EVALUATION OF A GREENHOUSE ECOSYSTEM TO MANAGE CRAFT BEVERAGE WASTEWATER

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Biosystems Engineering – Master of Science

2023

ABSTRACT

A greenhouse ecosystem, often referred to as a Living Machine©, is a technology for biological wastewater treatment using plants in a greenhouse structure. It has a small footprint relative to traditional onsite systems, has been shown to manage high strength wastewater, and can provide a high level of treatment to allow for reuse for purposes such as irrigation, toilet flushing, and landscaping. Craft beverage wastewater (water from wineries, breweries, and cideries) is considered high strength and contains chemical oxygen demands (COD) close to 20,000 mg/L, total nitrogen up to 80 mg/L, and total phosphorus up to 70 mg/L. Due to the variability of the wastewater in both flow and composition, it is hard to treat with a conventional wastewater treatment system. The ability of this system to treat craft beverage wastewater is determined through this project.

The experimental system consisted of three parallel systems, with one always serving as a control to treat representative synthetic winery wastewater. Each system had three reactors in series with 5 species of plants native to Michigan. The first two reactors had 12-hour aeration cycles. Synthetic wastewater was prepared to test the ability of the system to treat a variety of wastewater characteristics that are found from these sources. Once the performance of the greenhouse ecosystem is understood for various wastewater characteristics, actual wastewater from the three sources will be tested. wastewater characteristics that were routinely measured included pH, dissolved oxygen, and conductivity, as well as visually inspecting the plants. Nitrate, nitrite, ammonia, total nitrogen, COD, and total phosphorus levels were measured weekly.

Results show the system is effective within the designed organic and hydraulic loadings. However, extreme events will cause disruptions and potential system failure. An example of an extreme event is having a high concentration of COD. Aeration was found to be a key factor in reducing the odor produced from treating the wastewater. The greenhouse ecosystem can also be scaled up, as the volume and flow are known for a specific loading rate.

Next steps include performing an economic analysis, performing optimization experiments to determine the best plants to use, monitoring more sample types (such as total Kjeldahl nitrogen and nitrogen gas), performing microbial analyses, and performing a field scale experiment.

ACKNOWLEDGEMENTS

Thank you to the Craft Beverage Council (GG 22*1568), and the USDA (MICL04225) for providing funding for this project. Thank you to the winery, brewery, and cidery contacted for providing wastewater for this project.

Thank you to the College of Agriculture and Natural Resources Statistics Consulting Center for assisting with the statistical analysis of the data collected in this project.

Thank you to my committee members: Dr. Safferman, Dr. Dechand, and Dr. Masten. I have learned so much from all of you and I am grateful for the opportunity to study with you. Thank you to all the undergraduate workers who have helped me with this study: Jackson Hotchkiss, Jordan Dashner, Kate Mann, Ben Bridge, Serena Hurst, Madison Pritchett, and Arianna Fobbs. Thank you to my co-graduate student Greg.

Thank you to all my friends and family who have listened to me talk about this project for two years, with a special shoutout to my parents, who pushed me in high school and helped me realize my potential.

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CHAPTER 1: DESIGN OF THE GREENHOUSE ECOSYSTEM

INTRODUCTION

Craft beverage wastewater refers to winery, brewery, and cidery wastewater and is considered high strength wastewater as it has high amounts of chemical oxygen demand (COD), nitrogen, and phosphorus. Michigan has around 600 craft beverage facilities[1].

The COD in winery wastewater comes from alcohols, sugars, acids, tannins, and lignin[2]. COD in brewery wastewater comes from sugars, starches, ethanol, and fatty acids[3]. There is no literature on the COD components in hard cider wastewater, so it is assumed that it is similar to winery and brewery wastewater. Other components in the wastewater that make it hard to treat are the cleaning/disinfection products. These include sodium hydroxide and potassium hydroxide[4]. Since the cleaning chemicals are caustic and used to clean food processing equipment, including disinfecting, they have a negative impact on the environment and plants. Wastewater treatment systems need to be able to treat high-strength wastewater with substantial amounts of cleaning chemicals.

Wineries produce wastewater during several stages, as shown in Figure 1. Most of the wastewater comes from washing the machinery to keep it sterile. The exact amount of wastewater to wine produced changes from winery to winery, but it can range from 0.2L to 14L discharged per liter of wine[5]. Wineries in Michigan can produce over 3.5 million gallons of wine per year[1].

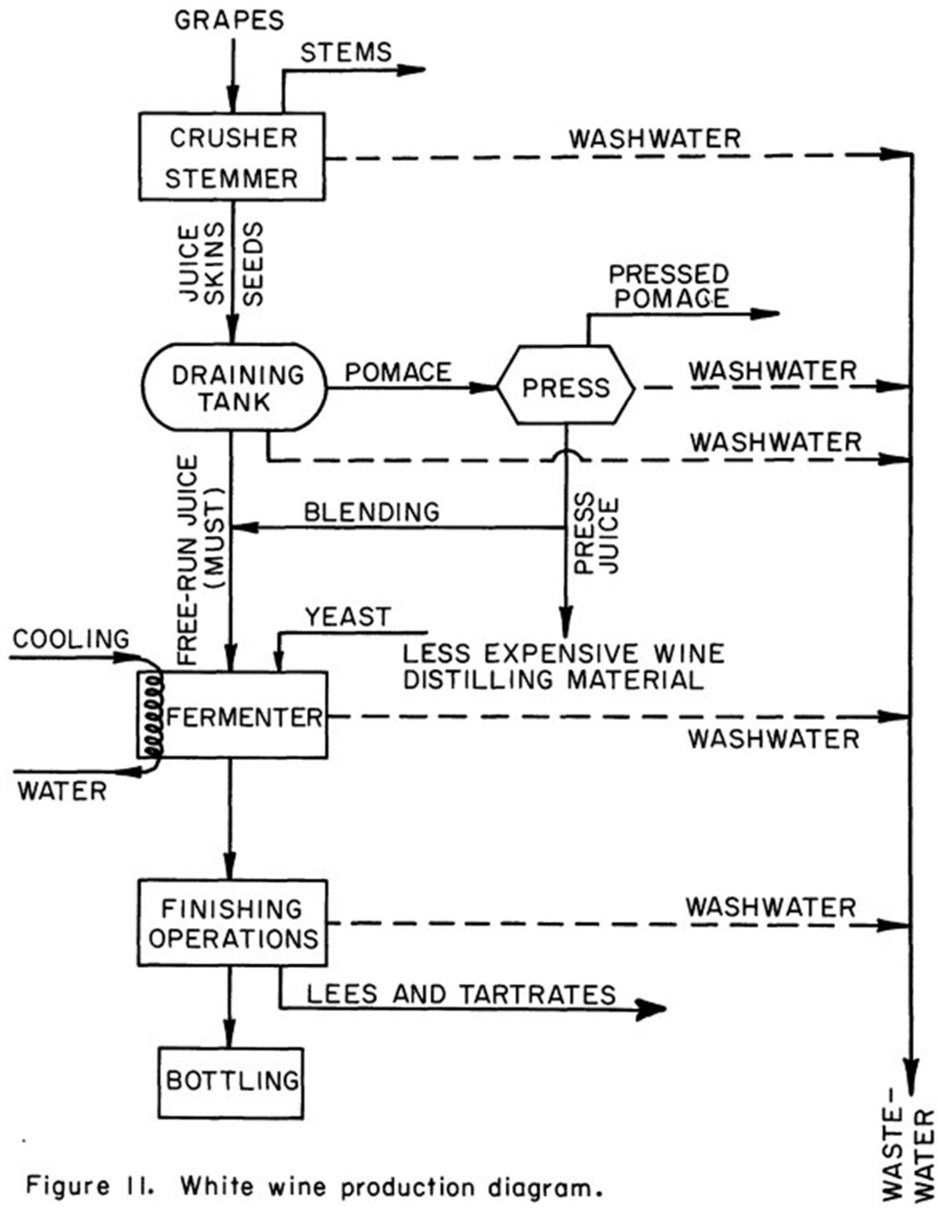

Figure 1: Wine production with locations of wastewater creation [6].

Breweries also produce wastewater at several stages, as shown in Figure 2. Wastewater is produced from cleaning as well, but since brewing requires more steps, there are more types of constituents in the wastewater. Brewery wastewater will also vary between brewery to brewery, but the average is 3.3 L of wastewater per L of beer produced. Breweries in Michigan produce over 1.2 million beer barrels in a year (39 million gallons)[7].

