

Lagoons: Under the Surface

An In-Depth Investigation of Bioremediation and Biological Factors Involved in Reducing Sludge at a Municipal Wastewater Treatment Facility Lagoon System

Heather Jennings, PE, Sr. Project Engineer, Probiotic Solutions®

Abstract

In this study, the operators of a municipal wastewater treatment facility with 4 lagoons had determined that their 2 primary lagoons—10-foot-deep, with 25-million-gallon holding capacity each—had reached sludge depths of 5–7 feet, putting the lagoons at risk of upset and seriously impacting the facility's wastewater processing capacity.

A bioremediation plan was implemented that included the use of a biostimulant to support microbial reduction of the organic solids in the system. Sludge judging was performed for the 2 primary lagoons at baseline and at quarterly intervals over a one-year period to measure the impact of the bioremediation plan on sludge reduction. This was supplemented with ATP analysis to identify live biomass energy levels and DNA analysis to identify presence of and changes in relative representation of bacteria species at various stages of plan implementation. It also documented bacteria response to changes in influent due to episodic loading from industry, including a near-septic event. A modified sludge judge was used to collect samples from each of 3 layers of the lagoon: bottom (sludge), middle (interstitial), and top (supernatant).

The results of the ATP and DNA analyses pointed out the often-misunderstood fact that **wastewater treatment facility lagoon sludge is not inert: it is the most biologically active layer of the water column** and can be efficiently controlled and reduced through proper bioremediation interventions. The ATP analyses also documented the lasting effects (3 months) of an episodic toxic loading on the 2 lagoons. The study reports the 47 most abundant bacteria species present at various quarterly samples and at each of the 3 water/sludge layers. Discussion is provided of the roles several of these bacteria species play in the bioremediation process.

At the end of the one-year bioremediation plan, sludge depth for the 2 lagoons had been reduced by an average of 45%, with sludge depth at some sample points completely reduced to zero. This represented 17,800 dry tons of sludge that did not need to be mechanically removed and hauled to a disposal location, **a potential savings to the treatment facility of over \$6 million.**

Keywords

bioremediation, ATP analysis, bioavailable nutrients, biological oxidation demand (BOD), bio-oxidation capacity, DNA analysis, dredging, lagoons, microbes, microbial nutrition, microorganisms, municipal wastewater treatment systems, nutritional biostimulants, sludge, sludge judge, sludge reduction, solids accumulation, wastewater treatment

Bioremediation is an efficient method for treating municipal wastewaters. Essential to successful bioremediation is that naturally occurring beneficial microorganisms (such as bacteria, fungi, and algae) have a supportive habitat and the specific bioavailable nutrients and biostimulants they need to actively process wastewater contaminants and convert them to water, harmless gas, and humus. Because poorly performing wastewater treatment systems can become a significant factor in treatment facility efficiency and annual operating costs, delivering bioavailable microbial nutrition and biostimulants can have a significant impact on cost savings, resource use, and plant capacity.

Problem

A California municipal wastewater treatment facility (WWTF) with 4 lagoons primarily processed municipal waste as well as several types of commercial/industrial waste, including waste from a tomato processing plant and a dairy processing plant. The WWTF operators determined that their 10-foot-deep lagoons had reached sludge depths of 5–7 feet, putting the lagoons at risk of upset and seriously impacting the facility's wastewater processing capacity. A bioremediation plan was requested to reduce sludge levels, increase capacity, and return the system to a state of biological health (see Figure 1, page 2).

Objective

In addition to meeting the WWTF's goal of using bioremediation to reduce sludge levels in its lagoons, the WWTF operator saw an opportunity to investigate and track specific changes in the primary lagoons' (ponds 1A and 1B) strata microbial life as remediation reduced the sludge and returned the system to a state of health. In addition to sludge judging, it was decided to utilize adenosine triphosphate (ATP) analysis to quantify the viability of the microbial population within the strata. Deoxyribonucleic acid (DNA) analysis was also used to investigate the significant bacterial populations contributing to the sludge reduction within the strata in ponds 1A and 1B.

Figure 1. Aerial Photograph of California Treatment Plant



Initial Status

The influent to the wastewater system was primarily municipal, with some commercial and industrial sources (25%) for a total of 2.8 million gallons a day. Ponds 1A and 1B—the two primary lagoons—performed in parallel, with the secondary and tertiary lagoons (ponds 2 and 3) running in series. Each primary lagoon was estimated to hold 25 million gallons of water, (1,270 ft x 325 ft x 10 ft) with berms sloped at a 1:3 ratio.

A sludge judge was performed on the primary lagoons as a baseline. The overall lagoon depth averaged 10 feet with 2 feet of freeboard. It was determined that the sludge blanket was greater than 5 feet in several locations in both primary lagoons, Pond 1A and Pond 1B. An example of the field notes can be seen with Figure 2 (p. 3). Water levels and sludge depths are recorded in tables 1a–1j and 2a–2j (pp. 20–23).

Due to the high average sludge-blanket depth and the loss of the primary lagoon's overall capacity, a biostimulant,

Bio Energizer® (BE), was selected to support the reduction of the organic solids in the system. BE is not a microbial product but a formulation of complexing agents, organic acids, natural biological stimulants, and nutrients that help balance the natural microbial ecosystem to increase bio-oxidation capacity. The biostimulant product, BE, was added via peristaltic pump to the inlets of both Pond 1A and Pond 1B. Initially the dose applied on November 29, 2018, was 7 ppm (7 gallons of product to 1,000,000 gallons of influent, assuming a typical Biochemical Oxygen Demand [BOD₅] of 240 mg/L) and was eventually decreased to 5 ppm in September 1, 2019. A maintenance dose of 3 ppm was started November 19, 2019.

Methodology

The sludge levels for Pond 1A and Pond 1B were analyzed by a baseline sludge judging and repeated quarterly throughout the year using 30 data points. Three cross-sections were selected, going from north to south in each primary lagoon depending on placement of electrical lines,

Figure 2. Sample Sludge Judge Field Note

Baseline

SLUDGE DEPTH ~~POND 2~~ Pond 1B

DATE 11/15/18 grit POND LEVEL

SP1 WL <u>8'11"</u> SLUDGE <u>40"</u> <i>mostly grit</i>	SP2 WL <u>8'</u> SLUDGE <u>16"</u>	SP3 WL <u>8'6"</u> SLUDGE <u>26"</u>
SP4 WL <u>8'7"</u> SLUDGE <u>32"</u>	SP5 WL <u>8'7"</u> SLUDGE <u>20"</u>	SP6 WL <u>8'10"</u> SLUDGE <u>37"</u>
SP7 WL <u>8'11"</u> SLUDGE <u>28"</u>	SP8 WL <u>9'</u> SLUDGE <u>36"</u>	SP9 WL <u>9'</u> SLUDGE <u>32"</u>
SP10 WL <u>9'</u> SLUDGE <u>34"</u>	SP11 WL <u>8'11"</u> SLUDGE <u>30"</u>	SP12 WL <u>9'</u> SLUDGE <u>23"</u>
SP13 WL <u>9'</u> SLUDGE <u>24"</u>	SP14 WL <u>8'9"</u> SLUDGE <u>25"</u>	SP15 WL <u>8'6"</u> SLUDGE <u>33"</u>
SP16 WL <u>9'</u> SLUDGE <u>20"</u>	SP17 WL <u>8'7"</u> SLUDGE <u>26"</u>	SP18 WL <u>8'8"</u> SLUDGE <u>26"</u>
SP19 WL <u>8'7"</u> SLUDGE <u>20"</u>	SP20 WL <u>8'6"</u> SLUDGE <u>15"</u>	SP21 WL <u>8'8"</u> SLUDGE <u>21"</u>
SP22 WL <u>8'8"</u> SLUDGE <u>21"</u>	SP23 WL <u>8'10"</u> SLUDGE <u>19"</u>	SP24 WL <u>8'8"</u> SLUDGE <u>24"</u>
SP25 WL <u>8'6.5"</u> SLUDGE <u>13"</u>	SP26 WL <u>9'</u> SLUDGE <u>13"</u>	SP27 WL <u>9'</u> SLUDGE <u>21"</u>
SP28 WL <u>8'7"</u> SLUDGE <u>21"</u>	SP29 WL <u>8'9"</u> SLUDGE <u>10"</u>	SP30 WL <u>9'2"</u> SLUDGE <u>22"</u>

WL = WATER LEVEL

11/15/18
Sunny, heavy
light breeze from
the North Temp 70°
Morning 40°

thick sludge not
as thick as
south though

CS3
WL = 8'11" 11/14/18

CS2

CS1
day sludge through
except at SP2

thicker sludge, you
can actually mold
it



aerators, and/or mixers (see tables 1a–1j and 2a–2j, pp. 20–23). Water profile samples were also collected along the cross-sections using a modified sludge judge. Taps and small valves were installed in the sludge judge every foot, with a delineated tape attached to accurately measure the sludge and layers to be sampled (see Figure 3). Samples were collected from the following three layers: bottom (sludge), middle (interstitial), and top (supernatant). The bottom layer was defined as the compressed, thick sludge that existed at the bottom of the lagoon and was the focus for the remediation. The middle layer was the interstitial layer between the bottom layer and the less turbid top layer or supernatant. The top layer was the supernatant of the lagoon.

Water-level samples were collected along with the compacted bottom sludge levels. Sludge levels were evaluated by estimating the average depth of the lagoon to be 10 feet. Assuming that the sludge judge would not be able to penetrate the compacted bottom sludge level, the sludge depth was calculated by subtracting the sludge level in the sludge

judge from the measured water level and then subsequently subtracting the result from 10 feet. This was the standard for sludge measurement until the compacted bottom sludge was digested and the sludge judge hit the bottom of the lagoon. At that point, sludge depths were recorded as zero.

During the quarterly sampling, samples from similar layers along the selected cross sections were collected with the modified sludge judge into 3 different 5-gallon buckets to create 3 composite samples for each cross section. The 5-gallon buckets were rinsed with potable water, rinsed with a 35% bleach solution, and rinsed again with potable water before each composite sample was collected. Ten sample points from each layer's 5-gallon bucket were selected for each composite sample, which were collected by using a sterile graduated pipet into sterile 30 ml conical centrifuge tubes, then capped and inverted 3 times to completely mix the new composite. Grab-samples of 10 ml were then collected from the centrifuge tubes into 15 ml sterile graduated test tubes that were sent to a professional DNA sequencing laboratory—MR DNA (Molecular Research) located in Shallowater, Texas—for a diversity assay. The same process was repeated for each cross-section to obtain 9 total composite samples for each primary lagoon. Taking 9 composite samples for each primary lagoon resulted in a total of 18 samples. A portion of the residual liquid sample collected in each 5-gallon composite bucket was then tested for additional data—such as pH, electrical conductivity and temperature—by using a Myron L Company Ultrameter II.

The remaining 20 ml portion of each composite 30 ml sample was evaluated with a LuminUltra QuenchGone21 Wastewater test protocol for the following:

- ◆ **Total ATP (tATP)**—Includes intracellular ATP plus extracellular ATP.
- ◆ **Dissolved ATP (dATP)**—Includes extracellular ATP only.
- ◆ **Cellular ATP (cATP)**—A measure of living biomass concentration or energy level.
- ◆ **Biomass Stress Index (BSI)**—A measure of living biomass relative health.

Per LuminUltra literature, ATP is quantified by measuring the light produced through its reaction with the naturally occurring firefly enzyme Luciferase using a Luminometer. The amount of light produced is directly proportional to the amount of ATP present in the sample. ATP measurements provided a useful method of monitoring the proportion of viable cells within each layer as well as the degree of stress each layer experienced throughout the evaluation period. The LuminUltra luminometer was calibrated before each

Figure 3. Modified Sludge Judge



sampling event to ensure accurate measurements of the composite samples.

Findings

Sludge Judge

Sludge levels were over 5 feet in 5 different locations in both Pond 1A and Pond 1B, but both had averages of 3.44 feet of sludge when the baseline sludge judge was completed. Originally, the influent was divided evenly between Pond 1A and 1B. However, after septicity issues in January 2019 developed in Pond 1A, additional flow was diverted to 1B so that it was handling 70% of the influent. The flow wasn't rebalanced evenly until November of 2019. At the end of the testing period, the average sludge depths removed were 1.68 ft for Pond 1A and 1.46 ft for Pond 1B. Pond 1B was still able to maintain reduction despite taking on the additional 20% of flow from Pond 1A.

By November 2019, Pond 1A had an average sludge level of 1.63 feet and there were 10 locations where a sludge level no longer existed (see figures 4e-i and 4e-ii, p. 11; Table 1j, p. 21). Pond 1A was still able to maintain reduction despite the septicity issues during the early quarter of the year. There was a mound of inorganic solids and grit located at the inlet of both primary lagoons that could not be biologically digested.

By November 2019, Pond 1B had an average sludge depth level of 1.99 feet. Five locations had sludge completely reduced. It is important to note that Pond 1B carried up to 70% of the flow off and on during the year, depending on Pond 1A's performance (see figures 5e-i and 5e-ii, p. 12; Table 2j, p. 23). Pond 1B also had a mound of inorganic solids and grit located at the inlet to the lagoon that could not be biologically digested.

pH and Temperature Analysis

Influent pH varied depending on the contributions from tomato and dairy processing plants. Industrial influent pH varied from 4.3 to 11.7, and municipal influent pH ranged from 6.6 to 9.5. It is suspected that a swing in the pH from 9.5 to 6.6 within one week contributed to the septicity of Pond 1A. Throughout the quarterly sampling, pH within each water cross-section became more acidic the further down the water column, in line with anaerobic treatment of sludge at the bottom of each primary lagoon. Cross sectional pH also became more acidic the closer to the effluent point of each primary lagoon where less aeration occurs, and more anaerobic activity happens. (see Table 3, p. 23).

Temperature sampling started to be captured during the May 15, 2019, sampling event. Findings were that the temperatures throughout cross sections during the May and August 2019 were warmer throughout the water column in the lagoons. Only in November 2019 had the bottom layers cooled off compared with their upper layers. Temperatures overall were within a couple of degrees of the ambient air even down to the bottom layer (see Table 4, p. 23).

ATP Analysis

Cellular ATP (cATP), which measures live biomass energy levels, was compared with Dissolved ATP (dATP), which represents the dead biomass for all the quarterly stratified samples. The ratio of cATP:dATP was calculated (see figures 6a-6c, pp. 13-14; figures 7a-7c, pp. 14-15; Table 5, p. 24; and Table 6, p. 25) as well as the Biomass Stress Index (BSI). BSI measured the relative living biomass health for each sample.

The baseline measured cATP and dATP showed that, typically, the bottom sludge layer of the primary lagoons was the most bioactive within the pond. The middle and top layers were active as well, although typically not to the same degree. Throughout the quarterly testing period the top and middle layers of the cross sections varied dramatically. During February 2019 sampling, most of the cross-sections for both Pond 1A and Pond 1B showed signs of suppression due to a suspected influent pH swing of 9 and 6.6 within a one-week period in January. Subsequently, Pond 1A turned a gray color, possibly went septic, and strained Pond 1B as well. The cATP:dATP during this time period was as low as 0.11 in 1A-2B and -0.07 at 1B-3B. The negative value at 1B-3B was attributed to the living bacteria lysing almost immediately with Luciferase in the LuminUltra protocol due to the cells being highly stressed. The fastest recovery was at Cross Section 3 for each pond, as it was located near the influent of the wastewater system, which consistently received new organic solids that mitigated the effects of the sludge removal.

