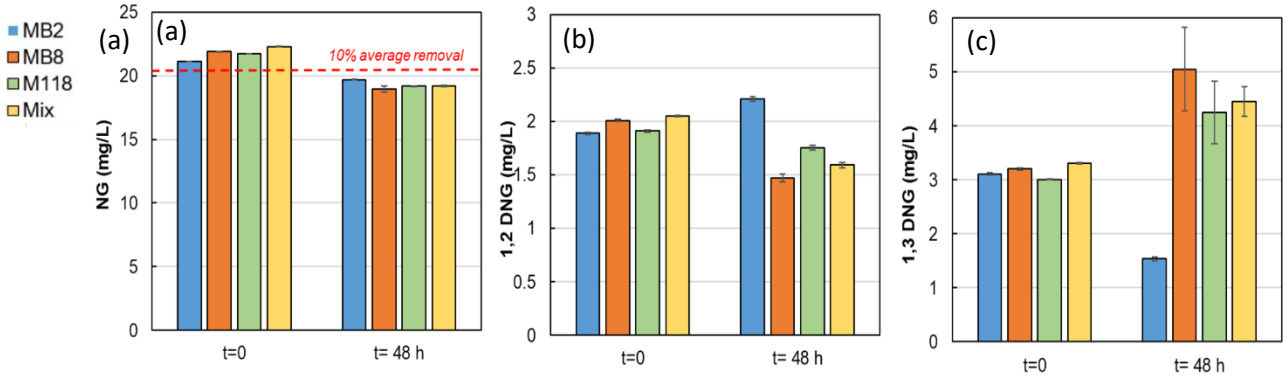

Figure 5. (a) NG, (b) 1, 2DNG and (c) 1,3 DNG concentrations at t= 0 and t= 48 h in BGPs samples (MB2, MB8, M118) oxygen uptake tests; Mix= MB2+MB8+M118. Error bars represent standard deviations from two replicates.

Evaluation of Bioaugmentation products (BGPs) for RFAAP Bioplant: microbial identification using next generation sequencing (NGS) technology

Introduction

As previously stated, listed BGPs in Table 8 and shown in Figure 6, are currently used at the RFAAP Bioplant to improve and support the performance of the plant's biological processes (i.e., RBC) for degradation of COD loads. As such, the products are also thought to support degradation of residual NG and derivative compounds (i.e., DNGs, MNGs) in the plant influent. In order to evaluate the extent of such performance and verify the claim, the team at Stevens designed and performed a series of qualitative oxygen (O_2) uptake tests based on the established methodology for BOD_5 assessment as described in the previous section. In addition, next generation sequencing technology was performed for each of the samples for identification purposes.

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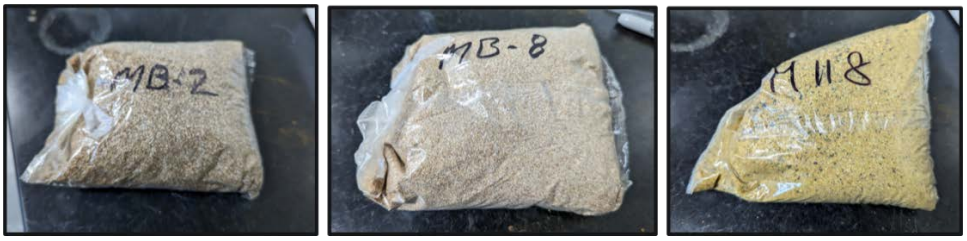

Figure 6. Appearance of bioaugmentation products received and analyzed by CES-SIT.

Methodology

Each BGP was activated by following standard procedure outlined for activation of the Polyseed® in BOD tests, and as previously described in functional analysis performed for the same BGP (i.e., mix fixed amount of product in 1 L of oxygen saturated dilution water for 1 hour, settle 30 minutes, decant, use supernatant as needed within 3 hours). After activation of the samples, the biomass was centrifuged at 3500 rpm, and the pellets were frozen until further analysis. DNA extraction and sequencing using high-throughput next generation sequencing (NGS) was performed by an external laboratory (Omega Bioservices, Norcross, GA). Shotgun metagenomic sequencing was chosen to sequence these complex microbial samples to gain insight into their microbial community biodiversity and function. In addition, this method allows culture-free identification and classification of complex microbial communities. The Ribosomal database project (RDP) was used within the Basespace app for identification of the sequences obtained.

Results

As stated before, these commercial products are used in the RFAAP Facility for aiding the degradation of residual NG and byproducts in the plant influent together with increasing the efficacy of BOD removal. According to the data sheet of MB-2 commercial product, a highly specialized blend of bacteria that could potentially remove BOD, COD, TOC, phenol, chlorinated hydrocarbons, aromatic and aliphatic hydrocarbons meta and ortho chlorinated phenols, propylene dichloride, styrene, formaldehyde, and alcohols. While MicroSolv 118 contains a specially formulated blend of microorganisms, micro/macro nutrients, fungi, and surface tension suppressants/penetrants, developed for pulp & paper wastewater applications where there are heavy influxes of cellulosic fibrous organic solids.