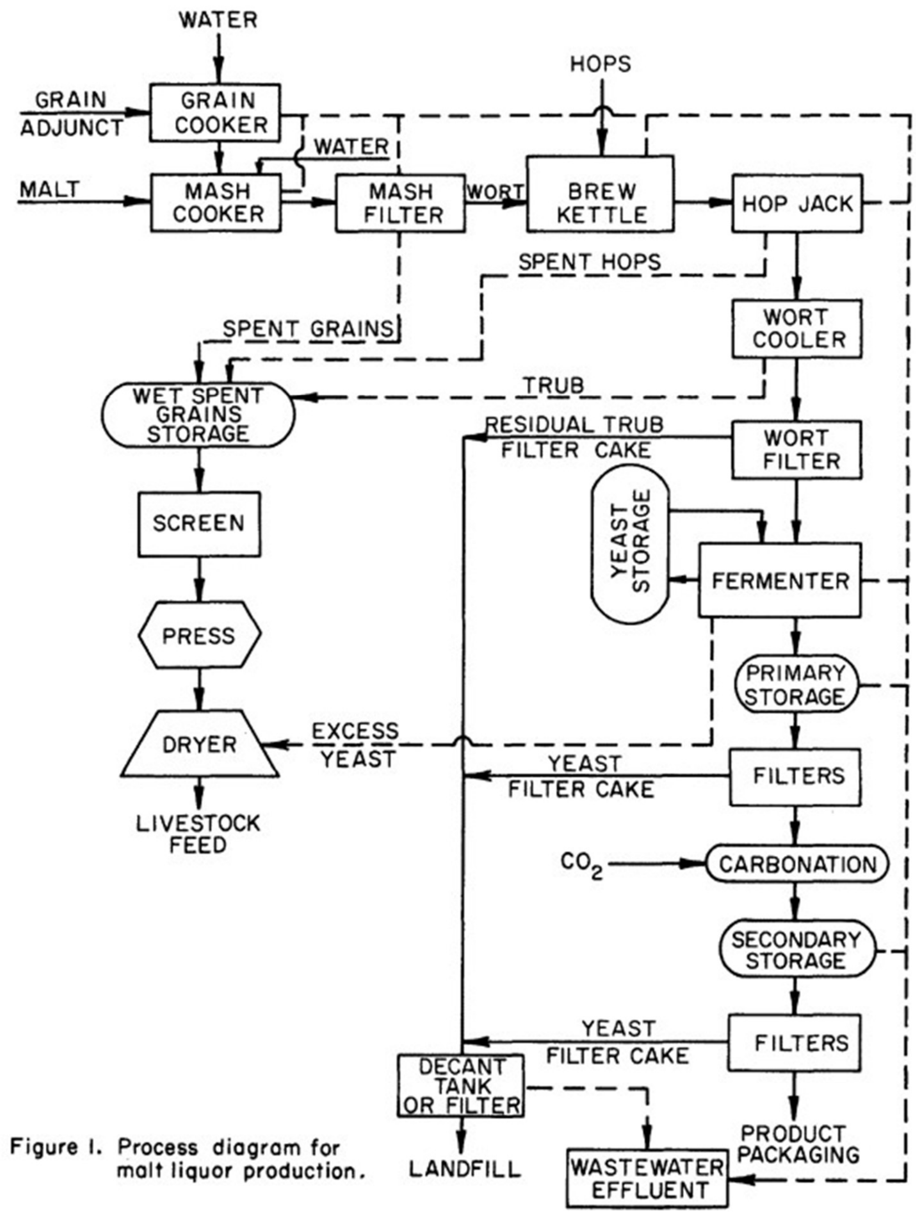

Figure 2: Process flow diagram of beer production [6]

In Michigan alone there are 630,000 gallons of hard cider produced in a year[1]. There is currently little to no literature on how much wastewater is produced per liter of hard cider produced but based on the brewery wastewater production (3.3 times as much wastewater as beer produced) and winery wastewater production (up to 14 times as much wastewater as wine produced), it can be assumed that the wastewater produced per liter is similar and varies depending on the season. The US EPA does not currently have a hard cider process flow diagram, but as it is fruit being turned into alcohol, it can be assumed to be similar to the winery production process as both are taking fruits and turning them into alcoholic beverages.

The high amount of wastewater produced in the craft beverage industry, as well as the high strength of the pollutants in the wastewater, means that it cannot be easily treated by a typical decentralized wastewater treatment plant. Wastewater treatment plants are designed for a specific range of values, so it is difficult and expensive to send water to wastewater treatment plants.

A greenhouse ecosystem, also known as a Living Machine ™ has been used in the past to treat onsite generated municipal and high strength wastewater. This system has been used to treat dairy wastewater[8], blackwater[9], and other types of high strength wastewater[10]. A greenhouse ecosystem has several benefits: less operation/maintenance, chemicals do not need to be added into the system, and it has a smaller footprint. A greenhouse ecosystem works by suspending plants in water media and allowing the microbes around the roots, as well as the plant uptake, to treat the pollutants within the wastewater. However, there is no literature on the use of a greenhouse ecosystem to treat craft beverage wastewater.

The need for this research is that there are not many cost effective, small-footprint treatment systems for craft beverage wastewater. The resulting objectives of this research follow.

- Design a greenhouse ecosystem for craft beverage wastewater, using literature values for other high-strength wastewater.
- Determine the characteristics of winery, brewery, and cidery wastewaters to develop synthetic wastewaters. Synthetic wastewater was used to determine the effect of specific constituents on treatment and plants. Actual winery, brewery, and cidery wastewater was used after the concept was proven to determine if any constituents that could be impactful were not included in the synthetic formulation.
- Choose the best native, non-invasive plants to use for the system.
- Develop a decision support tool for the selection of the best onsite wastewater treatment technology for site-specific craft beverage producers.

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CHAPTER 2: CRAFT BEVERAGE WASTEWATER TREATMENT USING A GREENHOUSE ECOSYSTEM

ABSTRACT

A greenhouse ecosystem, often referred to as a Living Machine ™, is a biological wastewater treatment process that uses plants in a greenhouse structure. It has a small footprint relative to traditional onsite wastewater systems, can manage high strength wastewater, and can provide a high level of treatment to allow for non-potable reuse. Craft beverage wastewater (CBW) is considered high strength because of its elevated levels of nitrogen, phosphorus, and biochemical oxygen demand. Due to the variability of the flow and composition, this wastewater is challenging to treat with a conventional onsite wastewater treatment system. A proof-of-concept study was conducted on the applicability of the greenhouse ecosystem to treat CBW.

The experimental system had three parallel trains, each with three reactors in series with 5 species of plants native to Michigan. The first two reactors in each train had 12-hour aeration cycles. One train always served as a control, receiving a consistent amount of synthetic winery wastewater (SSWW). The SSWW's characteristics were altered to represent the variability of craft beverage wastewater in the other two trains. Once the performance of the greenhouse ecosystem was understood, actual wastewater from a winery, cidery, and brewery were tested as to ensure the SSWW was representative. Wastewater characteristics that were measured daily included pH and dissolved oxygen. Visual inspection of the plants also occurred daily. Nitrate, nitrite, ammonia, total nitrogen (TN), chemical oxygen demand (COD), and total phosphorus (TP) levels were measured approximately once a week.

Results show that the system is effective at removing the nutrients and COD. The high amounts of COD caused a system failure in the COD spiked wastewater and in the cidery wastewater, but the system was able to recover after additional nutrients were added to COD spiked wastewater. The SSWW moving through Train 1 produced the best plant growth, which the high amounts of COD had the worst plant growth.

The highest removal rate of COD was during the cidery and winery wastewater, with 98% removal. The highest removal rate of TN was also in cidery wastewater, with a removal rate of 88%. The highest removal rate of nitrate was 97% in the brewery wastewater. The highest removal rate of nitrite was in the brewery wastewater with over 99% removal. The highest

removal rate of ammonia was above 99% removal in the SSWW, the winery wastewater, and the salt and nutrient spike. The highest removal rate of TP was 91% in the brewery wastewater.

INTRODUCTION

A greenhouse ecosystem (commonly known as a Living Machine ™) for wastewater treatment is a biological system with plants and microbes that are specifically selected to treat wastewater [1], [2] . Plants with large root systems typically have better treatment[3], [4]. The greenhouse ecosystem is intended to be a small-footprint option as well as being a more economical option than conventional onsite wastewater treatment systems for high-strength wastewater and has been demonstrated on blackwater[5][6], dairy wastewater[7], and poultry wastewater[8].

A greenhouse ecosystem typically has anaerobic and aerobic zones to allow for carbon oxidation, nitrification, and denitrification, as well as a clarifier and a final polishing reactor[9]. Plants also contribute to nitrogen uptake. Phosphorus removal is typically achieved by precipitation and plant uptake [10]. The wastewater can be treated to an extent that allows for reuse as irrigation water or toilet flushing. Another benefit of a greenhouse ecosystem is that it does not require any chemicals to be added for treatment.

Craft beverage wastewater is high strength wastewater with an average COD of around 3,000 mg/L[11]. Table 1 summarizes these characteristics. Winery and cidery wastewater are also unique in that for about half of the year, there is little to no wastewater being produced. Their main production period is after fall harvest. Beer is different as the wastewater is produced more consistently throughout the year [12]. Craft beverage wastewater includes a wide range of components because it is produced from excess raw products, cleaning equipment, processing, packaging preparation, and off-specification product that is discharged to the drain.

Within table 1, the Michigan Department of Environmental Quality [11] had 5 sample collection sites. Skornia et. al. [13] collected wastewater from one site and they noted that the values collected were lower than the average national values but matched values from other studies in Michigan. They also performed a literature review with national averages. Bakare et. al. [14] had 1 sample collection site and used literature to confirm the values. Brito et. al. [15] had 3 sample collection points and had sources they compared the results to. Hard cider wastewater was difficult to characterize as there is little to no literature found with characterization values of the wastewater; most studies examined the solids portions produced.