Sampling points 1A-1T and 1B-1T showed a more delayed response, with suppression occurring during the May sampling event. This is interesting, as these samples represent the supernatant effluent to Pond 2 and point to the longer lasting impacts of toxic upsets in the system. The corresponding BSI also indicated that the bacteria were under a great amount of stress during the system upset. The lowest BSI values were 0.08 and 0.14 for Pond 1A 1A-3B and Pond 1B 1B-3B near the inlets to the lagoons. The highest values were at Cross Section 1 for Pond 1A and Cross Section 2 for

Pond 1B. The BSI for Pond 1A at 1A-1T was significantly higher (883.60) compared with the highest value for Pond 1B at 1A-2M (277.8). When all the quarterly BSI measurements for Pond 1A and Pond 1B were averaged, Pond 1A's was 59.5 while Pond 1B was 25.5, verifying that Pond 1A was in general more stressed than Pond 1B even though Pond 1B treated 20% more of the inlet loading.

DNA Analysis

Quarterly diversity assays were performed with bTEFAP[®] Illumina 20k inhouse 16s rRNA sequencing on all 18 samples to identify the bacteria species. The top 20 species were selected quarterly for comparison with the baseline analysis. In a high-level review of the DNA data evaluated, it was found that the application of a biostimulant contributed to a more diversified microbial population that in turn helped lagoon systems recover from toxicity events and periodic turnover. Turnover occurs when lagoons have not only thermal stratification throughout the water column, but density as well. Prevailing winds throughout the season will cause these layers to mix and dissolved oxygen layers to drop. This action brings anaerobic microbiology to the surface of the wastewater system and can inhibit lagoon influent treatment. The impact of fungi, algae, and associated enzymes were not evaluated during this study.

During the Baseline DNA analysis in November 2018, it was found that the predominant species of bacteria—*Pseudomonas syringae* and *Pseudomonas rhizosphaerae*—represented 45% and 14% of the total bacteria sampled (see Fig. 8a, p.

16; Table 7a, p. 26). These specific species are predominantly associated with plants and not with wastewater treatment. The assumption was made that these were present due to the tomato processing plant that seasonally cans a variety of tomato products during the summer. There was another spike of 31% in *Pseudomonas syringae* in August of 2019 corresponding with the 2019 tomato processing season (see Fig. 8d, p. 17; Table 7d, p. 27). In February 2019, the bacteria started to naturally select toward BOD and phosphorus-consuming bacteria despite the suspected toxicity and suppressed cATP conditions. Phosphorus-consuming bacteria were predominantly found in the western portion of the primary lagoons near ponds' 1A and 1B effluent to Pond 2. In May, the bacteria selected away from BOD and phosphorus-consuming bacteria. This is believed to reflect not only a turnover within both primary lagoons, but also a delayed response to the suspected septic conditions—although cATP started recovering. In August, the bacteria returned to the microbial population that existed in February of that same year (see figures 8a–8e, pp. 16–18; tables 7a–7k, pp. 26–31). November reflected similar species that were present after seasonal turnover seen in May.

Other pseudomonas species that are associated with breaking down sugar, carbohydrates, and nitrification were found throughout the year of sampling. Acinetobacter species were also present throughout all the sampling events and *Rhodocyclus tenuis* appeared after biostimulant application. These are closely associated with polyphosphate uptake and phosphorus removal. Anaerobic organic digesting bacteria such as *Bacteroids* spp., *Bellilinea* spp. and *Clostridium* spp. were consistent throughout the sampling periods, corresponding with interstitial and sludge strata sampling. Filamentous anaerobic bacteria such as *Levilinea* spp. and *Longilinea* spp. were also found within the interstitial and sludge strata sampling. *Syctrophus* sp. and spp., known for the oxidation of butyrates and other fatty acids, were found throughout the sampling series and specifically

Before
BIO ENERGIZER[®]

After
BIO ENERGIZER[®]

BIO ENERGIZER[®]

- Reclaim Capacity
- Bio-oxidation of sludge 1/5 the cost of dredging
- Reduce Odors & Sludge

FOR MORE INFORMATION:
WWW.PROBIOTIC.COM
480.961.1220

Probiotic SOLUTIONS[®]

Microplex[®] JS
Plant in upset mode?
Have you had a die-off?
try MICROPLEX[®]JS to bring
your plant back.
RESEED with
MICROPLEX[®]JS

corresponded with interstitial and sludge strata sampling. The other top 20 species identified can be found in Figures 8a–8e (pp. 16–18) and Tables 7a–7j (pp. 26–30) with a summary in Table 7k (p. 31). Most of these other species were not considered to individually make up a significant population for organic solids degradation, but they supported overall treatment.

Nitrifiers such as *Nitrobacter*, *Nitrosomonas*, *Nitrospirillum* and other species were found throughout the year of treatment for the lagoons. They contributed to the reduction of ammonia and the removal of nitrogen from the influent but were not in the top 20 species identified during the quarterly sampling periods. Table 7l (p. 32) is a summary of the total number of bacteria contributing to nitrifying efforts. There is a significant increase of nitrifying bacteria in May 2019 (5.4 times) and November 2019 (4.6 times) compared with the baseline, while there was a loss of nitrifying bacteria in February 2019 due to the known septicity of the Pond 1A and the additional strain on Pond 1B. In August 2019, it is believed that aeration issues and a suspected low-level toxic event impacted the nitrifying bacteria as they dropped to almost a fourth of the baseline totals. By November 2019, the lagoons appeared to have recovered from the issues found in August 2019. Pond 1B consistently performed as well as Pond 1A or better despite treating a greater percentage (60%–70%) of the influent.

Economics

The baseline sludge levels were over 5 feet in 5 different locations in both Pond 1A and Pond 1B. On average, both ponds had 3.44 feet of sludge when the baseline sludge judge was completed. In November 2019, the average amounts of sludge removed were 1.68 ft for Pond 1A and 1.46 ft for Pond 1B which accounts for reductions of 48% and 42% respectively. These percentages represent about 9,375,000 gallons of sludge or about 2,800 truckloads removed with very little capital investment. These calculations can be found in Table 8 (p. 32). Reviewing lab data collected during the testing period, it was found that there was an average yearly removal rate of 99.8% of BOD. Additionally, the daily BOD or solids loading throughout treatment into Pond 1A and 1B was calculated using average flow rate during treatment and the assumption of 6% solids into the following equation:

$$\begin{aligned}\text{Influent 6\% Solids/Day} &= (\text{Vol. MGD}) \times (\% \text{ Solids}) \\ &= 2.62 \text{ MGD} \times 0.06 \times = 0.16 \text{ MG/Day}\end{aligned}$$

$$\text{Solids gal/Year} = 0.16 \text{ MG/Day} \times 365 \text{ Day/Year} = 57.4 \text{ MG/Year}$$

An additional 57.4 MG/Year of solids were treated in addition to what was removed. When adding the gallons of sludge reduced to the gallons of solids treated within the system, the total is 66.8 MG for the treatment time. Converting these gallons to 6% dry tons, it comes out to 17,800 dry tons. At \$340 dollars (from previous Customer Study) for removal of dry tons, there is a savings of \$6,066,000. This calculation excludes down time required to dredge the lagoons. This total was compared to an investment in product of \$350,000, and it was found that the facility product investment was 5.8% of the potential dredging costs.

Conclusions

A municipality was dealing with sludge build up in its two primary lagoons that was reducing capacity of the system to treat the incoming influent. A biostimulant was used to reduce the sludge in situ without taking the system down for mechanical treatment. During the treatment, several parameters—sludge judge, and ATP and DNA analysis—were used to evaluate the quarterly sludge reduction and health of the two primary lagoons. Sludge judging provided not only the water and sludge levels of each lagoon but, with a modified sludge judge, samples were collected from the following three layers: bottom (sludge), middle (interstitial), and top (supernatant). At the end of the treatment period, it was found that Pond 1A's and Pond 1B's sludge were reduced 48% and 42%, respectively.

Through ATP analysis, it was found that the bottom sludge layer was the most biologically active layer of the lagoons, which refutes the idea that the sludge layer is inert and can only be removed mechanically. A delayed response to a suspected toxicity event in December 2018 was captured as Pond 1A went to septic conditions, necessitating Pond 1B to take additional flow. Pond 1A and Pond 1B both showed a significant decrease in cATP in February 2019 due to the toxicity. Bacterial recovery did not fully occur till 6 months later in August 2019, which is substantially longer than anticipated as the color and appearance of the lagoon improved sooner.

DNA analysis allowed for the trending of 47 of the top different bacteria during the quarterly samples, out of the hundreds of bacteria found to be present in the two lagoons. Originally, the primary bacteria found in the baseline November 2018 sampling were plant-based due to the tomato processing that occurred during the summer. In February 2019, the bacteria started to naturally select toward BOD and phosphorus-consuming bacteria despite the suspected toxicity and suppressed cATP conditions. Phosphorus-accu-

mulating bacteria that were not present during the base-line analysis gained prominence during treatment, which was unexpected as lagoons are not known for phosphorus removal. *Pseudomonas* bacteria were identified as supporting nitrogen removal. Unsurprisingly, the work of the sludge reduction was accomplished by a variety of anaerobic bacteria.

A simple cost analysis was performed, evaluating not only the existing sludge quantities but the incoming potential sludge (see Table 8, p.32). It was found that 9,376,000 gallons of sludge were treated in place as well as an additional 57.4 MG/Year of solids, which at 6% solids came to 17,800 dry tons, at \$340 dollars per ton or a total of \$6,066,000.00. This total was compared with a product investment of \$350,000, 5.8% of the potential dredging costs. This is a considerable savings for a municipality with little upfront capital spending capacity.

Bibliography

- ◆ Akpor OB, Ogundeji MD, Olaohu TD, and Aderiye BI. (2014). Microbial roles and dynamics in wastewater treatment systems: An overview, *Int. J Pure & Applied Bioscience*, 2(1):156–168.
- ◆ Grau P. (1991). Criteria for nutrient balanced activated sludge systems. *Wat Sci Tech*, 24(3/4):251–258.
- ◆ Tracy DR, Whalen PA, Grandstaff J. (2008). *Prevent Upsets Before It's Too Late—What Your Biomass Can Tell You, If You Look*, LuminUltra Technologies, Ltd., Hopewell Regional Wastewater Treatment Facility. Available at https://www.researchgate.net/publication/233578027_Prevent_Upsets_Before_It's_Too_Late_-_What_Your_Biomass_Can_Tell_You_If_You_Look.
- ◆ Xie Bing, Xu Ya Tong. (2007). *Origins and Methods of Biological Wastewater Treatments*. China Light Industry Press.

Publication No. PS-201209-01

BIOREMEDIATION 3.0
THE NEXT GEN OF WASTEWATER TREATMENT

Celebrating 100 Years of Activated Sludge...
Introducing technology for the next 100 Years

Probiotic SOLUTIONS

To see compelling case studies, scan this code:



Powered By
MICRO CARBON TECHNOLOGY®

◆ Nano Molecular Carrier
◆ Bioavailable Nutrients

◆ Significant Cost Savings
◆ Simple Liquid Application

◆ Organic Based Solution
◆ Distributor Inquiries Welcome

800.961.1220



www.probiotic.com



info@probiotic.com

Figures

<u>List of Figures</u>	<u>Page</u>
Figure 1. Aerial Photograph of California Treatment Plant.....	2
Figure 2. Sample Sludge Judge Field Note	3
Figure 3. Modified Sludge Judge	4
Figure 4a(i). Pond 1A 11/29/18 Baseline, Sludge Depth Top View, West to East.....	10
Figure 4a(ii). Pond 1A 11/29/18 Baseline, Sludge Depth Cross Section	10
Figure 4b(i). Pond 1A 2/20/19 Sludge Depth Top View, West to East.....	10
Figure 4b(ii). Pond 1A 2/20/19 Sludge Depth Cross Section	10
Figure 4c(i). Pond 1A 5/14/19 Sludge Depth Top View, West to East.....	10
Figure 4c(ii). Pond 1A 5/14/19 Sludge Depth Cross Section	10
Figure 4d(i). Pond 1A 8/15/19 Sludge Depth Top View, West to East	10
Figure 4d(ii). Pond 1A 8/15/19 Sludge Depth Cross Section.....	10
Figure 4e(i). Pond 1A 11/13/19 Sludge Depth Top View, West to East	11
Figure 4e(ii). Pond 1A 11/13/19 Sludge Depth Cross Section.....	11
Figure 5a(i). Pond 1B 11/29/18 Baseline, Sludge Depth Top View, West to East.....	11
Figure 5a(ii). Pond 1B 11/29/18 Baseline, Sludge Depth Cross Section.....	11
Figure 5b(i). Pond 1B 2/20/19 Sludge Depth Top View, West to East.....	11
Figure 5b(ii). Pond 1B 2/20/19 Sludge Depth Cross Section	11
Figure 5c(i). Pond 1B 5/14/19 Sludge Depth Top View, West to East.....	11
Figure 5c(ii). Pond 1B 5/14/19 Sludge Depth Cross Section	11
Figure 5d(i). Pond 1B 8/15/19 Sludge Depth Top View, West to East	12
Figure 5d(ii). Pond 1B 8/15/19 Sludge Depth Cross Section.....	12
Figure 5e(i). Pond 1B 11/13/19 Sludge Depth Top View, West to East	12
Figure 5e(ii). Pond 1B 11/13/19 Sludge Depth Cross Section.....	12
Figure 6a. Pond 1A ATP, Cross-Section 1: Supernatant, Interstitial, Sludge Layer	13
Figure 6b. Pond 1A ATP, Cross-Section 2: Supernatant, Interstitial, Sludge Layer	13
Figure 6c. Pond 1A ATP, Cross-Section 3: Supernatant, Interstitial, Sludge Layer	14
Figure 7a. Pond 1B ATP, Cross-Section 1: Supernatant, Interstitial, Sludge Layer	14
Figure 7b. Pond 1B ATP, Cross-Section 2: Supernatant, Interstitial, Sludge Layer.....	15
Figure 7c. Pond 1B ATP, Cross-Section 3: Supernatant, Interstitial, Sludge Layer.....	15
Figure 8a. Microorganisms Identified by DNA Testing, 11/29/18	16
Figure 8b. Microorganisms Identified by DNA Testing, 2/20/19.....	16
Figure 8c. Microorganisms Identified by DNA Testing, 5/14/19	17
Figure 8d. Microorganisms Identified by DNA Testing, 8/15/19	17
Figure 8e. Microorganisms Identified by DNA Testing, 11/13/19	18