Microbial composition results obtained from sequencing and analysis are presented in figures 7, 8 and 9 for MB-2, MB-8 and Microsolv 118, respectively. The lowest taxonomy division was achieved, corresponding to genus and species. Overall, the main genus and species identified were similar for the three products. Figure 7 shows that the species identified in this MB-2 blend are *Ralstonia sp. MD27 (*30%*), Comamonas sp.Z1* (10 %), *Bacillus spp.* (accounting for 14 %),

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several species of genus *Pseudomona (spp.)* (representing 15 %) and genus Citrobacter (18 %). Figure 8 shows that the species identified in this MB-8 blend are *Ralstonia sp. MD27 (*17 %*), Comamonas sp. Z1* (12 %)*, Bacillus spp.* (3 %)*, Pseudomonas spp.* (representing 10 %) and genus Enterococcus (30 %). Whereas Figure 9 shows that the species identified in MicroSolv 118 blend are *Ralstonia sp. MD27* (28%)*, Comamonas sp. Z1* (23 %), *Bacillus spp.* (8 %), *Pseudomona spp.* (representing 3 %), *and* genus Citrobacter (18 %).

According to literature, *Bacillus* and *Pseudomona* strains have been confirmed to degrade NG aerobically individually (3, 4, 5, 6) or by synergistic effect (7). Some of those studies revealed that these bacteria can use NG as a sole nitrogen source since they contain NG reductase, an enzyme able to catalyze the NADPH-dependent denitration of NG, yielding nitrite. Moreover, some other strains of bacteria (8) or fungi (9) have been studied to aerobically degrade NG, whereas other studies demonstrated NG anaerobic degradation (10, 11).

Additionally, some studies have shown the efficacy of *Ralstonia sp. MD27* as a biopolymer-degrading bacterium (12) while others demonstrated *Comamonas sp.* can metabolize heterocyclic aromatic organic compounds (13, 14). Furthermore, *Citrobacter koseri* is responsible for the degradation of plastics such as polyvinyl chloride (PVC) (15).

Overall, the current identification study indicates that the commercial bioaugmentation products used at RFAAP Bioplant are targeted correctly. In addition, due to their similar microbial composition there might be no need to add all three simultaneously (preferably MB-2). Conversely, the functional studies performed previously simulating plant conditions indicated that there is no degradation of NG and byproducts; most likely because the conditions are not adequate for promoting degradation. For instance, Vanitha et al. (2019) demonstrated that optimum NG degradation, using *Bacillus sp. and Pseudomonas sp*. strains, proceeds at neutral pH and temperature of 30 degrees Celsius with addition of extra organic carbon sources.

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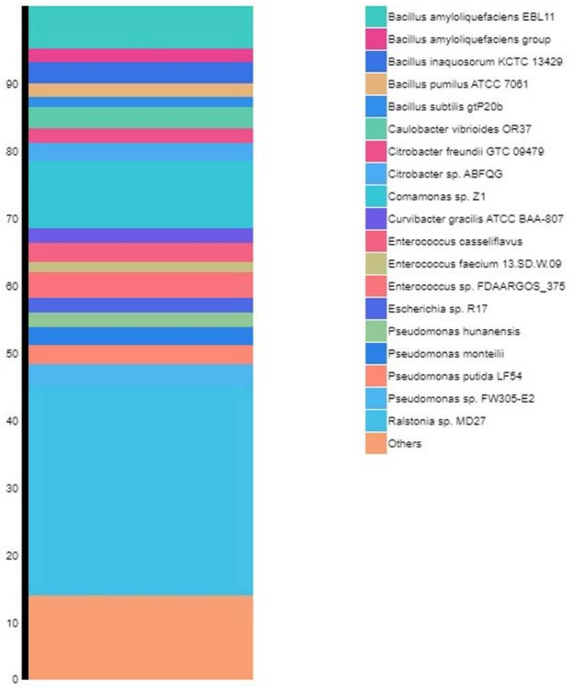

Figure 7. Microbial composition and relative abundance in MB-2 bioaugmentation product.