Table 1. General Craft Beverage Wastewater Characteristics

Parameter (mg/L)	**Winery** [11], [13]		**Brewery** [14], [15]		**Cidery** [16], [17]	
	Average	**Range**	**Average**	**Range**	**Average**	
Chemical oxygen demand (COD)	3,236	320-296,119	11,214	800-20,000	8,000	>170,000
Biochemical oxygen demand (BOD)	2,046	125-130,000	2,746	1,200-3,600	4,800	
pH	6.2	3-12.9	6.74	5-11	N/A	
Sodium	279	7-470	N/A		N/A	
Total solids	11,311	1,602-79,635	5,600 (TSS)	5,100-8,750	6,000	
Total phosphorus (TP)	5.26	3.3-188.3	16-68	9-50	N/A	
Total nitrogen (TN)	7.6	10-415	8.1	12-31	N/A	

The variability of craft beverage wastewater flow and components makes it difficult to use a conventional onsite wastewater treatment system such as a septic tank and drain field. Building a traditional activated sludge or fixed film treatment plant on-site takes a lot of space and is expensive.

The Michigan craft beverage industry is extensive, with over 300 breweries, over 200 wineries, and over 90 cideries[18]. This ranks Michigan 6th in the nation for craft breweries, 9th in the nation for wineries[18], and the top producer of hard cider[19]. Michigan craft beverage production facilities are typically in rural areas not served by a centralized wastewater facility. These facilities need an onsite system as trucking the wastewater to a treatment facility is not an economical option. There is much recent attention on the management and regulation of craft beverage wastewater as more facilities are built. Current regulations depend on the region and site-specific factors.

The greenhouse ecosystem has been demonstrated on high-strength food processing wastewater but literature on its application to craft beverage wastewater was not found. This research is a proof-of-concept study on its applicability to this industry. The objectives include the following.

- Design a greenhouse ecosystem for craft beverage wastewater, using literature values for other high-strength wastewater.

- Choose the best native, non-invasive plants to use for the system.
- Determine the characteristics of winery, brewery, and cidery wastewaters to develop synthetic wastewaters. Synthetic wastewaters were used to determine the effect of specific constituents on treatment and plants. Actual winery, brewery, and cidery wastewater was used after the concept was proven to determine if any constituents that could be impactful were not included in the synthetic formulation.
- Develop a qualitative decision support tool for the selection of the best onsite wastewater treatment technology for site-specific craft beverage producers.

METHODS

This section details the literature gathered for the experimental design, synthetic wastewater recipe, quality assurance and quality control, and the statistical analysis methods for analyzing the data.

Experimental Design

The desired water loadings were based on chemical oxygen demand (COD) as that is the pollutant of most concern within the wastewater. A loading of 0.61 kg/m^3-d was determined from the typical concentration in winery wastewater (Table 1) and literature loading values for a greenhouse ecosystem receiving high-strength wastewater (Table 2).

Table 2: Literature Loading Values for High-Strength Wastewater

COD Concentration-Loading value	Hydraulic residence time	Wastewater type	Technology	Reference
440 +/- 217 mg/L*	5 days	Black water	Bio-contact oxidation; constructed wetland	[20]
392 +/- 174 mg/L* on startup 723+/- 409 mg/L* once stable	6, 5, 4 days	Black water	Living Machine ™	[21]
0.004 kg/m^2/d	7, 10, 9 days (compared 3 technologies)	Sewage	2 biofilters, surface flow wetland, stabilization pond	[22]
0.03 kg/m^2/d	5	Brewery wastewater	Hydroponics with grass	[23]
425.7 mg/L*	3.27 days	Black water	Living Machine ™	[24]
0.092 kg COD/m^2/d	3-5 days in septic tank	Winery wastewater	Constructed wetland	[25]

*These values did not have loading values reported.

This project had three treatment trains consisting of three reactors each. The reactors were 55-gallon drums with 50 gallons marked. These reactors were found to have a depth adequate for unrestricted root growth for the selected plants. From the selected loading, 0.61 kg/m3-d, the flow rate to each reactor was 5.4 gal/day.

Grow lights were used to simulate the greenhouse ecosystem. Figure 1 shows the grow light frame, reactors, and the plants, and Figure 2 is an overhead view of the system. The water is placed into the refrigerator before it goes through the system. This is done to prevent microbial growth from occurring and taking pollutants before the water was pumped through the system. The water was then pumped into each of the train's Reactor 1. The water was then pumped from Reactor 1 into Reactor 2, and from Reactor 2 to Reactor 3. There was a hole drilled into the side of the Reactor 3's to allow for the water to come out of the reactor by gravity outflow. This reduced the need for an extra pump.

Figure 3: Entire system together with plants, grow lights, and water moving through the system.

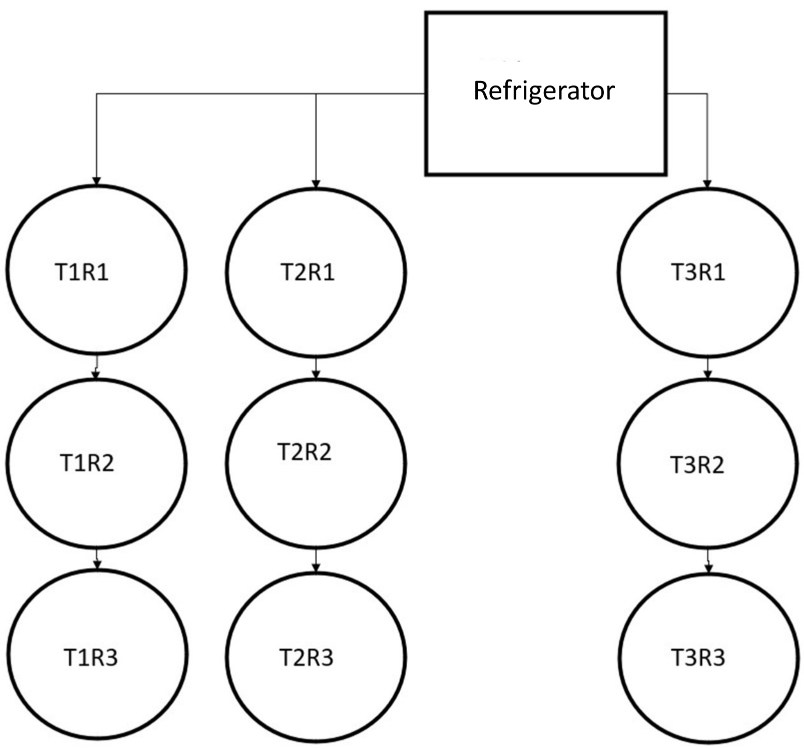

Figure 4: System Schematic with reactor labels. Arrows show direction of water movement.

Plant Decisions

The plants were chosen by performing a literature review. Austin et. al. [26] had an extensive list of effective plants that they experimented with, so their list was checked for those native to Michigan, as their experiment took place in Vermont. The following are criteria the plants had to meet to be chosen: cold tolerance (to allow for treatment over winter months), pruning ability, native to Michigan, and non-invasive in the US. The selected plants were *Schoenoplectus pungens*[27] (three square bulrush, also known as *Scirpis pungens*), *Acorus americanus* (American sweetflag), *Decodon verticillatus* (swamp loosestrife), *Typha augustifolia* (cattail)[28], [29], *Penstemon digitalis* (foxglove beard tongue), and a volunteer species (a plant that grew on its own in the system and was not initially planted) *Spirodela polyrhiza* (duckweed species)[30].

Swamp loosestrife has not been studied widely for wastewater treatment, but a similar plant called purple loosestrife has been. However, purple loosestrife is invasive in Michigan. Foxglove beard tongue also has not been studied widely for wastewater treatment, but it has been studied and determined to be an effective metal accumulator[31]. As some craft beverage wastewaters contain heavy metals, such as copper, it was added to attempt to uptake these heavy metals.

These plants were all collected from Cardno Native in Walkerton, Indiana, except the cattail and duckweed, which were collected from a local pond. A plastic chicken wire fence was used to keep the plants anchored in place. The fence was attached to the reactors by drilling holes through the lip of the reactor and using zip ties to keep the fence at the top of the water. The plants arrived in soil media, so as much soil was removed as possible to expose the roots. Holes in the fence were expanded to be slightly smaller than the root ball and the roots were placed into the hole. One of each plant species was placed into every drum. This was done so all drums would have a similar starting point.

The plants were monitored visually to determine health. Photos were taken of the plants every day the experiment was operated to allow for comparisons of health over time. The three growth indicators that were monitored were the leaf color, the leaf texture, and plant elongation. Plant leaves will change colors if they are stressed. Common colors are yellow or brown. The leaf texture will change if the plants become dehydrated or absorb too much water. They will become dry and wrinkled if the leaves dry out or become soft if they absorb too much water.

Plant elongation is when the plants stretch out because they cannot find enough light. This leads to yellowing leaves near the base of the plant that will be more likely to fall off.

<u>Wastewater Characteristics</u>

The influent wastewater was stored in a 6-gallon bucket in a refrigerator immediately before being pumped into the first reactor in each train. The pumps used were Cole-Parmer No. 7553-80, 1-100 RPM with Masterflex ® Model 7017-20 pump heads and Masterflex® tubing.