Figure 4a(i). Pond 1A 11/29/18 Baseline, Sludge Depth Top View, West to East

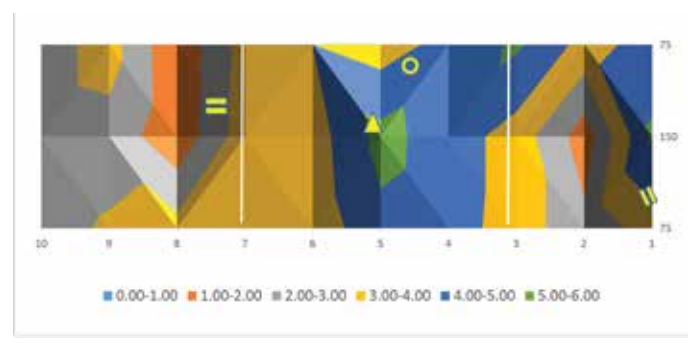


Figure 4a(ii). Pond 1A 11/29/18 Baseline, Sludge Depth Cross Section

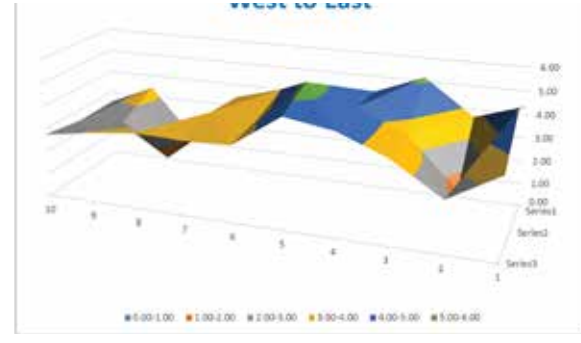


Figure 4b(i). Pond 1A 2/20/19 Sludge Depth Top View, West to East

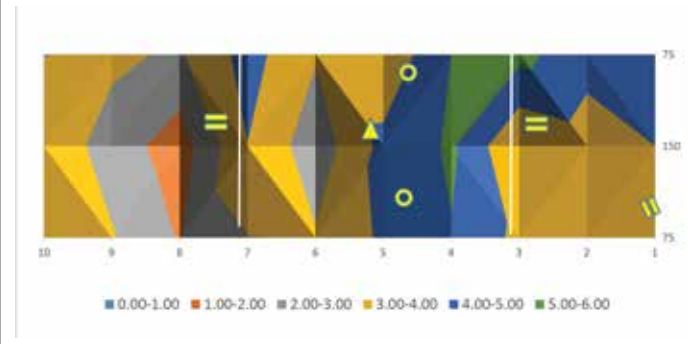


Figure 4b(ii). Pond 1A 2/20/19 Sludge Depth Cross Section

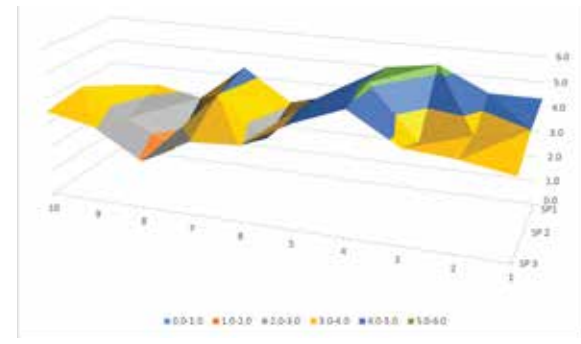


Figure 4c(i). Pond 1A 5/14/19 Sludge Depth Top View, West to East

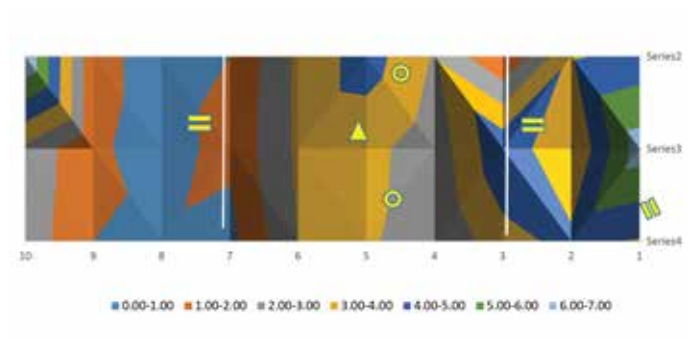


Figure 4c(ii). Pond 1A 5/14/19 Sludge Depth Cross Section

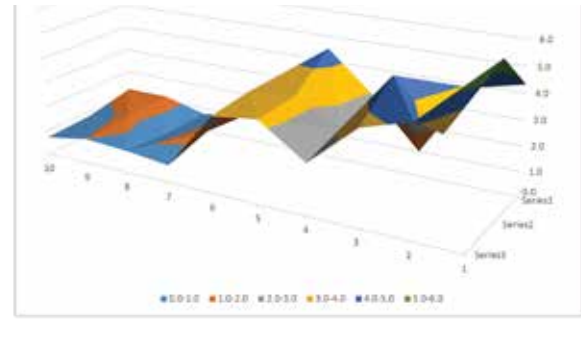


Figure 4d(i). Pond 1A 8/15/19 Sludge Depth Top View, West to East

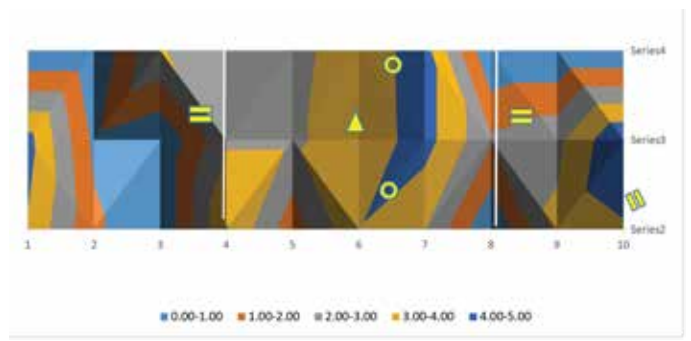


Figure 4d(ii). Pond 1A 8/15/19 Sludge Depth Cross Section

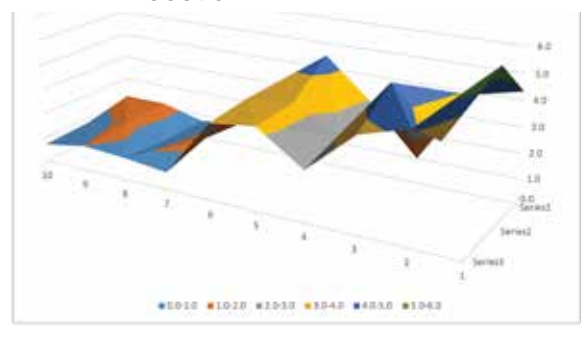


Figure 4e(i). Pond 1A 11/13/19, Sludge Depth Top View, West to East

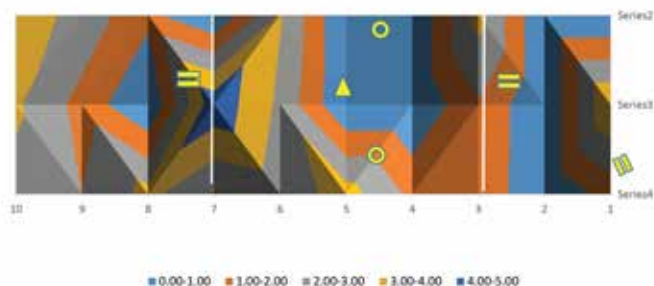


Figure 4e(ii). Pond 1A 11/13/19 Baseline, Sludge Depth Cross Section

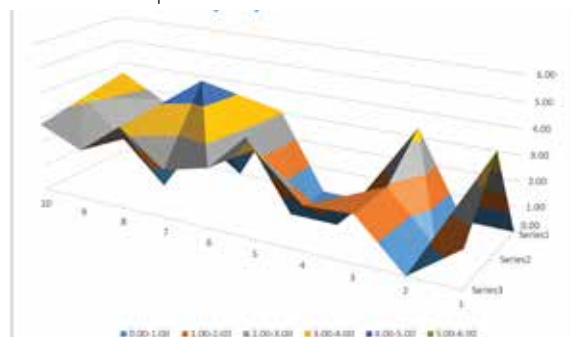


Figure 5a(i). Pond 1B 11/29/18 Sludge Depth Top View, West to East

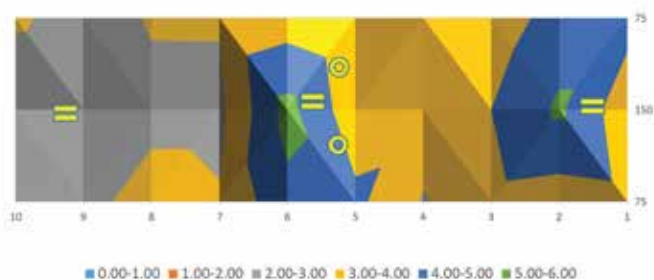


Figure 5a(ii). Pond 1B 11/29/18 Sludge Depth Cross Section

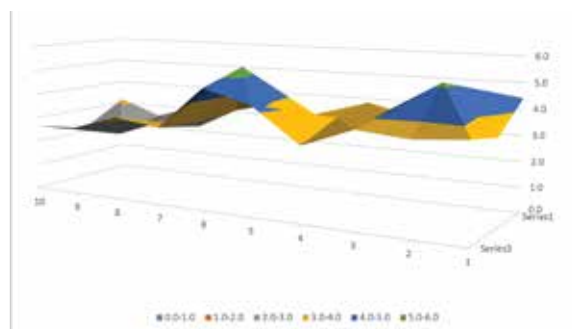


Figure 5b(i). Pond 1B 2/20/19 Sludge Depth Top View, West to East

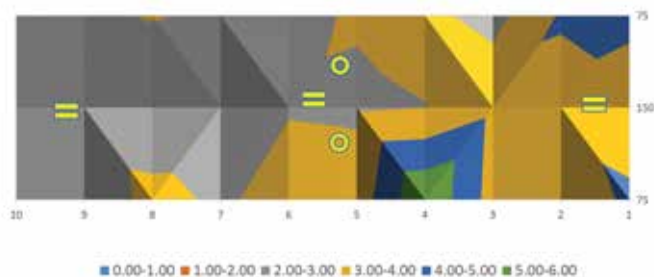


Figure 5b(ii). Pond 1B 2/20/19 Sludge Depth Cross Section

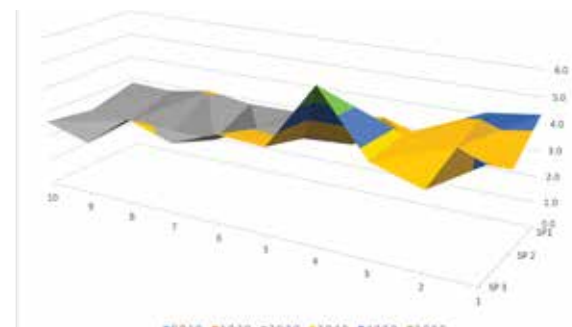


Figure 5c(i). Pond 1B 5/14/19 Sludge Depth Top View, West to East

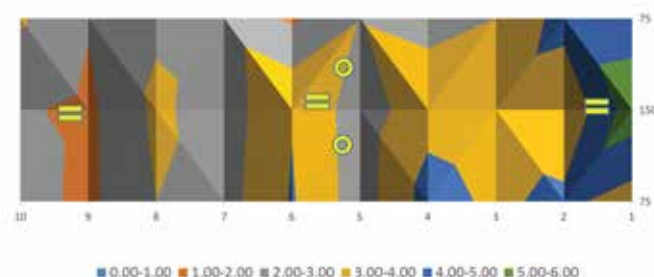


Figure 5c(ii). Pond 1B 5/14/19 Sludge Depth Cross Section

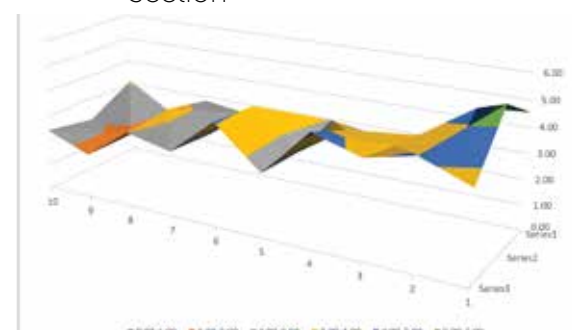


Figure 5d(i). Pond 1B 8/15/19 Sludge Depth Top View, West to East

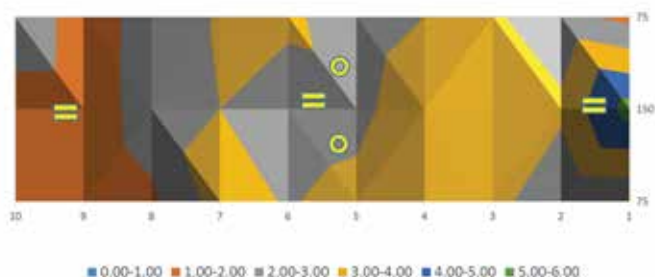


Figure 5d(ii). Pond 1B 8/15/19 Sludge Depth Cross Section

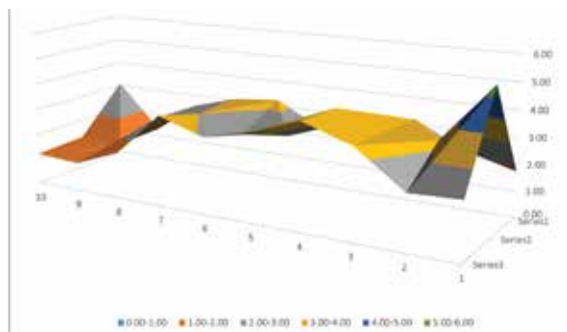


Figure 5e(i). Pond 1B 11/13/19 Sludge Depth Top View, West to East

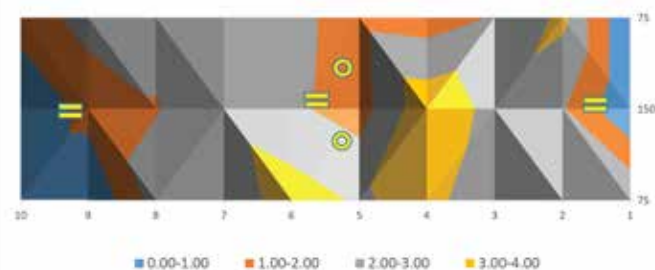


Figure 5e(ii). Pond 1B 11/13/19 Sludge Depth Cross Section

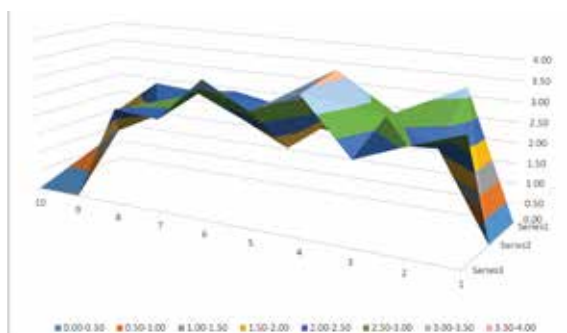


Figure 6a. Pond 1A ATP, Cross-Section 1:
Supernatant, Interstitial, Sludge Layer

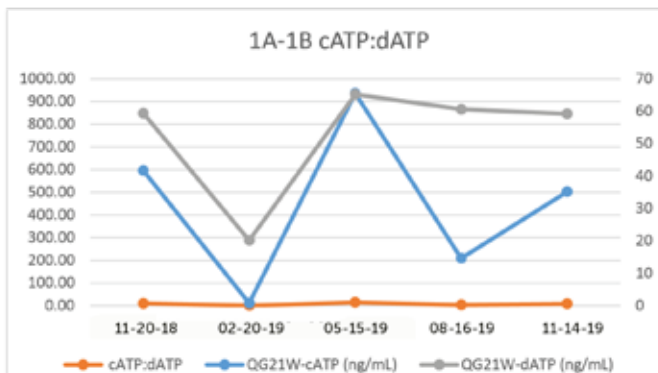
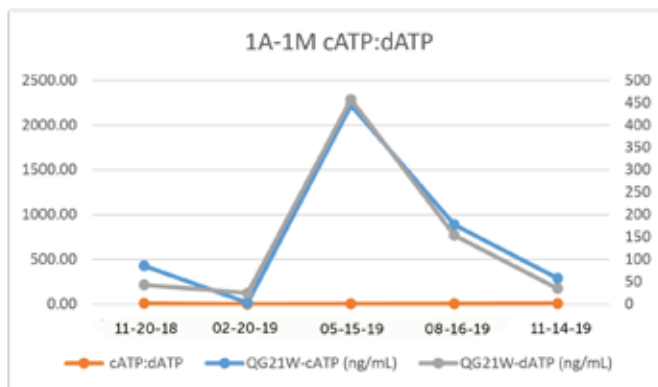
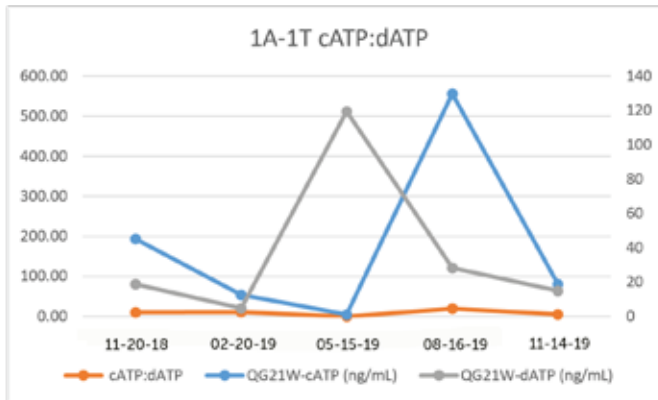