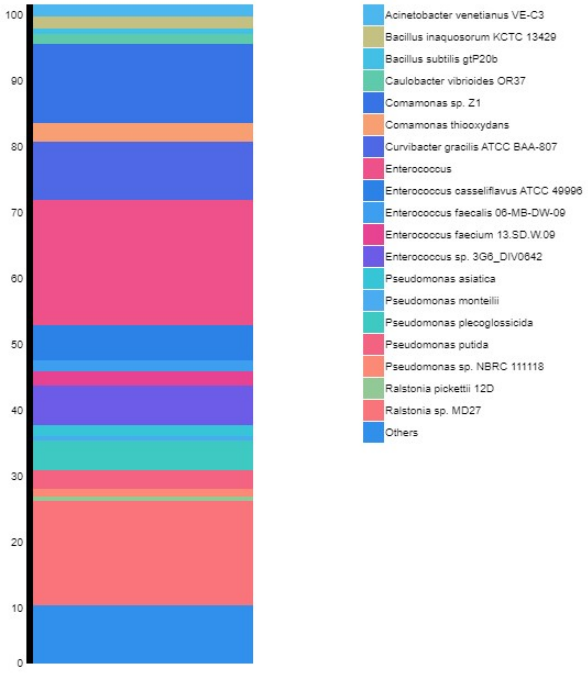

Figure 8. Microbial composition and relative abundance in MB-8 bioaugmentation product.

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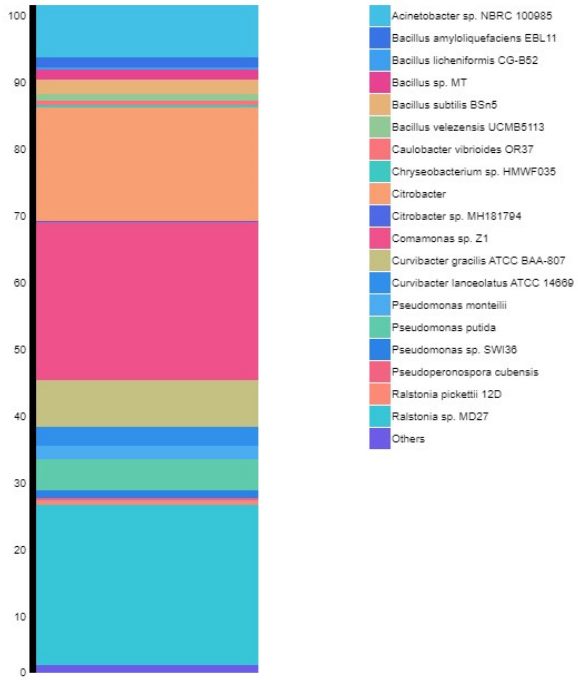

Figure 9. Microbial composition and relative abundance in Microsolv 118 bioaugmentation product.

Summary, conclusions, and recommendations

The analytical work results can be summarized as follows:

- SVI values fall in the lower end of the recommended range
- Biomass appears to be viable
- Oxygen uptake much higher in the EQ basins than in the RBCs
- 83% BOD_5 removal and a BOD_5 effluent satisfying VPDES daily limits
- TCOD and SCOD data need further analysis
- Solvent evaporative processes at the EQ basis might be playing a significant role in SCOD removal
- Microtox toxicity found for a few streams (pH effects, NG and derivatives present in samples)
- Bioaugmentation products appear not to enhance NG removal (<10% degradation mostly to 1,3 DNG) although might enhance COD removal. This is most likely due to Bioplant operational conditions which are not adequate for promoting NG degradation

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- High concentrations of graphite found in the EQ basins sludge
- DNT (mix of 2,4 and 2,6 DNT) only detected in UMS-6 sample; 96 ppb

Moreover, it is recommended that the operator should assess the feasibility of increasing phosphorous amendment to the Bioplant as the values measured in the active treatment areas were non-detected. The analysis of the flow through the Bioplant during 2023 shows a wide variation. The average and median flows were 0.92 and 0.9 mgd, respectively; however, the standard deviation was 0.15 mgd and the maximum and minimum values were 1.4 mgd and 0.53 mgd, respectively. It is possible that those swings also have an impact on the Bioplant level of treatment.

Regarding the recommendations made in the DBOC JV/Haskell, the tiered approach valued around $70M addressing the acid and industrial streams systems is sound and will help improve the current plant operation. As high NG concentrations that correlate well with microbial toxicity were found, it is recommended that the assessment of improvements in the NG interceptors as recommended in page 18 of the report is prioritized. However, a techno economic analysis should be performed as the facility is ageing and any major modification to the current plant will require Virginia DEQ approval and might face regulatory challenges. All equipment is subject to Virginia DEQ Sewage Collection and Treatment Regulations (SCAT), Section 9VAC25-790-450 “Treatment Works Design” Part D, “Flooding” which reads: “All mechanical and electrical equipment that could be damaged or inactivated by contact with or submergence in water (motors, control equipment, blowers, switch-gear, bearings, etc.) shall be physical located **above the 100-year level** or otherwise protected against the 100-year flood/wave action damage…”

A review of FEMA maps performed by Haskell shows the Bioplant is in the Zone AE Floodway and the 100-year flood plain; thus, any major modification likely will require relocation of major process units at which time cost estimations will likely rise and open the possibility of considering the design of a new, state of the art, wastewater treatment plant able to process the industrial streams and the acid streams (upon neutralization) with the associated reduction of nutrient discharges to the New River.

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