The same type of pump was used to transfer the water from the first reactor into the second reactor and second to the third. The pumps operated on different timelines to avoid overtopping the reactors. The first pump ran from 9 AM-7 PM, the second from 9 AM-8 PM, and the third from 9 AM-9 PM. There was a gravity outlet on the side of the third reactor that allows for water to move into a storage carboy.

All trains were initally opertated identially. After roughly a month and a half with the original synthetic wastewater to allow the plants to equlibriate. The feed to trains 2 and 3 were then changed to determine the effect of specific wastewater constiuents and simulate the strenths of brewery and cidery wastewater,. Thereafter, actual wastewater was used in trains 2 and 3 to determine if any wastewater consituents that were not in the synthetic formulaiton effected performnace and plant health. Details are provided in Table 3.

Table 3: Dates and Phases of Operation

Research Phase	3/20/23-4/6/23	4/13/23-5/17/23	5/31/23-6/22/23	7/14/23-8/21/23	10/20/23-11/15/23
Phase 1: equilibrium (all trains managed identally, receiving SSWW)	3/20/23 3/23/23 3/30/23 4/6/23				
Phase 2: • Train 1: SSWW • Train 2: COD spike (winery) • Train 3: Nutrient spike (brewery)		4/13/23 4/20/23 4/27/23 5/3/23 5/10/23 5/17/23			
Phase 3: • Train 1: SSWW • Train 2: COD and Nutrient spike (winery) • Train 3: Nutrient and Salt spike (cidery)			5/31/23 6/6/23 6/14/23 6/22/23		
Phase 4: • Train 1: SSWW • Train 2: Acutal winery wastewater • Train 3: Actual cidery wastewater				7/14/23 7/21/23 7/28/23 8/1/23 8/4/23 8/11/23 8/21/23	
Phase 5: • Train 1: SSWW • Train 2: Actual brewery wastewater • Train 3: SSWW, recovery					10/27/23 11/3/23 11/8/23

Note: SSWW is Standard Synthetic Wastewater, which simulated wastewater from a winery.

The extensive literature review (Table 1) conducted to determine the components of winery, cidery, and brewery wastewater was used to develop the synthetic formulation. This formula required 95% ethanol, diluted white grape juice, sodium phosphate, and nitrogen fertilizer. The diluted grape juice had a 1:20 ratio of juice:water. Table 4 is the exact formulation for each phase of the research.The white grape juice and ethanol mimics white wine, since white wine is produced the most often in Michigan.

A new batch of wastwater was produced every 3 days and stored at a temperature between 35-40 °F. The wastewater changeover was done in the mornings before 9am before the pumps turn on, or in the evenings after the pumps turn off. Between each batch, the buckets were washed with a phosphate free detergent.

Table 4: Synthetic Wastewater Recipes

Formulation	95% Ethanol	Diluted juice	Nitrogen Fertilizer	Sodium Phosphate	Salt
Standard Synthetic Winery wastewater (SSWW)	46 mL	75 mL	1032 mg	170 mg	0 mg
COD Spike	75 mL	570 mL	1032 mg	170 mg	0 mg
Nutrient Spike	46 mL	75 mL	3100 mg	1040 mg	0 mg
COD and Nutrient Spike	75 mL	570 mL	5160 mg	850 mg	0 mg
Salt Spike	46 mL	75 mL	3100 mg	1040 mg	15,600 mg

The standard synthetic winery wastewater (SSWW) recipe was designed and confirmed with in-house lab testing to obtain an average COD of around 1,000-2,000 mg/L. The amount of nitrogen and phosphorus added were 10 mg/L and 5 mg/L, respectively. The COD spike recipe was designed and confirmed at 5,000-6,000 mg/L. [11]The nutrient spike recipe aimed for 30 mg/L as both nitrogen and phosphorus, as 30 mg/L in the wastewater. The nutrient-salt spike recipe was the same recipe used for the nutrient spike, but added 15,600 mg of salt.

The COD in the SSWW was based on the average amount of COD in Michigan wineries. The amount of COD in the COD spiked wastewater comes from the max wastewater COD from 5 Michigan wineries[11]. The COD and nutrient spike was designed to have the same COD as the COD spike alone. The nutrient spike was to mimic brewery wastewater, as brewery wastewater has more excess nutrients than the winery wastewater does. The COD spiked recipe had a COD concentration of up to 5 times greater than the base synthetic wastewater, so the fertilizer and sodium phosphate were also multiplied by 5. The amount of salt comes from 280 mg of sodium in a liter, which is the average amount of salt in Michigan Wineries. There was little to no literature on hard cider wastewater characteristics, so no recipes could be designed for it.

Aeration was added after a month of operation to reduce odors. The aeration provided an anaerobic Reactor 1, aerobic Reactor 2, and anoxic Reactor 3. The amount of oxygen in the water was measured using a DO probe. The anaerobic drum being the first drum, even with aeration, is likely because of the high amounts of COD in the wastewater that the aeration is not effective to make an aerobic zone. Having an anaerobic reactor and an aerobic reactor allows for nitrification and denitrification. A 1744 GPM aeration pump was used to provide aeration to the first two reactors in each train. The air pump used was a Simple Deluxe pump, model number LGPUMPAIR110. The air was pumped air through a manifold to the 9 reactors. An aeration stone was placed at the bottom. Each aeration stone provided roughly 194 GPM of air.

Train 1 Reactor 3 and Train 3 Reactor 3 had no aeration and Train 2 Reactor 1 and Train 3 Reactor 1 had twice the aeration going into them by having two aeration stones. This was determined as the project continued because of issues with odor and lessening treatment efficiency. The trains were not operated identically because each of the wastewater types have different aeration requirements. The control ran well with only one stone in the first two reactors. There were no odor issues from the control wastewater. However, there were odor complaints about winery and cidery wastewater, so another aeration stone was added to each Reactor 1.

Daily sampling for pH, temperature, and dissolved oxygen was performed in each reator. Two probes were used, a HACH pH probe and a HACH LBOD probe. The HACH pH probe is a HACH sensION+ ™ portable pH meter with a Sension+5051T pH electrode[32]. The HACH DO probe is a HACH HQ1130 DO/1 Channel handheld device with an LDO 10101 probe[33]. Measurements until August were used with the HQ40d handheld device. The manufacturer tates these two devices are identical to each other.

Water samples were taken on average 3-5 times per sampling phase to measure nitrate, nitrite, total nitrogen (TN), total phosphorus (TP), and COD. HACH TNT kits were used to measure these parameters and a HACH DR6000 UV VIS Spectrophotometer was used to obtain the concentrations. The kit detection limits and methods used are in table 5.

Table 5: HACH Kits, Limits and EPA Methods

HACH TNT Kit	Limits	Method	References
TNT 823 (ultra high range COD)	250-15,000 mg/L COD	HACH method: 10212 EPA Reactor Digestion Method	[34]
TNT 826 (low range total nitrogen)	1-16 mg/L N	HACH method: 10208 Not EPA approved or equivalent	[35], [36]
TNT 831 (low range ammonia)	1-12 mg/L NH_3-N	HACH method: 10205 EPA method equivalent to 350.1, 351.1, 351.2	[36], [37]
TNT 835 (low range nitrate)	0.23-15.50 mg/L NO_3-N 1-60 mg/L NO_3	HACH method: 10206 EPA method approved, compared to 40 CFR 141	[36], [38]
TNT 839 (low range nitrite)	0.015-0.6 mg/L NO_2-N 0.05-2 mg/L NO_2	HACH method: 10207 EPA Diazotization method	[39]
TNT 844 (low range phosphorus)	0.5-5.0 mg/L PO_4-P 1.5-15.0 mg/L PO_4	HACH method: 10209/10210 Not EPA approved or equivalent	[36], [40]

A description of the facilites where the acutal wastewater used in Phases 4 and 5 were collected from follows.

- Winery:
  - Small scale
  - Current treatment: septic tank (25-30 gal), air treatment, bark bed
  - Comingled wastewater
  - No screening
  - Wastewater collected from the septic tank
- Cidery:
  - Medium scale
  - Current treatment: two 2,000-gallon septic tanks, then pumped into an aboveground 20,000 gallon tank and shipped away
  - Non-comingled wastewater
  - Screening
  - Wastewater collected from septic tank

- Brewery:
  - Large scale
  - Current treatment: wastewater pumped directly to 20,000 gallon aboveground tank and shipped to a wastewater treatment facility
  - Non-comingled wastewater
  - No screening
  - Wastewater collected from spigot near bottom of 20,000 gallon tank

Quality assurance and quality control

Water samples were collected by taking a plastic sample collection bottle and putting it into the center of the reactors. Samples were either analyzed immediately or placed into a fridge until analysis could be performed later that day. Analysis was always done on the same day as collection as to avoid having to use a preservative. Three replicates were chosen using a random number generator, representing a 25% replication rate. A standard and blank (DI water) were also performed for all tests (TN, TP, nitrate, nitrite, ammonia, and COD) at a rate of one standard and one blank for every seventeen samples. If the replicates were not within 10% of each and/or the standards and blanks were not within 10% of the expected vale the analyses were rerun.