Figure 6b. Pond 1A ATP, Cross-Section 2:
Supernatant, Interstitial, Sludge Layer

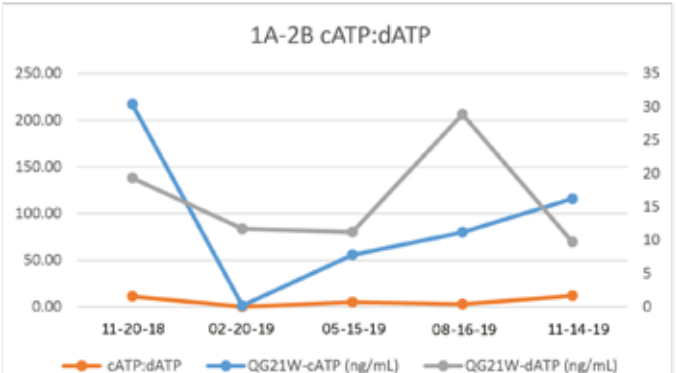
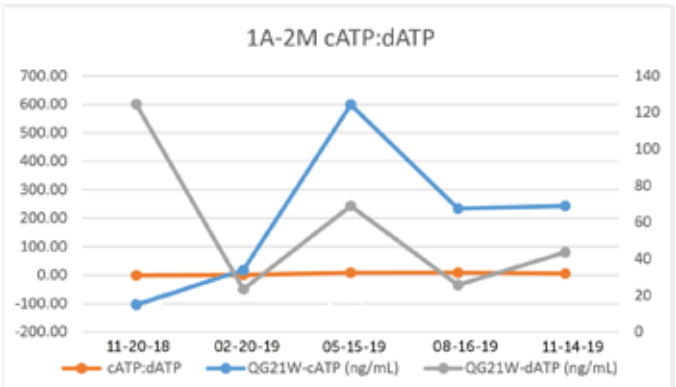
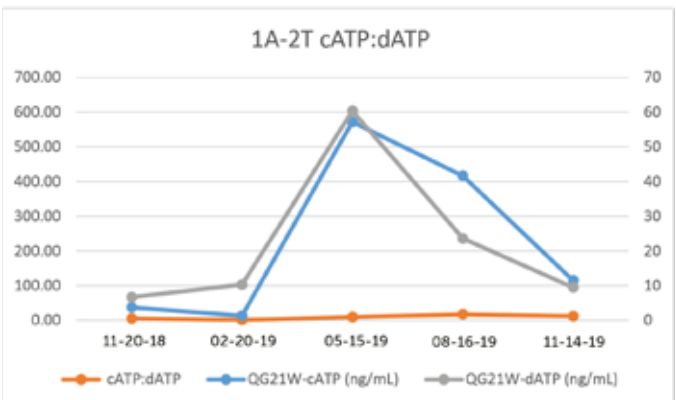


Figure 6c. Pond 1A ATP, Cross-Section 3:
Supernatant, Interstitial, Sludge Layer

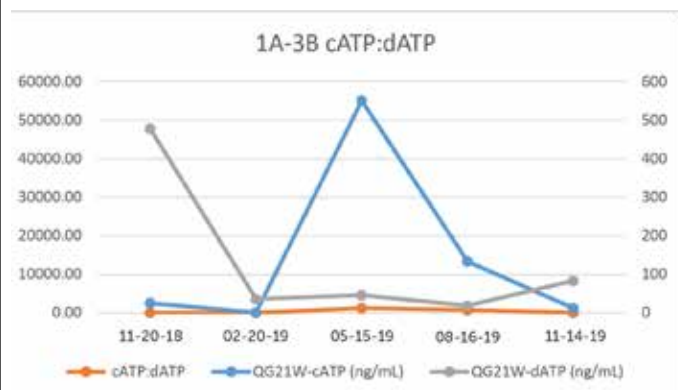
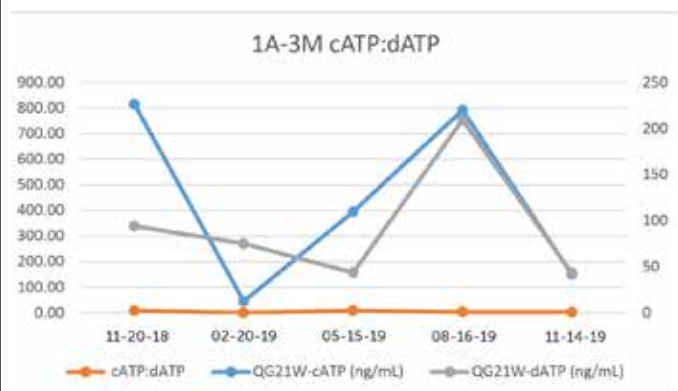
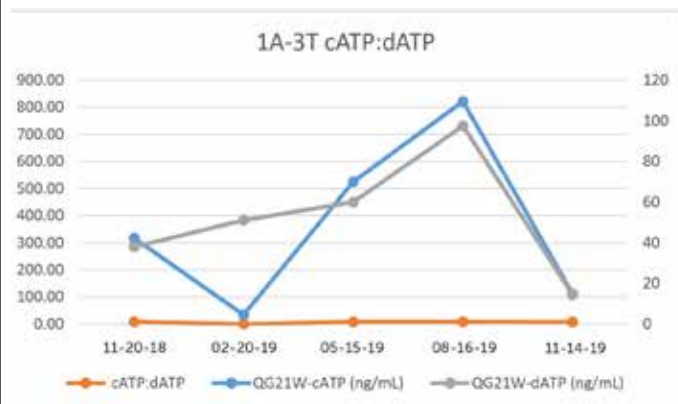


Figure 7a. Pond 1B ATP, Cross-Section 1:
Supernatant, Interstitial, Sludge Layer

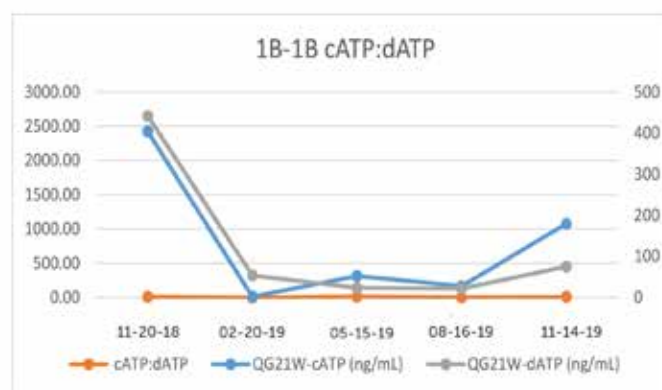
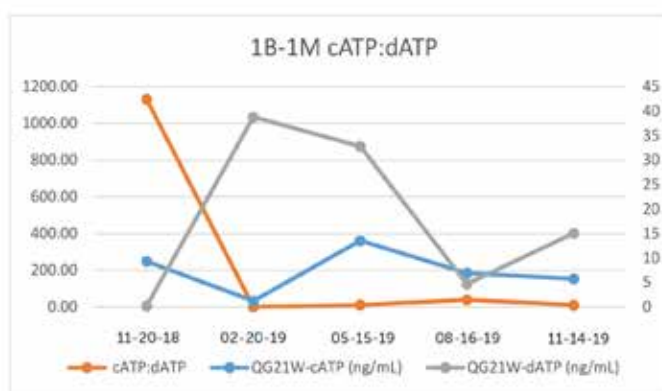
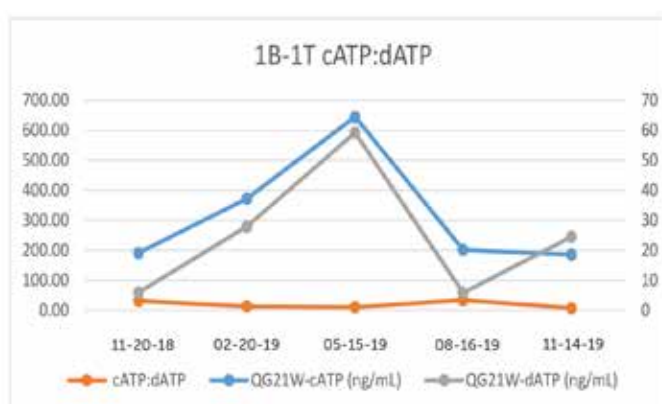


Figure 7b. Pond 1B ATP, Cross-Section 2: Supernatant, Interstitial, Sludge Layer

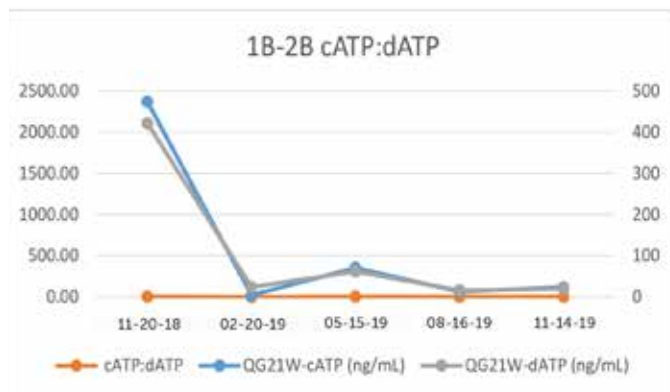
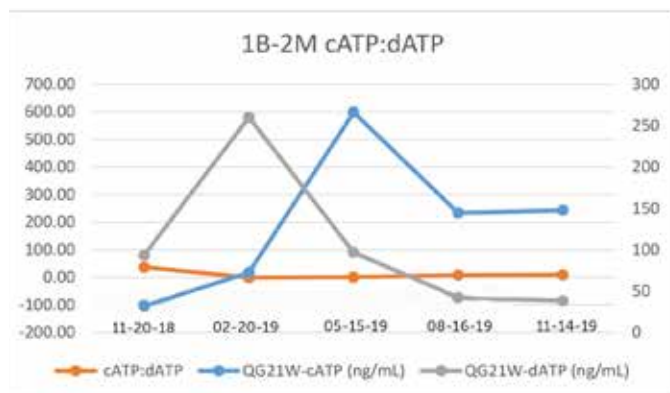
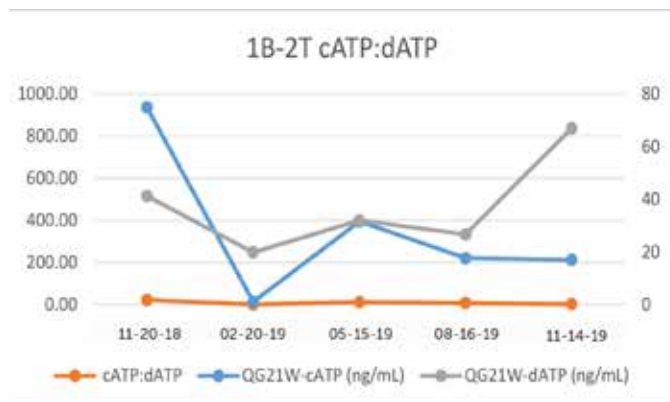


Figure 7c. Pond 1B ATP, Cross-Section 3: Supernatant, Interstitial, Sludge Layer

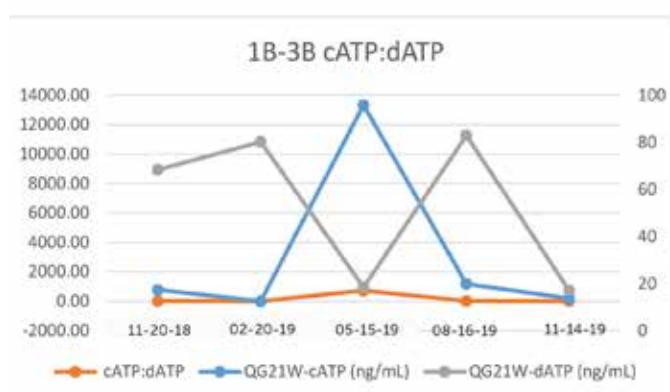
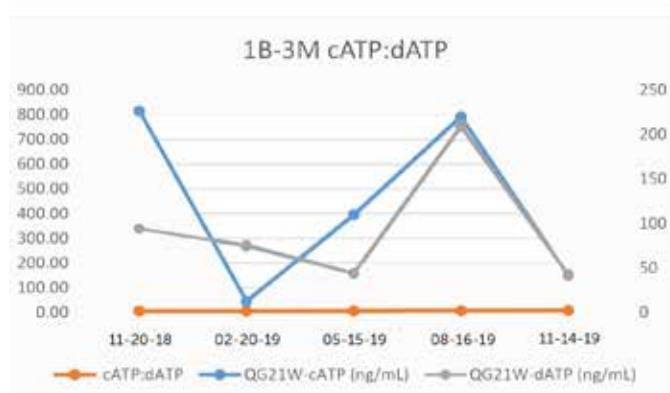
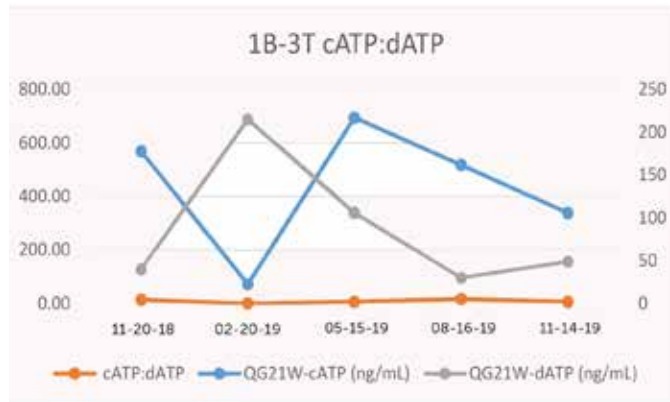