Statistical analysis

Data were analyzed using the R statistical software and the code is provided in Appendix 7. A linear mixed effect model was employed to test for the fixed effects of phases, train and drum, and their interactions on response variables using the lme4 package. Responses include COD, total phosphorus, total nitrogen, nitrate, nitrite, and ammonia. Sample ID nested within phases were considered random effects. Model residual diagnostics were performed using residual vs. fitted plots for constant variance, and QQ plots for normality assumptions. Data was log transformed to meet model assumptions. Mean shifting outliers were removed using the car package. If ANOVAs revealed significant main effects at alpha level of 0.05, post hoc tests were conducted using the emmeans package. Results were back transformed to the response scale and estimated marginal means were reported.

In each graph, there may be removals of 100% or 0%. The 100% removal occurred when the effluent value was 0 mg/L or not detected. The 0% removal occurred either when there was addition of nutrients (such as nitrate increasing due to nitrification) or when the influent value was marked as not detected and then had an effluent value as 0 (as happened often with ammonia).

The average values of the data are presented. The averages below the detection limit are marked as Below Detection Limit (BDL) in the tables. The averages below the detection limit were able to be calculated as the spectrophotometer produces a value for every sample run, but these values cannot be used as it is considered inaccurate. In the statistical analysis for the data BDL, the value midway between 0 and the minimum detection limit was used instead of the spectrophotometer reading. In several cases, BDL values were negative, and a negative concentration does not exist.

The percent removal standard deviation was calculated using the propagation of error equation, shown below. As some of the values for the effluent are BDL, the value midway between the value and the detection limit was used. The values used in the standard deviation for the removal percentages are in the tables next to the BDL indicator.

$$S_R = R\sqrt{\frac{S_{eff.}{}^2}{Eff.conc.} - \frac{S_{inf.}{}^2}{Inf.conc.}} \quad (1)$$

COD AND NUTRIENT ANALYSIS

The wastewater analysis is broken down into 6 sections: chemical oxygen demand, total nitrogen, nitrate, nitrite, ammonia, and total phosphorus. Within each section, the average influent and effluent values will be displayed for each phase, along with the number of data points, standard deviation, and percent removal. The phases and removals will be compared to each other. The actual wastewaters used will also be compared to each other.

The raw data for all constituents is in Appendices 1-6. Appendix 8 has the visual data provided by the statistical analysis, which is what was used to determine if data is statistically significant. Data in the tables is marked as statistically different by using superscripts. Data with no superscripts indicates that there is not a statistical difference between the values. Organic nitrogen estimates do not have statistical differences between the values as no statistical analyses were performed on them.

<u>Chemical Oxygen Demand</u>

The COD influent, effluent, number of samples taken, and percent removal are shown in Table 6. During Phase 1, the influent wastewater to all trains were the same and had a COD that ranged from 600 mg/L to 3000 mg/L. The broad range was from the wastewater prepping procedure as the COD degraded over the three-day storage period, even though stored at a low temperature. The range of the wastewater is beneficial in proving the concept, however, as this type of range is not unrealistic at actual processors. Regardless, the variability did not prevent the wastewater from being treated to close to or below 250 mg/L (detection limit of the TNT 823 HACH kit) for all trains. The COD was roughly halved between the influent and Reactor 1 for the control train (1100 mg/L to 300 mg/L), and then halved again between reactors 1 and 2 (260 mg/L to 150 mg/L). Reactor 2 and Reactor 3 had similar values (150 mg/L and 140 mg/L, respectively). The average removal percentage across the entire train was 88%. This trend of at least halving the COD between the influent and Reactor 1 and halving again between Reactor 1 and Reactor 2 was observed in all types of wastewaters analyzed.

Table 6: COD Analysis Statistics

	mg/L COD	Mass, kg/d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	1118	2.29E-02	19	754	88%	20.9
Train 1 SSWWW Effluent	BDL 125	2.56E-03	20	87		
Train 2 SSWWW Influent	1202	2.46E-02	4	593	67%	12.5
Train 2 SSWWW Effluent	393	8.05E-03	4	145		
Train 2 COD Spike Influent	4503[a]	9.22E-02	6	6039	88%	80.0
Train 2 COD Spike Effluent	560[b]	1.15E-02	6	378		
Train 2 COD and Nutrient Spike Influent	4469[a]	9.15E-02	3	543	96%	7.8
Train 2 COD and Nutrient Spike Effluent	BDL 125[b]	2.56E-03	3	10		
Train 2 Winery Wastewater Influent	7043[a]	1.44E-01	7	9074	98%	106.1
Train 2 Winery Wastewater Effluent	BDL 125[b]	2.56E-03	7	20		
Train 2 Brewery Wastewater Influent	12981[a]	2.66E-01	3	1093	97%	11.2
Train 2 Brewery Wastewater Effluent	334[b]	6.84E-03	3	115		
Train 3 SSWWW Influent	1082	2.22E-02	4	691	74%	15.9
Train 3 SSWWW Effluent	284	5.82E-03	4	82		
Train 3 Nutrient Spike Influent	1384[a]	2.83E-02	6	1043	89%	25.6
Train 3 Nutrient Spike Effluent	BDL 125[b]	2.56E-03	6	69		
Train 3 Salt and Nutrient Spike Influent	1408[a]	2.88E-02	3	1043	90%	25.2
Train 3 Salt and Nutrient Spike Effluent	BDL 125[b]	2.56E-03	3	23		
Train 3 Cidery Wastewater Influent	15222[a]	3.12E-01	7	18495	98%	147.7
Train 3 Cidery Wastewater Effluent	303[b]	6.20E-03	7	267		
Train 3 Recovery Influent	1137	2.33E-02	2	626	89%	16.5
Train 3 Recovery Effluent	BDL 125	2.56E-03	2	12		

Train 2 had a COD spike and then a COD + Nutrients spike in phases 2 and 3, respectively. During Phase 2, the COD spike representing when a bad batch of wine/beer/cider is dumped down the drain, the average effluent concentration almost doubled that during Phase 1.

This was because plant health was rapidly declining during this period (Figure 3). This was considered a system failure, and the loading was too high for this strength of wastewater.

Figure 5: Train 2, Reactor 1, COD Spike only. April 25, 2023. Example of unhealthy plant.

In Phase 3, additional nutrients were added at the same ratio to the COD (3 times higher than the control winery synthetic wastewater). The treatment efficiency almost instantly increased, and plant health improved. The wastewater at the beginning of the train was around 4500 mg/L and was treated to below detection limit in every analysis. Figure 4 shows the increase in plant health after the change. The cattails grew back the fastest, then the American sweetflag, three-square bulrush, and swamp loosestrife.

Figure 6: Train 2, Reactor 1, COD and nutrient spike. June 15, 2023. Plant growth increase upon the addition of nutrients.

COD removal is more consistent with higher amounts of nutrients in the water. This is likely due to the microbes and plants having more food to be able to break down the COD. Plant nutrient availability is often expressed as a carbon:nitrogen:phosphorus (C:N:P) ratio. This was calculated from the average values of the COD, TN, and TP from the COD and nutrient spike wastewater. These values were 4469 mg/L COD, 16.6 mg/L TN, and 6.41 mg/L TP. The C:N:P ratio was 697:2.6:1. The C:N ratio was 269:1 and the C:P ratio was 697:1. There have been multiple studies that attribute better COD removal from a higher amount of nitrogen, but with a ratio of up to 5:1 [41]–[43]. Since the COD is so much higher than the N and P, COD removal and plant health will likely be better if there are more nutrients in the wastewater.

Phase 2 demonstrated that wastewater with an optimal C:N:P can be effectively treated with the greenhouse ecosystem. The TP, and nitrite were treated to below 5 mg/l and 0.5 mg/L, respectively, TN was consistently above 5 mg/L, but less than 10 mg/L, 88% of the COD spike alone was removed, but 96% was removed when adequate nutrients were provided.

During Phase 3, Train 3 had a nutrient spike and a nutrient and salt spike. COD removal was more uniform with just the nutrients, but the removal rates were similar with 89% removal during the nutrient spike and 90% during the salt and nutrient spike. There was no plant health decrease during just the nutrient spike, but there was yellowing and slight curling of the leaves when salt was added. Excess salt leads to a nutrient and mineral imbalance, which impacts the plants by yellowing the leaves, stunting growth, and curling them[44], [45].

The actual winery wastewater had a 98% removal of COD. The highest COD within the winery wastewater was 25,000 mg/L. All values in the winery effluent were below detection limit. This was expected, and it follows the removal patterns for the SSWW.

The actual cidery wastewater had a 98% removal of COD as well. The highest COD within the cidery wastewater was 51,000 mg/L and the effluent values were less than 900 mg/L. The highest value of the COD came from the cidery dumping a bad batch of cider into the drain, which has a COD of roughly 91,000 mg/L. This increase of the COD has a negative impact on the plants (Figure 5). The C:N:P ratio (using the highest values for each one) was calculated to be 871:0.77:1. Nitrogen is a limiting factor here (as it is less than the phosphorus amount) and so the plants do not have enough nutrients to effectively remove the pollutants and maintain health.

Figure 7: Train 3, August 11, 2023. During hard cider spike.

Actual brewery wastewater had a high odor, but it smelled like beer. A very thick film appeared on top of the wastewater in Train 2 Reactor 1 and 2. The plants did not survive in Train 2 Reactor 1. The film appeared within a few days of the brewery wastewater. The aeration stones were changed right before the brewery water was introduced and so were working at full potential. The brewery wastewater was already bubbly during collection due to the carbonation and so adding in aeration increased the amount of bubbles in the system. The bubbles then hardened on top of the water, creating the film. It would likely be a long-term issue and more studies are needed for determination of effect on plants. Films grew on top of the surface of the water in the previous phase so this may have influenced this occurrence with the actual brewery wastewater. Figure 6 shows the film on top of the water in Train 2 Reactor 1 and Figure 7 shows the film on Train 2 Reactor 2. Regardless, excellent COD removal resulted with an overall removal of 97%, with influent values ranging from 12,000-14,000 mg/L.

When comparing brewery wastewater to winery and cidery, there seems to be a distinction. Brewery wastewater has a higher overall COD but has less big COD spikes. Winery and cidery wastewater have a lower average COD (7,000 mg/L for winery and 9,000 mg/L for cidery, excluding the data point that came from dumping a batch of hard cider), but the range is much bigger for both (range of 500-25,000 mg/L for winery, and 800-51,000 mg/L for cidery). This is likely because the wastewater is better equalized at the brewery as it is stored in a 20,000-gallon tank, compared to the 30-gallon septic tank and 2,000-gallon septic tank used at the winery and cidery, respectively. The brewery wastewater is also stronger at the sample collection location than the cider location or brewery location.

Figure 8: Train 2 Reactor 1 with brewery wastewater film on top of water surface.

Figure 9: Train 2 Reactor 2 with brewery wastewater film on top of water surface.

Total Nitrogen

There were four different nitrogen measurements taken: total nitrogen, nitrate, nitrite, and ammonia. All nitrogen data is expressed as mg/L-N. The constituents in the wastewater that add nitrogen are malt (cereal grains that have been prepared for brewing by soaking in water)[46], adjuncts (other ingredients added to the beer that can be fermented)[47], and the nitric acid used for cleaning[48].

Table 7 shows the total nitrogen data. Regarding Train 1, although data is available for each research phases, the feed was consistent throughout the entire research period and data is supplied for the entire research, which will be used for comparisons. The 64% decrease in the amount of total nitrogen through the system in the control train was due to reduction in the nitrate, nitrite, and ammonia within the system, which will be discussed in more detail in following sections. The removal rate of nitrate was lower consistently in Train 1, the control, than in the other drums. Interestingly, there was also an increase in TN in the control's Reactor 1 in almost half of the research phases. This could be attributed to an increase in organic matter, which is measured by total Kjeldal nitrogen. This was not measured, but it can be estimated using an equation.

Table 7: TN Analysis Statistics.

	mg/L N	Mass, kg/gal-d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	5.7	1.17E-04	19	3.6	64%	1.17
Train 1 SSWWW Effluent	2.0	4.19E-05	20	1.5		
Train 2 SSWWW Influent	3.7	7.48E-05	4	3.5	59%	1.09
Train 2 SSWWW Effluent	1.5	3.07E-05	4	0.4		
Train 2 COD Spike Influent	16.7	3.43E-04	5	18.7	31%	1.65
Train 2 COD Spike Effluent	11.5	2.35E-04	5	8.8		
Train 2 COD and Nutrient Spike Influent	16.6	3.41E-04	4	6.5	11%	0.18
Train 2 COD and Nutrient Spike Effluent	14.8	3.03E-04	4	0.6		
Train 2 Winery Wastewater Influent	22.2	4.55E-04	5	2.1	20%	0.95
Train 2 Winery Wastewater Effluent	17.9	3.66E-04	7	20.5		
Train 2 Brewery Wastewater Influent	22.23	4.55E-04	0	3.72	53%	1.62
Train 2 Brewery Wastewater Effluent	10.51	2.15E-04	3	9.62		
Train 3 SSWWW Influent	2.98	6.10E-05	4	2.62	32%	0.49
Train 3 SSWWW Effluent	2.03	4.14E-05	3	0.38		
Train 3 Nutrient Spike Influent	10.77	2.21E-04	5	7.45	76%	1.74
Train 3 Nutrient Spike Effluent	2.60	5.32E-05	5	0.46		
Train 3 Salt and Nutrient Spike Influent	14.59	2.99E-04	4	7.45	64%	1.39
Train 3 Salt and Nutrient Spike Effluent	5.28	1.08E-04	4	2.26		
Train 3 Cidery Wastewater Influent	45.33	9.28E-04	6	59.28	88%	7.95
Train 3 Cidery Wastewater Effluent	5.27	1.08E-04	7	4.22		
Train 3 Recovery Influent	7.02	1.44E-04	2	3	0%	0.00
Train 3 Recovery Effluent	10.69	2.19E-04	2	2		

Note: For the brewery wastewater influent, the percent removal was calculated from the Train 2 Reactor 1 values.

The influent for the brewery wastewater was over the analytical detection limit of 16 mg/L of nitrogen. However, since the effluent total nitrogen was around 10 mg/L, it can be determined that there is effective treatment through the system, although all 3 reactors are needed to achieve this removal.

Train 3 had a nutrient spike, a salt spike, and the actual cidery wastewater. All the final TN values for the nutrient spike were below 3 mg/L, and it was very consistently treated. The TN removal for the nutrient and salt spike was less consistent, however, was below 6 mg/L. The recovery phase had an increase in the amount of TN, resulting from an increase in Nitrate. As the ammonia decreased, denitrification was inhibited, and the plant must have been contributing to the organic nitrogen level.

The experimental system was designed for nitrification, denitrification, and plant uptake. Nitrification is when ammonia is converted to nitrite, then nitrate, and takes place when there are higher amounts of oxygen (aerobic conditions)[49]. Denitrification is when nitrate is converted into nitrogen gas (N_2) and happens when there is low oxygen (anaerobic conditions)[49]. Additionally, plant uptake of ammonia and nitrate can also occur. Another pathway of nitrogen removal is anammox, which is when nitrite and ammonia are converted directly into N_2. Interestingly, ethanol and other alcohols, as well as high concentrations of nitrite, can inhibit the anammox procedure[50]. Ethanol is the alcoholic component in craft beverages, but in high enough quantities it is used as an antibacterial agent and so can be toxic to the microbes that perform anammox.

The most common type of nitrogen within the drums is nitrate. As nitrate is only produced in the water by the process of nitrification, that indicates that there is enough dissolved oxygen in the water to allow this process to occur. The total nitrogen is the highest in the first reactors, but the other forms of nitrogen did not increase in the first reactor. The estimated organic nitrogen increased in the first reactor as well. This could be from N_2 fixation, which occurs under anaerobic conditions[51]. The bacteria fix the nitrogen from the air into the first reactors, which could increase the nitrogen, and the plants then uptake that nitrogen. This could be a reasonable explanation as the dissolved oxygen in the first reactors were always the lowest. There is little to no nitrite accumulating in the reactor.

There was almost always no nitrite within the system, which is likely due to it being consumed during nitrification. There is also likely little to no N_2 or N_2O being produced. The nitrogen decreases as the water moves through the train, but based on the concentration of nitrite, the nitrification process is not occurring. There was no nitrite going into the system, and there was no nitrite detected in the other reactors, so it is being used up much faster than it can accumulate.

Organic Nitrogen

Organic nitrogen can be estimated by using the following equation. The total nitrogen, ammonia, nitrate, and nitrite are all known values, so these can be used to estimate the organic nitrogen. Table 8 shows the estimated organic nitrogen calculated. In several cases, the removal is considered to be 0% as the estimated organic nitrogen increased.

$$Organic\ Nitrogen = Total\ Nitrogen - Nitrate - Nitrite - Ammonia \tag{2}$$

Based on the values and removal rates estimated, there is a lot of variability in the amount of organic nitrogen. The influent concentrations did not get very high, however. The highest influent concentrations were between 11-13 mg/L.

There were three cases when organic nitrogen increased through the system: the COD spike, the winery wastewater, and the brewery wastewater. The COD spike is when the plant health declined rapidly, so the plants decomposed and released the nutrients into the water. This could also be due to microbiological activity increasing within the drums. The increase in organic nitrogen in the winery wastewater is likely due to the lack of screening. The wastewater collected from the septic tank had to be strained to remove the leftover fruit. The fruit that made it into the septic tank was in the process of decomposing, and likely provided food for microbial activity within the drums. Brewery wastewater has a high solids content, which is composed of the dead yeasts from the brewing process. The dead yeast More microbiological testing would be needed to allow for conclusions to be confirmed.