Figure 8a. Microorganisms Identified by DNA Testing, 11/29/18

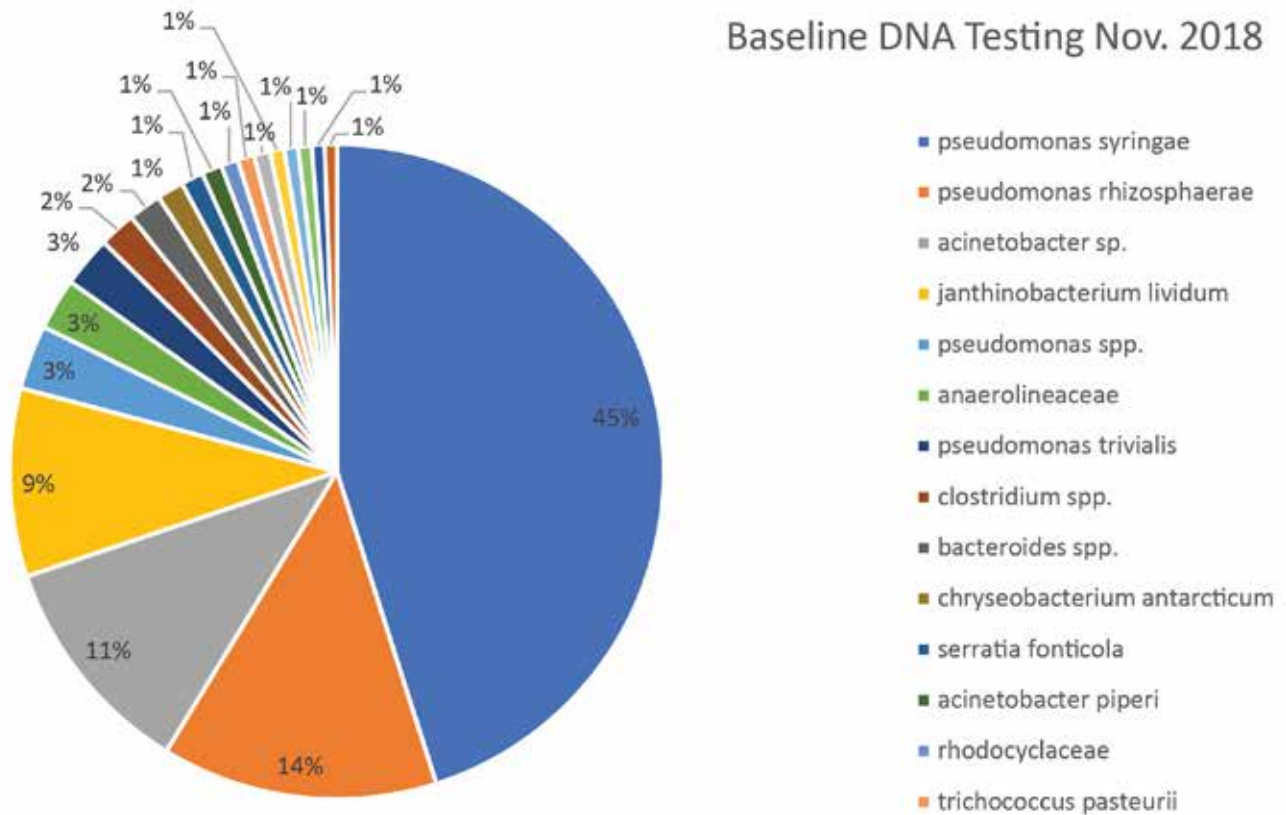


Figure 8b. Microorganisms Identified by DNA Testing, 2/20/19

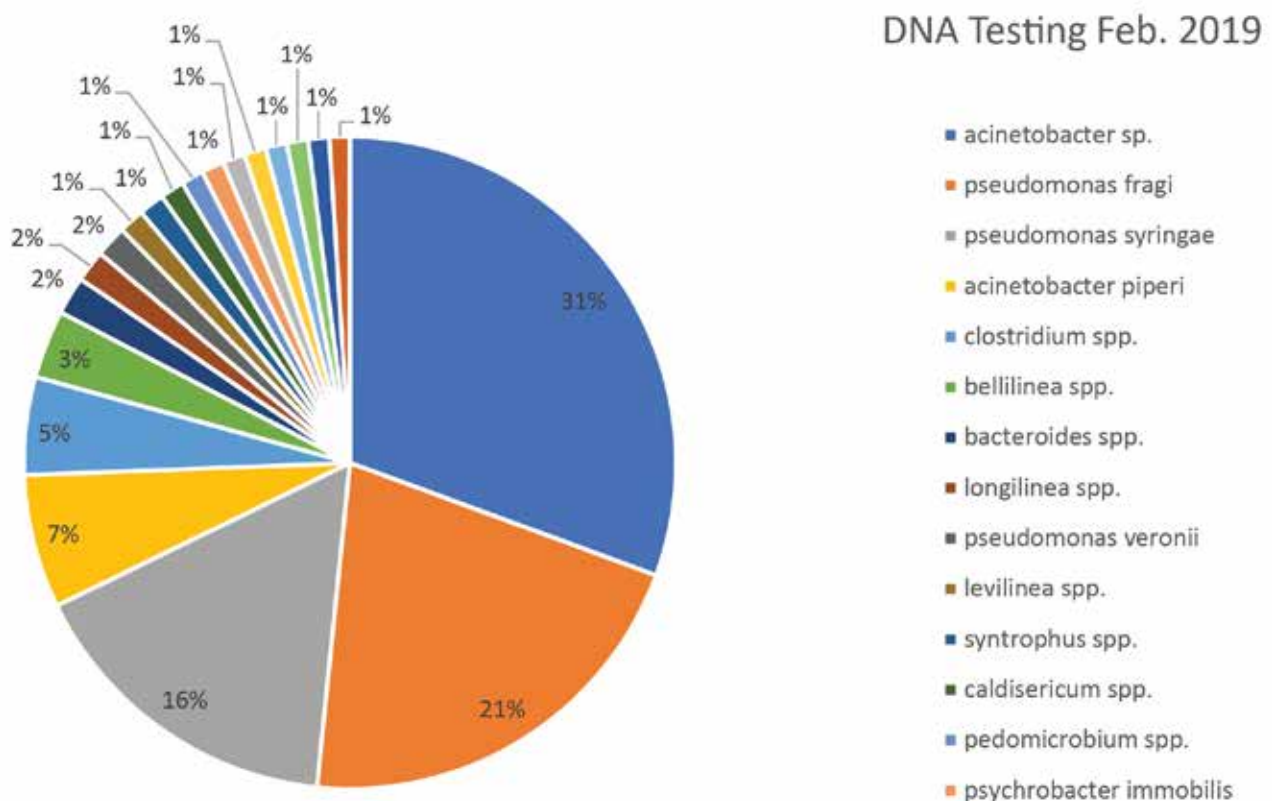


Figure 8c. Microorganisms Identified by DNA Testing, 5/14/19

DNA Testing May 2019

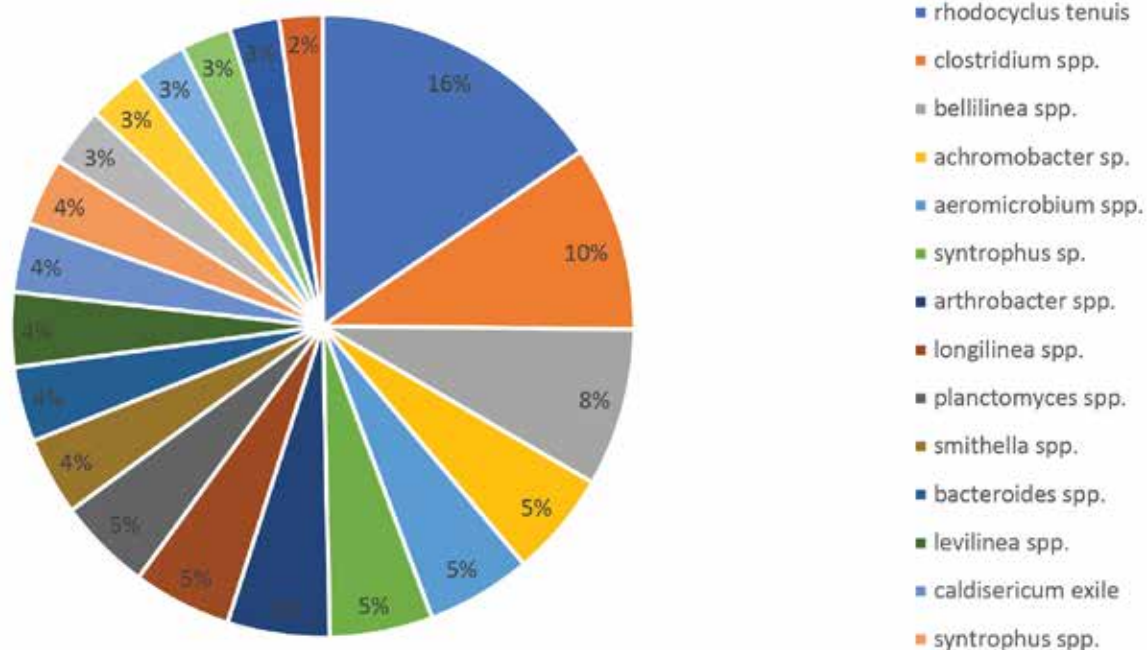


Figure 8d. Microorganisms Identified by DNA Testing, 8/15/19

DNA Testing Aug. 2019

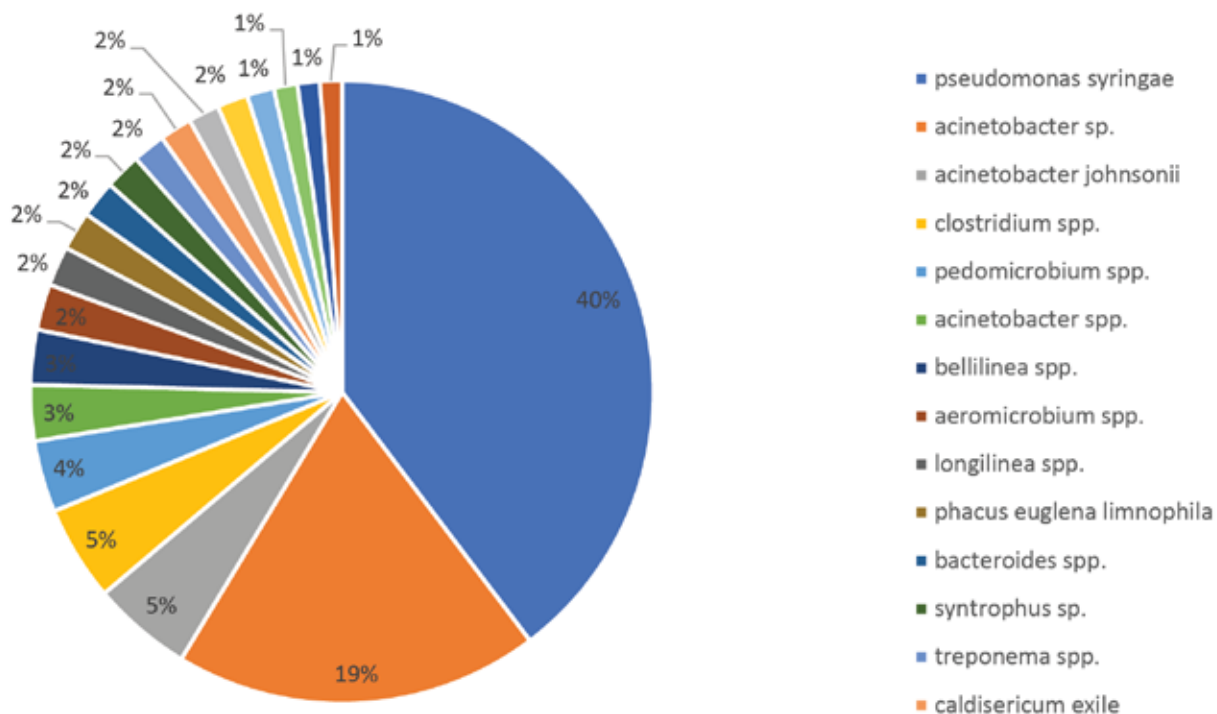
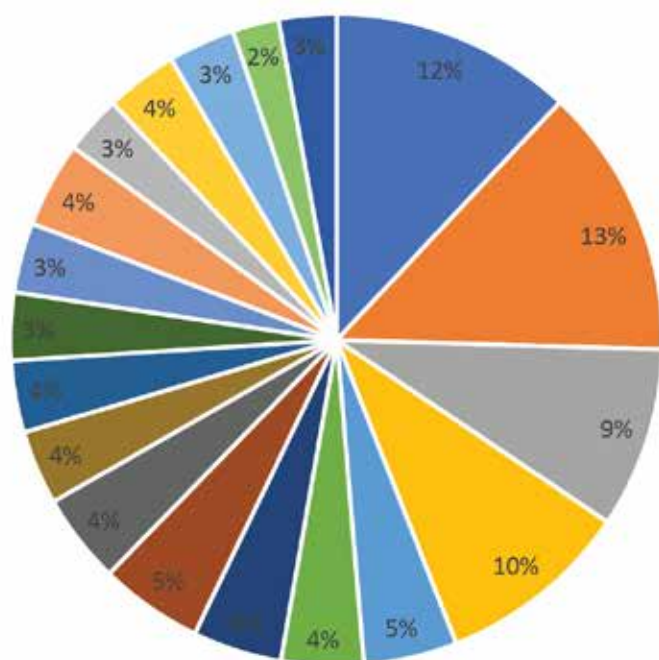


Figure 8e. Microorganisms Identified by DNA Testing, 11/13/19

DNA Testing Nov. 2019



- clostridium spp.
- achromobacter sp.
- bellilinea spp.
- thiodictyon bacillosum
- planctomyces spp.
- syntrophus sp.
- rhodocyclus tenuis
- rhodoferax albidiferax sp.
- methylocaldum spp.
- levilinea spp.
- blastopirellula spp.
- longilinea spp.
- methylocaldum sp.
- leptocinlis ovum

Tables

<u>List of Tables</u>	<u>Page</u>
Table 1a. 11/14/18 Pond 1A Baseline Water Column in Feet, West to East.....	20
Table 1b. 11/14/18 Pond 1A Baseline Sludge Depth in Feet, West to East.....	20
Table 1c. 2/21/19 Pond 1A Water Column in Feet, West to East.....	20
Table 1d. 2/21/19 Pond 1A Sludge Depth in Feet, West to East.....	20
Table 1e. 5/14/19 Pond 1A Water Column in Feet, West to East.....	20
Table 1f. 5/14/19 Pond 1A Sludge Depth in Feet, West to East.....	20
Table 1g. 8/15/19 Pond 1A Water Column in Feet, West to East.....	21
Table 1h. 8/15/19 Pond 1A Sludge Depth in Feet, West to East.....	21
Table 1i. 11/13/19 Pond 1A Water Column in Feet, West to East.....	21
Table 1j. 11/13/19 Pond 1A Sludge Depth in Feet, West to East.....	21
Table 2a. 11/14/18 Pond 1B Baseline Water Column in Feet, West to East.....	21
Table 2b. 11/14/18 Pond 1B Baseline Sludge Depth in Feet, West to East.....	21
Table 2c. 2/20/19 Pond 1B Water Column in Feet, West to East.....	22
Table 2d. 2/20/19 Pond 1B Sludge Depth in Feet, West to East.....	22
Table 2e. 5/14/19 Pond 1B Water Column in Feet, West to East.....	22
Table 2f. 5/14/19 Pond 1B Sludge Depth in Feet, West to East.....	22
Table 2g. 8/15/19 Pond 1B Water Column in Feet, West to East.....	22
Table 2h. 8/15/19 Pond 1B Sludge Depth in Feet, West to East.....	22
Table 2i. 11/13/19 Pond 1B Water Column in Feet, West to East.....	23
Table 2j. 11/13/19 Pond 1B Sludge Depth in Feet, West to East.....	23
Table 3. pH of Pond 1A and Pond 1B, by Date and Sample Location.....	23
Table 4. Temperature(°F) West to East (Cross Sections 1–3), by Date and Sample Location.....	23
Table 5. Pond 1A LuminUltra GQ21W Tests Performed, Sampling West to East (Cross Sections 1–3).....	24
Table 6. Pond 1B LuminUltra GQ21W Tests Performed, Sampling West to East (Cross Sections 1–3).....	25
Table 7a. 11/29/2018 Pond 1A Microorganism Counts from DNA Testing, Baseline, by Species and Sample Date.....	26
Table 7b. 2/25/2019 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site.....	26
Table 7c. 5/15/2019 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site.....	27
Table 7d. 8/15/2019 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site.....	27
Table 7e. 11/13/2019 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site.....	28
Table 7f. 11/29/2018 Pond 1B Microorganism Counts from DNA Testing, Baseline, by Species and Sample Date.....	28
Table 7g. 2/25/2019 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site.....	29
Table 7h. 5/15/2019 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site.....	29
Table 7i. 8/15/2019 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site.....	30
Table 7j. 11/13/2019 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site.....	30
Table 7k. Summary of Microorganism Counts from DNA Testing, by Species, Date, Pond, and Percentage of Sampling Event.....	31
Table 7l. Quarterly Nitrifying Microorganism Counts.....	32
Table 8. Sludge Reduction Summary.....	32

Table 1a. 11/14/18 Pond 1A Baseline, Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.67	4.84	6.50	
2	6.33	8.50	7.75	CS3A
3	4.84	6.42	6.41	
4	5.66	5.50	5.58	
5	6.50	4.67	5.25	
6	6.00	6.50	6.67	CS2A
7	6.58	6.42	6.75	
8	8.84	8.75	6.67	
9	6.59	7.33	6.92	CS1A
10	7.46	7.50	7.25	
WEST				

LEGEND: CS = Cross Section

Table 1b. 11/14/18 Pond 1A Baseline, Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.33	5.16	3.50	
2	3.67	4.50	2.25	CS3A
3	5.16	3.58	3.59	
4	4.34	4.50	4.42	
5	3.50	5.33	4.75	
6	4.00	3.50	3.33	CS2A
7	3.42	3.58	3.25	
8	1.16	1.25	3.33	
9	3.41	2.67	3.08	CS1A
10	2.54	2.50	2.75	
WEST				

LEGEND: CS = Cross Section

Table 1c. 2/21/19 Pond 1A Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.65	6.00	6.75	
2	5.65	6.47	6.42	CS3A
3	4.70	6.95	6.25	
4	5.00	4.87	5.05	
5	6.82	5.72	5.83	
6	6.92	7.58	6.92	CS2A
7	5.62	6.02	7.03	
8	7.17	8.53	8.08	
9	6.78	7.43	6.92	CS1A
10	7.03	6.28	6.53	
WEST				

LEGEND: CS = Cross Section

Table 1d. 2/21/19 Pond 1A Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.35	4.00	3.25	
2	4.35	3.53	3.58	CS3A
3	5.30	3.05	3.75	
4	5.00	5.13	4.95	
5	3.18	4.28	4.17	
6	3.08	2.42	3.08	CS2A
7	4.38	3.98	2.97	
8	2.83	1.47	1.92	
9	3.22	2.57	3.08	CS1A
10	2.97	3.72	3.47	
WEST				

LEGEND: CS = Cross Section

Table 1e. 5/14/19 Pond 1A Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.67	3.50	6.08	
2	6.12	7.05	5.73	CS3A
3	9.00	5.22	6.32	
4	7.10	7.60	7.98	
5	5.50	6.67	6.73	
6	6.80	6.80	6.98	CS2A
7	9.00	8.80	9.30	
8	9.25	9.25	9.10	
9	8.80	8.90	6.00	CS1A
10	2.55	7.20	7.38	
WEST				

LEGEND: CS = Cross Section

Table 1f. 5/14/19 Pond 1A Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.33	6.50	3.92	
2	3.88	2.95	4.27	CS3A
3	1.00	4.78	3.68	
4	2.90	2.40	2.02	
5	4.50	3.33	3.27	
6	3.20	3.20	3.02	CS2A
7	1.00	1.20	0.70	
8	0.75	0.75	0.90	
9	1.20	1.10	1.00	CS1A
10	7.45	2.80	2.62	
WEST				

LEGEND: CS = Cross Section

Table 1g. 8/15/19 Pond 1A Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	6.08	5.63	8.70	
2	8.80	9.20	9.30	CS3A
3	7.20	10.70	6.95	
4	5.95	7.13	7.47	
5	8.60	7.13	7.27	
6	6.03	6.60	6.53	CS2A
7	6.95	5.58	5.58	
8	10.00	7.85	9.00	
9	6.77	7.25	9.00	CS1A
10	6.13	5.00	7.74	
WEST				

LEGEND: CS = Cross Section

Table 1h. 8/15/19 Pond 1A Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	3.92	4.37	0.00	
2	1.20	0.80	0.00	CS3A
3	0.00	0.00	3.05	
4	4.05	2.87	2.53	
5	1.40	2.87	2.73	
6	3.97	3.40	3.47	CS2A
7	3.05	4.42	4.42	
8	0.00	2.15	0.00	
9	3.23	2.75	0.00	CS1A
10	3.87	5.00	0.00	
WEST				

LEGEND: CS = Cross Section

Table 1i. 11/13/19 Pond 1A Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	8.70	6.00	8.53	
2	8.60	9.40	9.25	CS3A
3	6.63	8.08	8.17	
4	8.70	8.90	8.25	
5	9.30	9.25	6.55	
6	6.92	7.22	7.62	CS2A
7	9.25	5.28	8.07	
8	8.77	8.80	6.70	
9	7.33	8.33	8.00	CS1A
10	6.43	6.85	7.27	
WEST				

LEGEND: CS = Cross Section

Table 1j. 11/13/19 Pond 1A Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	0.00	4.00	1.47	
2	0.00	0.00	0.00	CS3A
3	3.37	1.92	1.83	
4	0.00	0.00	1.75	
5	0.00	0.00	3.45	
6	3.08	2.78	2.38	CS2A
7	0.00	4.72	1.93	
8	1.23	0.00	3.30	
9	2.67	1.67	2.00	CS1A
10	3.57	3.15	2.73	
WEST				

LEGEND: CS = Cross Section

Table 2a. 11/14/18 Pond 1B Baseline Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.59	6.67	6.33	
2	5.91	6.91	5.75	CS3A
3	6.59	6.00	6.33	
4	6.17	6.42	7.08	
5	7.00	6.67	5.75	
6	7.33	6.41	6.50	CS2A
7	6.91	7.25	6.92	
8	6.92	7.25	6.67	
9	7.46	7.92	7.25	CS1A
10	6.83	7.92	7.34	
WEST				

LEGEND: CS = Cross Section

Table 2b. 11/14/18 Pond 1B Baseline Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.41	3.33	3.67	
2	4.42	5.17	3.50	CS3A
3	3.41	4.00	3.67	
4	3.83	3.58	2.92	
5	3.00	3.33	4.25	
6	3.50	5.33	4.75	CS2A
7	3.09	2.75	3.08	
8	3.08	2.75	3.33	
9	2.54	2.08	2.75	CS1A
10	3.17	2.08	2.66	
WEST				

LEGEND: CS = Cross Section

Table 2c. 2/20/19 Pond 1B Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.65	6.82	5.75	
2	5.90	6.38	7.00	CS3A
3	7.33	6.25	6.42	
4	6.77	7.02	4.05	
5	6.93	7.13	6.62	
6	7.17	7.08	6.50	CS2A
7	7.18	7.91	7.20	
8	6.93	8.00	6.62	
9	7.23	7.78	7.92	CS1A
10	7.17	7.65	7.38	
WEST				

LEGEND: CS = Cross Section

Table 2d. 2/20/19 Pond 1B Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.35	3.18	4.25	
2	4.10	3.62	3.00	CS3A
3	2.67	3.75	3.58	
4	3.23	2.98	5.95	
5	3.07	2.87	3.38	
6	2.83	2.92	3.50	CS2A
7	2.82	2.09	2.80	
8	3.07	2.00	3.38	
9	2.77	2.22	2.08	CS1A
10	2.83	2.35	2.62	
WEST				

LEGEND: CS = Cross Section

Table 2e. 5/14/19 Pond 1B Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	5.45	4.42	6.47	
2	5.60	6.92	5.75	CS3A
3	7.00	6.58	6.18	
4	7.33	6.33	5.62	
5	7.05	7.55	7.50	
6	8.17	6.05	5.92	CS2A
7	7.42	7.52	7.40	
8	7.17	6.80	7.00	
9	7.92	8.82	8.25	CS1A
10	6.88	7.88	7.58	
WEST				

LEGEND: CS = Cross Section

Table 2f. 5/14/19 Pond 1B Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	4.55	5.58	3.53	
2	4.40	3.08	4.25	CS3A
3	3.00	3.42	3.82	
4	2.67	3.67	4.38	
5	2.95	2.45	2.50	
6	1.83	3.95	4.08	CS2A
7	2.58	2.48	2.60	
8	2.83	3.20	3.00	
9	2.08	1.78	1.75	CS1A
10	3.12	2.12	2.42	
WEST				

LEGEND: CS = Cross Section

Table 2g. 8/15/19 Pond 1B Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	8.27	4.60	7.83	
2	7.77	6.77	7.92	CS3A
3	6.83	6.68	6.58	
4	7.00	6.08	6.00	
5	7.68	7.38	6.63	
6	6.77	7.58	7.08	CS2A
7	6.93	7.00	6.53	
8	7.45	7.40	8.22	
9	8.70	8.75	8.90	CS1A
10	6.92	8.72	8.80	
WEST				

LEGEND: CS = Cross Section

Table 2h. 8/15/19 Pond 1B Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	1.73	5.40	2.17	
2	2.23	3.23	2.08	CS3A
3	3.17	3.32	3.42	
4	3.00	3.92	4.00	
5	2.32	2.62	3.37	
6	3.23	2.42	2.92	CS2A
7	3.07	3.00	3.47	
8	2.55	2.60	1.78	
9	1.30	1.25	1.10	CS1A
10	3.08	1.28	1.20	
WEST				

LEGEND: CS = Cross Section

Table 2i. 11/13/19 Pond 1B Water Column in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	8.60	6.50	7.00	
2	6.78	7.90	7.43	CS3A
3	7.95	7.28	7.92	
4	8.50	6.32	6.58	
5	8.30	8.42	7.13	
6	7.80	7.83	6.50	CS2A
7	7.58	7.32	7.63	
8	7.72	8.05	7.53	
9	7.63	8.63	9.25	CS1A
10	8.50	8.75	9.00	
WEST				

LEGEND: CS = Cross Section

Table 2j. 11/13/19 Pond 1B Sludge Depth in Feet, West to East

Sample Point	Zone 1	Zone 2	Zone 3	Cross Section
EAST				
1	0.00	0.00	3.00	
2	3.22	2.10	2.57	CS3A
3	2.05	2.72	2.08	
4	1.50	3.68	3.42	
5	1.70	1.58	2.87	
6	2.20	2.17	3.50	CS2A
7	2.42	2.68	2.37	
8	2.28	1.95	2.47	
9	2.37	1.38	0.00	CS1A
10	1.50	0.00	0.00	
WEST				

LEGEND: CS = Cross Section

Table 3. pH of Pond 1a and Pond 1B, by Date and Sample Location

Date	Pond 1A									Pond 1B								
Sample Loc.	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B
11/15/2018	8.2	7.7	7.12	7.2	6.98	6.74	6.9	6.96	6.76	—	—	—	—	—	—	—	—	—
11/16/2018	—	—	—	—	—	—	—	—	—	7.3	7.41	7.13	7.49	7.11	6.74	7.64	6.8	6.88
02/20/2019	7.1	7.1	6.9	6.7	6.9	6.8	6.99	6.99	7.04	7.8	7.9	8.1	7.6	7.4	7.0	7.5	7.3	7.1
05/15/2019	7.7	7.7	7.2	7.6	7.0	7.0	7.5	7.0	6.9	8.0	7.9	7.5	7.81	7.16	7.1	7.4	7.0	7.0
08/16/2019	6.8	6.7	6.2	7.0	6.7	5.9	6.8	6.5	6.3	7.2	7.4	7.3	7.2	7.1	6.6	7.0	6.7	6.5
11/14/2019	6.7	6.8	6.1	7.1	6.7	6.3	6.7	6.8	6.8	7.9	7.6	7.3	7.2	7.4	6.8	7.0	6.7	6.5

LEGEND: T = Top, Supernatant; M = Middle, Interstitial; B = Bottom, Sludge Layer

Table 4. Temperature (°F) West to East (Cross Sections 1–3), by Date and Sample Location

Date	Pond 1A									Pond 1B								
Sample Loc.	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B
05/15/2019	64.8	65.0	65.6	70.2	70.5	70.3	74.3	75.2	74.4	73.6	73.7	75.2	64.6	66.3	66.4	79.1	77.7	74.9
08/16/2019	91.0	88.9	87.2	91.5	92.8	89.3	97.4	96.7	93.7	81.7	82.2	81.2	87.2	85.8	85.7	90.7	92.6	88.6
11/14/2019	68.1	67.2	68.5	62.7	63.3	63.9	64.1	63.7	64.3	64.4	64.4	64.6	65.2	65.3	65.7	66.3	67.2	67.2

LEGEND: T = Top, Supernatant; M = Middle, Interstitial; B = Bottom, Sludge Layer

Table 5. Pond 1A LuminUltra QG21W Tests Performed, Sampling West to East (Cross Sections 1–3)

Sample Location	Date	QG21W-AVSS (mg/L)	QG21W-BSI (%)	QG21W-cATP (ng/mL)	QG21W-dATP (ng/mL)	QG21W-tATP (ng/mL)	cATP-dATP
1A-1T	11/20/2018	96.82	8.87	193.64	18.85	212.49	10.27
	02/20/2019	27.05	8.17	54.09	4.78	58.87	11.32
	05/15/2019	-52.96	883.60	4.7	119.44	124.14	0.04
	08/16/2019	278.08	4.84	556.15	28.32	584.47	19.64
	11/15/2019	40.46	15.63	80.91	14.99	95.9	5.40
1A-1M	11/20/2018	214.92	9.2	429.84	43.54	473.38	9.87
	02/20/2019	7.99	61.26	15.98	25.27	41.24	0.63
	05/15/2019	-82.77	156.74	2221.42	457.28	2678.70	4.86
	08/16/2019	444.05	14.79	888.11	154.12	1042.22	5.76
	11/15/2019	144.74	10.74	289.47	34.82	324.3	8.31
1A-1B	11/20/2018	297.69	9.06	595.38	59.33	654.71	10.04
	02/20/2019	5.46	64.88	10.91	20.17	31.08	0.54
	05/15/2019	21.97	59.74	935.63	65.19	1000.82	14.35
	08/16/2019	104.66	22.45	209.32	60.58	269.9	3.46
	11/15/2019	64.03	10.54	502.46	59.18	561.64	8.49
1A-2T	11/20/2018	18.77	15.17	37.54	6.71	44.25	5.59
	02/20/2019	6.61	43.83	13.22	10.32	23.54	1.28
	05/15/2019	286.07	9.55	572.14	60.38	632.52	9.48
	08/16/2019	208.34	5.36	416.68	23.62	440.30	17.64
	11/15/2019	57.52	7.62	115.05	9.49	124.54	12.12
1A-2M	11/20/2018	-51.85	597.00	-103.70	124.56	20.86	-0.83
	02/20/2019	8.89	56.85	17.79	23.44	41.23	0.76
	05/15/2019	299.64	10.33	599.28	69.01	668.29	8.68
	08/16/2019	117.01	9.91	234.03	25.73	259.76	9.10
	11/15/2019	121.66	15.21	243.33	43.65	286.97	5.57
1A-2B	11/20/2018	108.49	18.17	216.98	19.31	236.29	11.24
	02/20/2019	0.64	90.08	1.29	11.70	12.99	0.11
	05/15/2019	27.81	16.79	55.63	11.22	66.85	4.96
	08/16/2019	39.94	26.56	79.88	28.88	108.76	2.77
	11/15/2019	57.89	7.76	115.79	9.74	125.53	11.89
1A-3T	11/20/2018	158.90	10.69	317.80	38.04	355.84	8.35
	02/20/2019	17.09	59.92	34.18	51.10	85.28	0.67
	05/15/2019	262.45	10.26	524.91	60.02	584.93	8.75
	08/16/2019	410.81	10.61	821.62	97.51	919.12	8.43
	11/15/2019	55.96	11.44	111.91	14.46	126.37	7.74
1A-3M	11/20/2018	407.5	10.36	815.01	94.24	909.25	8.65
	02/20/2019	22.41	62.61	44.83	75.06	119.89	0.60
	05/15/2019	197.59	9.54	395.19	43.63	438.82	9.06
	08/16/2019	396.05	20.90	792.09	209.33	1001.43	3.78
	11/15/2019	75.10	22.30	150.20	43.11	193.32	3.48
1A-3B	11/20/2018	1246.68	16.08	2493.36	477.86	2971.22	5.22
	02/20/2019	14.73	54.88	29.45	35.82	65.27	0.82
	05/15/2019	27527.60	0.08	55055.24	45.74	55100.97	1203.66
	08/16/2019	591.82	6.55	1183.64	82.91	1266.55	14.28
	11/15/2019	140.70	7.32	281.4	22.23	303.63	12.66

Table 6. Pond 1B LuminUltra QG21W Tests Performed, Sampling West to East (Cross Sections 1–3)