Table 8: Estimated Organic Nitrogen Analysis Statistics

	mg/L ON-N	Mass, kg/gal-d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	4.50	9.21E-05	16	4.14	77%	1.543
Train 1 SSWWW Effluent	1.05	2.16E-05	16	0.51		
Train 2 SSWWW Influent	2.63	5.39E-05	4	3.62	49%	1.103
Train 2 SSWWW Effluent	1.35	2.75E-05	4	0.37		
Train 2 COD Spike Influent	0.00	0.00E+00	2	N/A	0%	0.000
Train 2 COD Spike Effluent	8.47	1.73E-04	2	6.03		
Train 2 COD and Nutrient Spike Influent	11.90	2.43E-04	4	5.59	21%	0.507
Train 2 COD and Nutrient Spike Effluent	9.39	1.92E-04	4	5.45		
Train 2 Winery Wastewater Influent	10.79	2.21E-04	6	15.59	0%	0.000
Train 2 Winery Wastewater Effluent	11.14	2.28E-04	6	22.77		
Train 2 Brewery Wastewater Influent	0.00	0.00E+00	3	N/A	0%	0.000
Train 2 Brewery Wastewater Effluent	10.26	2.10E-04	3	9.83		
Train 3 SSWWW Influent	2.02	4.13E-05	4	2.52	33%	0.649
Train 3 SSWWW Effluent	1.35	2.77E-05	4	0.99		
Train 3 Nutrient Spike Influent	9.81	2.01E-04	2	4.56	81%	1.267
Train 3 Nutrient Spike Effluent	1.89	3.87E-05	2	0.81		
Train 3 Salt and Nutrient Spike Influent	13.80	2.82E-04	4	4.56	88%	1.494
Train 3 Salt and Nutrient Spike Effluent	1.72	3.51E-05	4	1.55		
Train 3 Cidery Wastewater Influent	13.49	2.76E-04	6	20.62	69%	4.192
Train 3 Cidery Wastewater Effluent	4.22	8.63E-05	6	4.90		
Train 3 Recovery Influent	5.26	1.08E-04	2	3	43%	0.537
Train 3 Recovery Effluent	2.99	6.13E-05	2	1		

Nitrate

Table 9 shows all nitrate data. Train 1, the control, had a low influent nitrate level that doubled in Reactor 2 and then substantially decreased in Reactor 3. Although a mass balance of nitrogen is not possible as the levels uptake by the plants and then returned to the waster was not measured, this pattern indicates a microbial nitrification/denitrification pathway was functioning.

This is reinforced because the first two reactors are aerated (the first reactor being anaerobic and the second being aerobic), causing nitrification, and reactor 3 was anoxic, resulting in denitrification. However, the removal of nitrate in Phase 1 for Train 1 was substantially less than for Trains 2 and 3. As all were receiving the same wastewater, SSWW, during this phase the reason is unclear.

Table 9: Nitrate Analysis Statistics

	mg/L NO3-N	Mass, kg/gal-d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	0.89	1.82E-05	18	0.54	35%	0.30
Train 1 SSWWW Effluent	0.58	1.18E-05	19	0.47		
Train 2 SSWWW Influent	1.04	2.13E-05	4	0.24	83%	0.20
Train 2 SSWWW Effluent	BDL 0.115	2.35E-06	4	0.02		
Train 2 COD Spike Influent	5.39[a]	1.10E-04	4	2.89	96%	1.21
Train 2 COD Spike Effluent	0.23[b]	4.76E-06	4	0.11		
Train 2 COD and Nutrient Spike Influent	4.73	9.68E-05	4	3.78	0%	0.00
Train 2 COD and Nutrient Spike Effluent	4.85	9.92E-05	4	5.03		
Train 2 Winery Wastewater Influent	3.51	7.19E-05	7	4.58	0%	0.00
Train 2 Winery Wastewater Effluent	8.01	1.64E-04	7	1.21		
Train 2 Brewery Wastewater Influent	11.50[a]	2.35E-04	2	1.13	97%	0.36
Train 2 Brewery Wastewater Effluent	0.38[b]	7.70E-06	2	0.10		
Train 3 SSWWW Influent	0.99	2.02E-05	4	0.31	81%	0.26
Train 3 SSWWW Effluent	BDL 0.115	2.35E-06	4	0.02		
Train 3 Nutrient Spike Influent	1.19	2.43E-05	4	0.60	25%	0.26
Train 3 Nutrient Spike Effluent	0.89	1.83E-05	4	0.86		
Train 3 Salt and Nutrient Spike Influent	0.79	1.61E-05	4	0.60	0%	0.00
Train 3 Salt and Nutrient Spike Effluent	3.56	7.29E-05	4	0.94		
Train 3 Cidery Wastewater Influent	5.34	1.09E-04	7	3.78	79%	1.44
Train 3 Cidery Wastewater Effluent	1.11	2.27E-05	7	0.84		
Train 3 Recovery Influent	1.76	3.60E-05	2	0	0%	0.00
Train 3 Recovery Effluent	7.69	1.57E-04	2	1		

Train 2 had a COD spike, a COD and nutrient spike, actual winery wastewater, and actual brewery wastewater. The nitrate followed similar patterns for the COD spike as it did for the control: increase in the first and second reactors, then decrease to the third reactor. However, a substantially higher removal was observed, 96% compared to 35% in the control. The removal rate of nitrate in Phase 2 specifically of the control was 40%, which is the lowest removal rate observed (the removal rate in Phase 1 of the control was 81%). The nitrate concentration dropped from the influent to Reactor 1 but increased in Reactor 2 and Reactor 3 during the COD and nutrient spike. The overall removal rate was 0 because nitrate increased overall. A similar trend occurred for the actual winery wastewater. This indicates that denitrification or plant uptake of nitrate were not occurring. However, nitrate was 97% removed from the brewery wastewater. The very high COD of the brewery wastewater may have been contributing to an anoxic reactor environment resulting in denitrification of all nitrate that was formed in aerobic zones within the reactors.

Train 3 had a nutrient spike, a salt spike, actual cidery wastewater, and recovery from cidery wastewater. Nitrate was not removed in the salt and nutrient spike and recovery phases, indicating that there was enough oxygen to convert the nitrogen forms to nitrate, but there was not enough plant or microbe removal to reduce the amount of nitrate. This makes sense for the salt spike as having a higher salinity water decreases the amount of oxygen. Nitrate was removed in the actual cidery wastewater phase, which had a very high COD, similar to the actual brewery wastewater.

<u>Nitrite</u>

All influent wastewater had nitrite values below the detection limit of 0.6 mg/L except for actual brewery wastewater, which was 0.85 mg/L N. All nitrite leaving the systems, in all phases, were below the detection limit. This is not unusual for wastewater as nitrite is often the limiting, intermediate compound formed in nitrification, the microbial conversion of ammonia to nitrate. Consequently, when formed, it is immediately converted to nitrate and does not accumulate.

<u>Ammonia</u>

Train 1 was the control, and the ammonia concentration is shown in Table 10. The detection limit was 1 mg/L. There was no ammonia in the SSWW, and none was produced from ammonification of organic nitrogen in the wastewater or from the plants in any of the reactors. The small amount of ammonia in the influent COD and nutrient spike was not removed through the reactors, for unknown reasons. However, in the actual winery wastewater, all the influent was removed. The poor overall removal in the COD and nutrient spike is unknown. The actual brewery wastewater had high ammonia, which could be related to the amount of yeast within the system, as yeast is a large part of the addition of nitrogen[48].Most of the ammonia was removed in the first reactor. The small amounts of ammonia in the influent of the salt and nutrient spike and cidery wastewater were removed to below detection limits by Reactor 2.

Table 10: Ammonia Analysis Statistics

	mg/L NH3-N	Mass, kg/gal-d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	BDL 0.5	1.02E-05	20	0.77	0%	0.00
Train 1 SSWWW Effluent	BDL 0.5	1.02E-05	21	0.03		
Train 2 SSWWW Influent	BDL 0.5	1.02E-05	4	0.14	0%	0.00
Train 2 SSWWW Effluent	BDL 0.5	1.02E-05	4	0.02		
Train 2 COD Spike Influent	BDL 0.5	1.02E-05	6	0.26	0%	0.00
Train 2 COD Spike Effluent	BDL 0.5	1.02E-05	6	1.21		
Train 2 COD and Nutrient Spike Influent	3.12	6.39E-05	4	3.25	5%	0.21
Train 2 COD and Nutrient Spike Effluent	2.95	6.04E-05	4	5.83		
Train 2 Winery Wastewater Influent	6.48[a]	1.33E-04	7	5.48	92%	1.99
Train 2 Winery Wastewater Effluent	BDL 0.05[b]	1.02E-05	7	0.01		
Train 2 Brewery Wastewater Influent	22.95*	4.70E-04	2	7.28	94%	2.09
Train 2 Brewery Wastewater Effluent	1.39	2.85E-05	2	1.92		
Train 3 SSWWW Influent	BDL 0.5	1.02E-05	4	0.10	0%	0.00
Train 3 SSWWW Effluent	BDL 0.5	1.02E-05	4	0.17		
Train 3 Nutrient Spike Influent	BDL 0.5	1.02E-05	6	0.32	0%	0.00
Train 3 Nutrient Spike Effluent	BDL 0.5	1.02E-05	6	0.19		
Train 3 Salt and Nutrient Spike Influent	3.76	7.70E-05	4	0.32	87%	0.14
Train 3 Salt and Nutrient Spike Effluent	BDL 0.5	1.02E-05	4	0.02		
Train 3 Cidery Wastewater Influent	1.75[a]	3.59E-05	7	3.35	71%	1.92
Train 3 Cidery Wastewater Effluent	BDL 0.5[b]	1.02E-05	7	0.64		
Train 3 Recovery Influent	BDL 0.5	1.02E-05	2	0	0%	0.00
Train 3 Recovery Effluent	BDL 0.5	1.02E-05	2	0		

*Note: This value is Train 1, Reactor 1 value as the influent values are much higher than the detection limit. Reactor 1 is not statistically different than Reactor 3, but it is likely that the actual influent value is statistically different than Reactor 3.