Sample Location	Date	QG21W-AVSS (mg/L)	QG21W-BSI (%)	QG21W-cATP (ng/mL)	QG21W-dATP (ng/mL)	QG21W-tATP (ng/mL)	cATP-dATP
1B-1T	11/20/2018	95.79	3.02	191.57	5.97	197.54	32.09
	02/20/2019	186.19	6.97	372.38	27.89	400.26	13.35
	05/15/2019	322.39	8.41	644.79	59.24	704.03	10.88
	08/16/2019	100.74	2.82	201.49	5.85	207.33	34.44
	11/14/2019	92.86	11.66	185.73	24.52	210.24	7.57
1B-1M	11/20/2018	124.46	0.09	248.92	0.22	249.14	1131.45
	02/20/2019	16.37	54.22	32.75	38.79	71.54	0.84
	05/15/2019	180.46	8.34	360.92	32.83	393.75	10.99
	08/16/2019	92.47	2.48	184.94	4.70	189.64	39.35
	11/14/2019	76.73	8.94	153.46	15.06	168.52	10.19
1B-1B	11/20/2018	1212.7	15.42	2425.39	442.08	2867.47	5.49
	02/20/2019	6.00	81.77	12.00	53.84	65.85	0.22
	05/15/2019	157.29	6.96	314.58	23.53	338.11	13.37
	08/16/2019	83.02	11.43	166.05	21.42	187.47	7.75
	11/14/2019	538.18	6.53	1076.37	75.23	1151.60	14.31
1B-2T	11/20/2018	468.60	4.22	937.19	41.26	978.45	22.71
	02/21/2019	6.94	58.90	13.88	19.90	33.78	0.70
	05/16/2019	197.50	7.50	395.00	32.01	427.02	12.34
	08/16/2019	110.36	10.78	220.72	26.67	247.39	8.28
	11/14/2019	106.24	23.98	212.47	67.02	279.49	3.17
1B-2M	11/20/2018	1739.99	2.62	3479.99	93.64	3573.63	37.16
	02/21/2019	-83.31	277.88	-166.62	260.29	93.67	-0.64
	05/16/2019	49.92	49.23	99.83	96.82	196.66	1.03
	08/16/2019	176.75	10.69	353.49	42.33	395.82	8.35
	11/14/2019	182.24	9.53	364.47	38.39	402.86	9.49
1B-2B	11/20/2018	1186.16	15.10	2372.32	422.09	2794.41	5.62
	02/21/2019	11.32	51.73	22.64	24.25	46.89	0.93
	05/16/2019	177.61	14.93	355.22	62.36	417.58	5.70
	08/16/2019	32.28	19.79	64.56	15.93	80.49	4.05
	11/14/2019	60.31	14.06	120.61	19.73	140.34	6.11
1B-3T	11/20/2018	284.52	6.55	569.04	39.91	608.95	14.26
	02/20/2019	36.28	74.78	71.56	215.20	287.75	0.33
	05/16/2019	347.30	13.25	694.61	106.05	800.66	6.55
	08/16/2019	258.95	5.47	517.90	30.00	547.89	17.26
	11/14/2019	169.40	12.61	338.80	48.89	387.69	6.93
1B-3M	11/20/2018	898.95	14.98	1797.91	316.66	2114.57	5.68
	02/20/2019	898.95	14.98	1797.91	316.66	2114.57	5.68
	05/16/2019	259.35	13.42	518.69	80.40	599.10	6.45
	08/16/2019	339.70	11.98	679.40	92.48	771.88	7.35
	11/14/2019	242.78	10.62	485.56	57.71	543.27	8.41
1B-3B	11/20/2018	388.05	8.10	776.10	68.38	844.48	11.35
	02/20/2019	-2.64	107.50	-5.28	80.24	74.96	-0.07
	05/16/2019	6664.99	0.14	13329.99	18.41	13348.40	724.06
	08/16/2019	591.82	6.55	1183.64	82.91	1266.55	14.28
	11/14/2019	94.4	8.30	188.8	17.08	205.88	6.93

Table 7a. 11/29/18 Pond 1A Microorganism Counts from DNA Testing, Baseline, by Species and Sample Site

Species	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	Total
pseudomonas syringae	34,070	42,709	41,948	56,642	38,730	46,009	55,856	41,421	37,086	394,471
pseudomonas rhizosphaerae	14,769	20,880	6033	20,855	10,270	10,513	31,812	14,529	8,014	137,675
acinetobacter sp.	44,679	21,740	30,017	482	6,891	3,970	8,119	9,479	3,595	128,972
janthinobacterium lividum	222	287	256	15,274	369	285	363	7,687	734	25,477
pseudomonas spp.	2,574	3,699	1,581	4,354	2,264	1,836	6,996	3,136	1,542	27,982
anaerolineaceae	26	592	1,842	86	2,255	3,163	72	1,594	2,428	12,058
pseudomonas trivialis	2,226	1,103	949	4,597	1,518	233	4,234	2,776	940	18,576
clostridium spp.	41	1,680	522	213	678	595	145	4,270	8,385	16,529
bacteroides spp.	18	164	968	54	2,325	1,990	55	1,865	1,967	9,406
chryseobacterium antarcticum	18	67	26	33	20	31	22	31	30	278
serratia fonticola	3,954	742	72	550	140	66	6,893	461	180	13,058
acinetobacter piperi	30	35	577	43	188	31	38	8,766	1,919	11,627
rhodocyclaceae	170	59	39	487	49	78	507	56	27	1,472
trichococcus pasteurii	99	262	861	56	421	699	281	1,012	945	4,636
burkholderia	105	53	43	403	40	86	346	35	11	1,122
hyphomicrobiaceae	11	315	357	73	1,664	467	18	125	158	3,188
longilinea spp.	3	314	556	13	726	907	10	1,361	1,479	5,369
syntrophus sp.	11	298	559	39	922	1,424	18	580	722	4,573
clostridiaceae	60	258	320	229	740	551	148	547	423	3,276
psychrobacter immobilis	780	1,007	408	31	199	476	1,060	508	649	5,118

Table 7b. 2/25/19 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	Total
acinetobacter sp.	22,972	18,652	23,023	10,879	24,412	14,768	24,149	28,019	48,733	215,607
pseudomonas fragi	67,309	15,043	3,226	27,008	4,109	559	30,337	4,400	3,322	155,313
pseudomonas syringae	40,691	9,927	2,137	19,797	2,694	648	25,041	3,742	2,288	106,965
acinetobacter piperi	14,939	9,577	5,445	757	2,918	998	1,515	4,261	3,710	44,120
clostridium spp.	3,906	1,363	2,360	3,917	4,493	4,926	1,392	10,647	7,388	40,392
bellilinea spp.	1,335	844	2,757	983	3,803	6,824	348	4,318	3,462	24,674
bacteroides spp.	818	334	1,978	518	1,892	3,152	329	1,784	1,023	11,828
longilinea spp.	401	419	1,358	248	2,207	3,384	154	2,264	1,717	12,152
pseudomonas veronii	3,405	519	204	596	222	88	1,148	342	137	6,661
levilinea spp.	525	298	1,068	244	1,477	2,550	147	1,385	1,388	9,082
syntrophus spp.	349	370	1,603	276	1,891	3,001	146	1,626	1,377	10,639
caldisericum spp.	633	384	555	432	1,784	1,663	127	857	372	6,807
pedomicrobium spp.	918	953	797	303	887	492	121	573	177	5,221
psychrobacter immobilis	165	67	56	85	69	46	568	190	220	1,466
planctomyces spp.	183	58	932	98	967	4,160	171	987	1,823	9,379
rhodocyclus tenuis	2,988	183	415	2,530	220	673	352	189	115	7,665
ancalomicrobium spp.	6,052	86	241	3,309	291	871	776	175	327	12,128
syntrophus sp.	356	203	1,204	239	1,328	2,141	158	1,283	945	7,857
syntrophorhabdus spp.	236	263	1,026	161	1,051	1,772	145	2,055	1,531	8,240
psychrobacter okhotskensis	449	60	90	158	142	102	567	203	415	2,186

Table 7c. 5/15/19 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	Total
rhodocyclus tenuis	6,323	512	2,688	36,038	1,179	2,175	20,949	1,143	3,224	74,231
clostridium spp.	4,232	7,669	4,742	10,636	4,398	3,418	9,951	20,315	21,013	86,374
bellilinea spp.	4,565	8,600	10,628	3,795	7,534	7,575	380	11,666	12,295	67,038
achromobacter sp.	9,012	483	763	19,759	1,051	322	9,970	326	704	42,390
aeromicrobium spp.	120	97	147	233	79	77	149	139	217	1,258
syntrophus sp.	373	1,404	6,460	495	5,703	5,807	260	5,735	15,961	42,198
arthrobacter spp.	5,316	1,006	1,682	12,180	828	1,590	22,250	806	802	46,460
longilinea spp.	2,404	6,198	6,040	1,474	5,245	3,865	258	7,640	11,571	44,695
planctomyces spp.	423	1,927	7,388	448	3,312	22,713	177	4,962	15,259	56,609
smithella spp.	125	1,634	5,801	329	3,764	3,846	123	6,039	8,845	30,506
bacteroides spp.	1,192	2,122	4,411	1,749	2,994	3,562	672	4,794	8,542	30,038
levilinea spp.	1,553	3,681	4,444	1,085	3,357	3,231	323	5,507	7,113	30,294
caldisericum exile	52	107	127	133	82	193	65	421	1,166	2,346
syntrophus spp.	1,641	4,068	5,279	909	4,143	3,550	223	4,495	6,832	31,140
thiodictyon bacillosum	2,191	1,225	1,498	5,430	1,533	459	5,606	943	1,696	20,581
lepocinclis ovum	669	182	124	3,841	135	58	1,796	85	141	7,031
pedomicrobium spp.	472	1,778	2,050	1,532	2,319	322	233	801	1,488	10,995
methylocaldum spp.	3,633	247	261	5,572	475	112	4,099	135	331	14,865
rikenella sp.	1,839	626	407	3,843	380	128	1,143	157	505	9,028
syntrophorhabdus spp.	193	1,135	2,534	500	2,275	2,074	123	3,636	8,309	20,779

Table 7d. 8/15/19 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	Total
pseudomonas syringae	7,925	13,561	6,642	12,598	14,291	470	12,025	681	8,423	76,616
acinetobacter sp.	2,879	1,029	531	11,429	4,362	9,493	5,951	275	130	36,079
acinetobacter johnsonii	7,653	1,642	437	133	2,155	297	69	29	105	12,520
clostridium spp.	159	712	779	153	163	505	1,280	4,125	2,657	10,533
pedomicrobium spp.	206	1,197	1,715	114	457	266	717	1,366	1,082	7,120
acinetobacter spp.	3,964	615	175	92	1,132	183	32	23	33	6,249
bellilinea spp.	69	343	1,138	45	177	1,175	455	1,252	542	5,196
aeromicrobium spp.	162	268	174	493	386	76	233	211	56	2,059
longilinea spp.	58	403	1,052	28	162	1,003	426	1,377	660	5,169
phacus euglena limnophila	62	19	8	81	5	7	16	9	10	217
bacteroides spp.	130	371	675	58	153	420	313	1,210	935	4,265
syntrophus sp.	16	143	772	14	72	801	158	877	556	3,409
treponema spp.	12	65	198	12	30	107	81	347	796	1,648
caldisericum exile	7	11	16	5	8	18	20	32	72	189
pseudomonas taiwanensis	197	1,022	607	199	636	5	514	33	39	3,252
trichococcus pasteurii	113	122	628	133	116	458	193	299	894	2,956
syntrophorhabdus spp.	18	136	527	23	94	435	311	951	617	3,112
acinetobacter piperi	483	281	261	258	538	53	16	12	24	1,926
rhodocyclus tenuis	72	65	194	256	390	154	125	159	269	1,684
pseudomonas veronii	228	144	304	557	614	5	158	13	27	2,050

Table 7e. 11/13/19 Pond 1A Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1A-1T	1A-1M	1A-1B	1A-2T	1A-2M	1A-2B	1A-3T	1A-3M	1A-3B	Total
clostridium spp.	26,827	14,132	9,467	42,333	6,943	2,405	30,123	21,197	36,640	5,101
achromobacter sp.	5,932	1,747	833	5,605	1,047	320	41,16	1,648	1,432	37,755
bellilinea spp.	2,824	13,112	21,496	6,654	16,921	13,428	4,496	16,602	30,983	595
thiodictyon bacillosum	7,611	2,601	1,925	11,192	2,305	775	5,301	4008	2,954	30,103
planctomyces spp.	416	1,289	9,678	662	15,146	40,545	673	3,125	27,368	352
syntrophus sp.	370	2,141	7,430	555	7,934	5,738	623	4,234	11,811	175
rhodocyclus tenuis	9,136	489	666	10,218	390	645	5,478	929	890	10,854
rhodoferax albidiferax sp.	165	209	392	251	204	172	291	359	282	16,470
methylocaldum spp.	6,949	998	257	6,251	273	123	5,168	1,098	657	11,822
levilinea spp.	1,372	3,286	8,386	1,818	7,058	4,386	2,086	4,970	12,193	230
blastopirellula spp.	604	4,460	7,906	838	5,043	6,031	1,517	4,721	9,071	229
longilinea spp.	543	3,446	9,148	676	7,302	3,335	898	3,926	10,774	345
methylocaldum sp.	7,422	1,048	447	8,197	578	314	5,475	1,199	743	8,791
lepocinclis ovum	78	109	157	157	104	65	302	148	228	12,809
syntrophus spp.	758	3,733	8,141	960	6,975	3,577	1,007	4,167	8,445	164
pedomicrobium spp.	436	800	4,898	576	2,583	320	844	2,063	2,622	1,080
bacteroides spp.	261	710	5,852	152	4,014	2,158	363	1,331	4,894	1,471
methanobacterium sp.	902	5,537	8,559	1,557	6,117	3,547	1,988	6,856	10,172	132
actinoplanes spp.	7,596	843	240	10,754	297	117	6,796	837	331	6,091
ralstonia spp.	324	2,279	5,559	525	4,077	4,669	1,272	2,682	8,097	158

Table 7f. 11/29/18 Pond 1B Microorganism Counts from DNA Testing, Baseline, by Species and Sample Site

Species	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B	Total
pseudomonas syringae	10,064	24,358	30,577	11,788	31,901	26,112	35,139	42,794	28,488	241,221
pseudomonas rhizosphaerae	1,938	4,793	4,852	1,383	5,303	5,998	8,709	10,878	11,004	54,858
acinetobacter sp.	1,920	3,251	3,284	1,504	1,292	4,904	6,499	1,112	3,549	27,315
janthinobacterium lividum	24,827	25,944	852	25,601	852	8,381	11,426	2,824	4,914	105,621
pseudomonas spp.	1,025	1,460	1,380	834	1,455	2,449	2,250	3,032	2,895	16,780
anaerolineaceae	35	53	5,811	129	5,291	4,824	2,398	2,965	2,495	24,001
pseudomonas trivialis	2,736	2,275	1,754	1,516	2,287	948	2,641	2,076	625	16,858
clostridium spp.	558	305	1,355	578	570	2,241	438	995	2,029	9,069
bacteroides spp.	403	243	2,034	712	2,809	1,967	1,590	2,773	1,312	13,843
chryseobacterium antarcticum	1,633	3,500	35	8,263	20	32	4,297	271	142	18,193
serratia fonticola	351	970	105	230	57	30	231	399	134	2,507
acinetobacter piperi	42	27	40	395	62	502	420	36	1,013	2,537
rhodocyclaceae	5,947	1,489	50	2,514	50	59	187	45	32	10,373
trichococcus pasteurii	136	229	1,766	800	726	903	672	1,084	777	7,093
burkholderia	6,160	1,423	44	2,260	68	43	198	25	15	10,236
hyphomicrobiaceae	51	32	1,652	57	1,589	1,004	871	945	396	6,597
longilinea spp.	7	8	1,166	15	795	825	290	680	405	4,191
syntrophus sp.	9	12	886	29	800	1,085	296	566	1,229	4,912
clostridiaceae	259	210	794	419	773	530	1,391	720	453	5,549
psychrobacter immobilis	64	485	145	686	280	509	906	81	254	3,410

Table 7g. 2/25/19 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B	Total
acinetobacter sp.	13,547	16,331	25,082	5,094	39,537	32,410	18,343	13,754	3,344	167,442
pseudomonas fragi	21,836	9,334	4,642	36,960	11,302	1,467	15,531	6,231	424	107,727
pseudomonas syringae	12,686	8,189	4,576	43,386	9,088	1,065	11,863	4,016	475	95,344
acinetobacter piperi	11,811	4,028	5,432	847	3,247	1,995	7,005	3,018	1,361	38,744
clostridium spp.	686	1,247	1,870	3,236	1,382	2,507	898	2,780	5,752	20,358
bellilinea spp.	252	1,290	2,298	2,769	1,868	2,008	847	2,688	3,701	17,721
bacteroides spp.	213	835	849	1,923	1,178	1,241	930	2,024	2,643	11,836
longilinea spp.	84	519	799	1,439	940	947	299	1,052	1,502	7,581
pseudomonas veronii	1,877	4,694	2,324	1,707	628	458	745	414	78	12,925
levilinea spp.	79	400	645	1,492	783	709	305	1,023	1,466	6,902
syntrophus spp.	78	281	541	664	555	661	212	800	1,414	5,206
caldisericum spp.	58	437	844	1,396	1,317	555	450	1,777	1,157	7,991
pedomicrobium spp.	72	470	1,215	806	1,217	615	355	2,873	1,281	8,904
psychrobacter immobilis	866	1,324	109	8,086	116	50	1,772	98	40	12,461
planctomyces spp.	51	197	477	336	318	733	226	593	1,483	4,414
rhodocyclus tenuis	1,079	1,208	204	2,369	71	118	580	157	232	6,018
ancalomicrobium spp.	41	63	55	522	63	91	61	83	125	1,104
syntrophus sp.	54	193	494	656	682	656	253	883	1,465	5,336
syntrophorhabdus spp.	72	241	492	435	325	557	234	904	1,573	4,833
psychrobacter okhotskensis	844	1,165	204	6,877	167	99	871	139	84	10,450

Table 7h. 5/15/19 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B	Total
rhodocyclus tenuis	51,347	42,208	9,168	32,338	977	3,380	9,411	905	1,844	151,578
clostridium spp.	1,153	1,039	8,307	1,315	6,001	13,210	7,681	6,705	8,271	53,682
bellilinea spp.	228	301	6,424	349	12,355	7,211	8,415	9,953	6,884	52,120
achromobacter sp.	6,293	9,513	4,078	7,403	386	951	7,415	651	319	37,009
aeromicrobium spp.	19,334	20,120	9,990	17,946	606	1,041	7,241	1,022	774	78,074
syntrophus sp.	143	207	4,733	202	8,950	9,174	1,193	4,720	6,294	35,616
arthrobacter spp.	3,919	2,644	3,090	6,348	858	2,121	7,207	3,108	1,385	30,680
longilinea spp.	143	162	3,289	181	7,549	4,671	2,979	6,344	4,383	29,701
planctomyces spp.	125	139	1,304	86	2,823	2,700	1,256	2,337	3,324	14,094
smithella spp.	125	165	4,321	146	8,018	5,946	1,208	4,698	3,765	28,392
bacteroides spp.	1,068	807	1,233	1,060	5,236	3,898	4,352	5,358	3,550	26,562
levilinea spp.	135	158	2,883	181	5,844	3,854	4,017	5,384	3,463	25,919
caldisericum exile	79	110	23,695	67	401	17,015	340	562	7,658	49,927
syntrophus spp.	96	146	2,824	156	5,041	4,424	2,016	3,265	2,428	20,396
thiodictyon bacillosum	4,004	4,913	2,656	4,242	1,404	1,267	3,695	1,096	832	24,109
lepocinclis ovum	10,235	7,533	973	7,059	249	186	8,105	312	87	34,739
pedomicrobium spp.	239	199	3,787	258	4,496	7,630	6,629	3,721	1,484	28,443
methylocaldum spp.	4,778	5,417	2,717	3,950	256	689	4,903	360	203	23,273
rikenella sp.	9,392	5,907	454	7,727	295	221	3,727	467	106	28,296
syntrophorhabdus spp.	102	118	1,508	81	2,301	2,681	1,228	1,814	2,183	12,016

Table 7i. 8/15/19 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B	Total
pseudomonas syringae	14,251	12,028	6,305	12,220	9,259	477	14,623	1,694	130	70,987
acinetobacter sp.	327	5,828	6,682	6,857	9,111	889	2,958	1,577	72	34,301
acinetobacter johnsonii	458	357	63	75	181	50	134	5,443	35	6,796
clostridium spp.	144	123	1,060	110	407	3,803	274	372	1,536	7,829
pedomicrobium spp.	816	404	924	385	630	267	536	1,005	1,741	6,708
acinetobacter spp.	223	218	38	57	86	23	69	3,805	15	4,534
bellilinea spp.	287	58	320	37	449	1,626	598	510	1,496	5,381
aeromicrobium spp.	1,180	1,624	1,274	1,320	768	217	283	76	43	6,785
longilinea spp.	98	17	74	13	422	646	239	217	762	2,488
phacus euglena limnophila	1,693	1,622	377	1,001	62	68	2,323	37	79	7,262
bacteroides spp.	204	64	64	54	440	423	238	344	1,067	2,898
syntrophus sp.	26	19	205	10	365	1,176	52	315	1,416	3,584
treponema spp.	62	41	1,096	27	67	2,453	56	122	778	4,702
caldisericum exile	10	24	1,727	27	96	3,827	12	25	277	6,025
pseudomonas taiwanensis	469	180	683	443	265	10	402	264	9	2,725
trichococcus pasteurii	58	40	180	78	222	703	576	86	1,051	2,994
syntrophorhabdus spp.	44	23	162	18	219	460	44	220	917	2,107
acinetobacter piperi	388	90	27	1,373	59	8	11	660	5	2,621
rhodocyclus tenuis	774	682	227	461	126	164	57	47	111	2,649
pseudomonas veronii	612	458	163	321	60	11	515	32	3	2,175

Table 7j. 11/13/19 Pond 1B Microorganism Counts from DNA Testing, by Species and Sample Site

Species	1B-1T	1B-1M	1B-1B	1B-2T	1B-2M	1B-2B	1B-3T	1B-3M	1B-3B	Total
clostridium spp.	8,925	6,904	6,946	7,545	20,329	6,921	12,146	14,431	279,315	363,462
achromobacter sp.	2,370	45,011	53,494	5,708	1,828	44,592	4,354	363	218,155	375,875
bellilinea spp.	12,353	1,145	721	12,881	15,840	1,401	16,560	13,082	201,094	275,077
thiodictyon bacillosum	5,247	28,881	29,520	4,518	4,018	21,027	3,819	1,130	166,935	265,095
planctomyces spp.	3,624	456	354	4,162	5,944	518	2,377	3,907	120,596	141,938
syntrophus sp.	4,283	368	269	13,400	11,591	438	5,754	6,083	83,197	125,383
rhodocyclus tenuis	1,473	12,528	13,721	1,782	1,983	10,126	645	389	82,342	124,989
rhodoferax albidiferax sp.	1,761	16,717	21,096	1,809	1,152	16,570	1,719	239	79,858	140,921
methylocaldum spp.	819	12,961	16,497	1,402	667	11,490	1,771	165	79,368	125,140
levilinea spp.	5,493	569	373	6,217	6,103	847	8,251	5,668	79,306	112,827
blastopirellula spp.	10,705	440	210	4,410	4,416	600	7,909	5,722	74,832	109,244
longilinea spp.	5,448	452	288	6,652	5,914	508	7,834	4,052	71,541	102,689
methylocaldum sp.	1,235	6,982	11,544	1,640	1,189	6,944	1,901	456	66,105	97,996
lepocinclis ovum	1,420	14,134	16,670	2,401	635	13,260	2,960	313	65,950	117,743
syntrophus spp.	6,284	356	251	4,779	4,782	394	5,421	2,986	63,180	88,433
pedomicrobium spp.	19,384	1,713	1,638	6,784	4,171	2,106	9,128	1,276	62,422	108,622
bacteroides spp.	3,350	1,145	2,261	11,282	6,405	2,972	6,977	5,333	60,931	100,656
methanobacterium sp.	3,153	241	80	1,527	2,074	260	2,696	2,615	58,013	70,659
actinoplanes spp.	628	8,086	6,606	690	720	5,666	1,231	261	57,790	81,678
ralstonia spp.	9,804	332	162	3,657	3,939	322	5,380	3,759	56,997	84,352

Table 7k. Summary of Microorganism Counts from DNA Testing, by Species, Date, Pond, and Percentage of Sampling Event

Species	Date	Pond A	Pond B	Total	% of Sample	Species	Date	Pond A	Pond B	Total	% of Sample	
achromobacter sp.	05/15/19	42,390	37,009	79,399	5.5	methylocaldum sp.	05/15/19	8,791	97,996	106,787	2.6	
	11/13/19	37,755	375,875	413,630	13.1			11/13/19	14,865	23,273	38,138	3.4
acinetobacter johnsonii	08/15/19	12,520	6,796	19,316	5.2	pedomicrobium spp.	02/25/19	5,221	8,904	14,125	1.1	
acinetobacter piperi	11/29/18	11,627	2,537	14164	1.0			05/15/19	10,995	28,443	39,438	2.7
	02/25/19	44,120	38,744	82,864	6.6			08/15/19	7,120	6,708	13,828	3.7
	08/15/19	1,926	2,621	4,547	1.2			11/13/19	1,080	108,622	109,702	3.5
acinetobacter sp.	11/29/18	128,972	27,315	156,287	11.1	phacus euglena limnophila	05/15/19	217	7,262	7,479	2.0	
	02/25/19	215,607	167,442	383,049	30.6	planctomyces spp.	02/25/19	9,379	4,414	13,793	1.1	
	08/15/19	36,079	34,301	70,380	18.9			05/15/19	56,609	14,094	70,703	4.9
aeromicrobium spp.	05/15/19	1,258	78,074	79,332	5.5			11/13/19	352	141,938	142,290	4.5
	8/15/19	2,059	6,785	8,844	2.4	pseudomonas fragi	05/25/19	155,313	107727	263,040	21.0	
anaerolineaceae	11/29/18	12,058	24,001	36,059	2.6	pseudomonas spp.	11/29/18	27,982	16,780	44,762	3.2	
ancalomicrobium spp.	02/25/19	12,128	1,104	13,232	1.1	pseudomonas syringae	11/29/18	394,471	241,221	635,692	45.1	
arthrobacter spp.	05/15/19	46,460	30,680	77,140	5.3			02/25/19	106,965	95,344	202,309	16.2
bacteroides spp.	11/29/18	9,406	13,843	23,249	1.6			08/15/19	76,616	70,987	147,603	39.7
	02/25/19	11,828	11,836	23,664	1.9	pseudomonas trivialis	11/29/19	18,576	16,858	35,434	2.5	
	05/15/19	30,038	26,562	56,600	3.9	pseudomonas veronii	02/25/19	6,661	12,925	19,586	1.6	
	08/15/19	4,265	2,898	7,163	1.9			08/15/19	2,050	2,175	4,225	1.1
	11/13/19	1,471	100,656	102,127	3.2	psychrobacter immobilis	11/29/19	5,118	3,410	8,528	0.6	
bellilinea spp.	02/25/19	24,674	17,721	42,395	3.4		02/25/19	1,466	12,461	13,927	1.1	
	05/15/19	67,038	52,120	119,158	8.2	psychrobacter okhotskensis	02/25/19	2,186	10,450	12,636	1.0	
	08/15/19	5,196	5,381	10,577	2.8	raistonla spp.	11/13/19	158	84,352	84,510	2.7	
	11/13/19	595	275,077	275,672	8.7	rhodocyclaceae	11/29/18	1,472	10,373	11,845	0.8	
blastopirellula spp.	11/13/19	229	109,244	109,473	3.5	rhodocyclus tenuis	05/25/19	7,665	6,018	13,683	1.1	
burkholderia	11/29/18	1,122	10,236	11,358	0.8			05/15/19	74,231	151,578	225,809	15.5
caldisericum exile	05/15/19	2,346	49,927	52,273	3.6			08/15/19	1,684	2,649	4,333	1.2
	08/15/19	189	6,025	6,214	3.6			11/13/19	10,854	124,989	135,843	4.3
caldisericum spp.	02/25/19	6,807	7,991	14,798	1.2	rhodoferax albidiferax sp.	11/13/19	16,470	140,921	157,391	5.0	
chryseobacterium antarcticum	11/29/18	278	18,193	18,471	1.3	rikenella sp.	05/15/19	9,028	28,296	37,324	2.6	
clostridiaceae	11/29/18	3,276	5,549	8,825	0.6	serratia fonticola	11/29/18	13,058	2,507	15,565	1.1	
clostridium spp.	11/29/18	16,529	9,069	25,598	1.8	smithella spp.	05/15/19	30,506	28,392	58,898	4.1	
	02/25/19	40,392	20,358	60,750	4.9	syntrophorhabdus spp.	02/25/19	8240	4,833	13,073	1.0	
	05/15/19	86,374	53,682	140,056	9.6			05/15/19	20,779	12,016	32,795	10.2
	08/15/19	10,533	7,829	18,362	4.9			08/15/19	3,112	2,107	5,219	1.4
	11/13/19	5,101	363,462	368,563	11.7	syntrophus sp.	11/29/18	4,573	4,912	9,485	0.7	
hyphomicrobiaceae	11/29/18	3,188	6,597	9,785	0.7		02/25/19	7,857	5,336	13,193	1.1	
janthinobacterium lividum	11/29/18	25,477	105,621	131,098	9.3		05/15/19	42,198	35,616	77,814	3.5	
lepocinclis ovum	05/15/19	7,031	34,739	41,770	2.9		08/15/19	3,409	3,584	6,993	0.3	
	11/13/19	12,809	117,743	130,552	4.1		11/13/19	175	125,383	125,558	11.7	
levilinea spp.	02/25/19	9,082	6,902	15,984	1.3	syntrophus spp.	02/25/19	10,639	5,206	15,845	1.0	
	05/15/19	30,294	25,919	56,213	3.6			05/15/19	31,140	20,396	51,536	3.5
	11/13/19	230	112,827	113,057	3.6			11/13/19	164	88,433	88,597	1.9
longilinea spp.	11/29/18	5,369	4,191	9,560	0.7	thiodictyon bacillosum	05/15/19	20,581	24,109	44,690	3.1	
	02/25/19	12,152	7,581	19,733	1.6			11/13/19	30,103	265,095	295,198	9.3
	05/15/19	44,695	29,701	74,396	5.1	treponema spp.	08/15/19	1,648	4,702	6,350	1.7	
	08/15/19	5,169	2,488	7,657	2.1	trichococcus pasteurii	11/29/18	4,636	7,093	11,729	0.8	
	11/13/19	345	102,689	103,034	3.3			08/15/19	2,956	2,994	5,950	1.6
methanobacterium sp.	11/13/19	132	70,659	70,791	2.2							

Table 7I. Quarterly Nitrifying Microorganism Counts

	Nov '18	Feb '19	May '19	Aug '19	Nov '19
Pond 1A Nitrifying Microorganism Count	389	907	6,349	330	3,692
Pond 1B Nitrifying Microorganism Count	2,071	1,090	7,045	334	7,673
Combined Ponds Nitrifying Micro. Count	2,460	1,997	13,394	664	11,365

Table 8. Sludge Reduction Summary

Parameter (Unit)	Pond 1A	Pond 1B
Length (feet)	1,266.77	1,266.77
Width (feet)	315.29	315.29
Baseline Average Sludge Depth (feet)	3.44	3.44
Average Sludge Reduction (feet)	1.68	1.46
Volume of Sludge Removed (cubic feet)	670,304.00	581,637.22
Volume of Sludge Removed (gallons)	5,013,873.91	4,350,646.39
Volume of Sludge Removed (short tons)	20,921.65	18,154.17
Volume of Sludge Removed (trucks)	1,494.40	1,296.73
% Removed	48.72	42.23



Probiotic
SOLUTIONS®
Enhancing Nature's Science™

Bio Huma Netics, Inc.
 1331 W. Houston Ave., Gilbert, AZ 85233
1.480.961.1220

www.Probiotic.com