Total Phosphorus

Table 11 shows the phosphorus data for all trains and phases. Train 1 was the control train throughout the experiment. It had the same synthetic wastewater going through every day. The influent wastewater has a variety of phosphorus concentrations, but the effluent levels were always less than the detection limit. Phosphorus in wastewater mainly comes from cleaning chemicals [48].

Table 11: Total Phosphorus Analysis Statistics

	mg/L P	Mass, kg/d	n	Std. Dev.	% removal	% removal std.
Train 1 SSWWW Influent	2.25	4.60E-05	20	0.45	79%	0.43
Train 1 SSWWW Effluent	BDL 0.25	5.12E-06	21	0.22		
Train 2 SSWWW Influent	2.30	4.71E-05	4	0.03	95%	0.32
Train 2 SSWWW Effluent	BDL 0.25	5.12E-06	4	0.16		
Train 2 COD Spike Influent	3.25[a]	6.66E-05	6	3.80	79%	1.73
Train 2 COD Spike Effluent	0.69[b]	1.42E-05	6	0.51		
Train 2 COD and Nutrient Spike Influent	6.41[a]	1.31E-04	4	1.68	75%	0.52
Train 2 COD and Nutrient Spike Effluent	1.63[b]	3.33E-05	4	0.26		
Train 2 Winery Wastewater Influent	9.40[a]	1.92E-04	7	7.51	88%	2.19
Train 2 Winery Wastewater Effluent	1.09[b]	2.24E-05	7	0.39		
Train 2 Brewery Wastewater Influent	89.72[a]	1.84E-03	3	61.20	91%	6.05
Train 2 Brewery Wastewater Effluent	7.83[b]	1.60E-04	3	4.18		
Train 3 SSWWW Influent	2.32	4.74E-05	4	0.24	88%	0.43
Train 3 SSWWW Effluent	BDL 0.25	5.12E-06	4	0.23		
Train 3 Nutrient Spike Influent	4.94[a]	1.01E-04	6	2.05	77%	0.97
Train 3 Nutrient Spike Effluent	1.12[b]	2.29E-05	6	0.89		
Train 3 Salt and Nutrient Spike Influent	6.31[a]	1.29E-04	4	2.05	66%	0.57
Train 3 Salt and Nutrient Spike Effluent	2.13[b]	4.35E-05	4	0.40		
Train 3 Cidery Wastewater Influent	23.90[a]	4.89E-04	7	23.94	88%	4.42
Train 3 Cidery Wastewater Effluent	2.99[b]	6.11E-05	6	2.16		
Train 3 Recovery Influent	2.44	4.99E-05	2	0	0%	0.00
Train 3 Recovery Effluent	2.87	5.87E-05	2	0		

The highest amount of phosphorus detected was in the brewery wastewater. 90 mg/L was detected by diluting the sample in distilled water and running the diluted sample. The next highest concentration was in the cidery wastewater. However, both concentrations were treated to below 10 and 3 mg/L, respectively. The phosphorus in the brewery wastewater could have been removed by sedimentation as there is a very high solids content. The phosphorus in the cidery wastewater was likely used by the plants and microbes around the roots as opposed to sedimentation, as the cidery wastewater did not have as many visible solids in it.

The phosphorus in Train 3 for the recovery phase was the only phase where there was an increase in the concentration. The phosphorus increased between the influent and Reactor 1, decreased in Reactor 2, and Reactor 3 had a similar concentration to Reactor 2. This could be due to the plants dying in Reactor 1 and the decomposed plant material dropping to the bottom of the drum, then being brought back up with aeration.

The removal rates in the trains are similar to each other, and consistently have good treatment. In several cases, the effluent levels of phosphorus are below detection limit, and in the cases where it's not, the effluent is between 1-2 mg/L, which can be easily polished. Based on the variables within this train, three cells are needed to get the phosphorus below the EPA limit of 1 mg/L.

PLANT HEALTH RESULTS

P. digitalus did not survive past the equilibrium stage with no wastewater in 6 of the 9 reactors, so it was discarded from all reactors. This may be due to acquiring them and placing them in water in December, which is out of its growing season.

S. polyrhiza was the volunteer plant (a plant that grew on its own in the system and was not initially planted) and it grew in most reactors during the equilibrium stage and covered most of the surface of the water. It died in the first reactor in all three trains, most likely due to the increased amount of pollutants, especially in the actual wastewater runs and high COD runs. It grew consistently well in the third reactors in all three trains. However, it died in Train 2 completely during the COD spike but grew back during the COD and nutrient spike. *S. polyrhiza* was the most sensitive plant in the system.

T. augustifolia grew quickly in all reactors. It spread quickly and consistently and had a large root system. It was inhibited during the COD spike and the cidery wastewater as well, but it was the first plant to be revived when a new variable was introduced. It also grew past its growing season in the fall, most likely due to the grow lights and consistent warmth.

A. americanus had continuous but slow growth over the course of the experiment. They were very hardy and grew back after the plants were inhibited. They were consistently green even at various parts of the year (fall and winter), which suggests they could continue growing for multiple years at a time.

D. verticillatus grew consistently over the course of the experiment. It was the first plant to start growing in the system though all plants were planted at the same time. *D. verticillatus* is a flowering plant and was specifically chosen for its flowers. It had consistent growth, but it did not flower over the course of the experiment, likely due to being stressed.

CONCLUSIONS

The main objective of this research was to determine the ability of a greenhouse ecosystem to treat craft beverage wastewater. Results show the system is effective within the designed organic and hydraulic loadings. However, extreme events will cause disruptions and potential system failure.

Particularly, COD spikes caused a system failure. COD with no extra nutrients essentially starved the plants of oxygen and their health declined. Plants use oxygen for aerobic respiration and if the oxygen is completely depleted for a long enough period of time, which differs based on one plant to another, then the cells will start to die[52]. Another possibility is that the microbes living around the roots are depleting the nutrients before the plant can uptake them[53]. The plants took a long time to recover during the recovery phase, in some cases over two months. Based on the COD results, only two reactors are needed. However, all three reactors are needed for nitrogen removal phosphorus removal.

Aeration is also recommended for all the reactors to minimize odors and pests. Odors resembling rotting fruit, likely due to the grape juice in the synthetic wastewater, were observed after 3 weeks. The smell improved within a week after adding aeration. The air bubbles also helped keep the water moving and made the trains more aerobic than they were before adding aeration.

As this greenhouse ecosystem has known flowrate, volume, and loading values, it can be scaled up to fit different facilities. However, there is a point at which the system will be too big. At that point, it is more economical to build a conventional treatment plant, or other type of treatment. Calculations and a life cycle assessment need to be performed to determine the most flow the greenhouse ecosystem could treat.

The treatment methods for the volatile organic carbon pollutants (in the case of craft beverage wastewater, it is ethanol) are microbial degradation, volatilization, and phytoremediation[54]. In natural systems, it is quickly volatilized at the water-surface interface and has a half-life of only 5 days[55]. Ammonia is also volatilized from wastewater, which is not desirable. Volatilization could possibly be an important removal mechanism of ethanol, especially since the hydraulic residence time is 9 days. However, the greenhouse ecosystem was not designed for volatilization and volatilization of ethanol was not studied within this research.

The main limitation of this project was the low budget. The quality of the instruments could've been upgraded if the budget was higher, as well as more sample analyses performed. However, the low budget was beneficial as most craft beverage facilities have lower budgets, and since the concept was proven with a lower budget, it will still be effective if there is a higher budget used.

There were a few lessons learned over the course of this project. The first is to include aeration at the very beginning of the project instead of waiting until the system had already been deprived of oxygen. This likely had an impact on all of the future analyses. The second is that there is a long start up period and so this needs to be factored into design plans when being used at an actual facility.

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CHAPTER 3: CRAFT BEVERAGE SITE SPECIFIC WASTEWATER TREATMENT SELECTION TOOL

INTRODUCTION

This chapter introduces a decision tool and the methodology that went into designing it to use for craft beverage wastewater treatment, with an emphasis on the greenhouse ecosystem. Data to collect before using the decision tool includes climate, land availability, land cost, hauling cost to process offsite, location within the community, highest daily flow rate, and pollutant characteristics.

There are several treatment methods that have been shown to treat craft beverage wastewater (CBW). The following is a list of the biological treatment processes that were compared in this chapter: anaerobic digestion, land application, constructed wetlands, drain fields, and treatment lagoons. Physical and chemical treatments can also be used to treat CBW, but they are more expensive and less developed than biological treatments, which is why only covering biological methods were compared. A brief overview of each treatment chosen is given in the following sub-sections and then a simplified decision tool is presented in the next section.

Anaerobic digestion

Anaerobic digestion is used to treat high strength wastewater and has the added benefit of potentially producing green energy. Anaerobic digestion has been shown to treat winery and brewery wastewater[1]–[4], removing over 90% of COD, even with influent values of over 300,000 mg/L [2], [4]. Recurring expenses to operate anaerobic digestion may be more economical than aerobic treatments because there is no aeration and less sludge, as well as being able to reuse the biogas, but capital costs may be higher. Operation and maintenance costs are more expensive because a skilled operator is needed as the digesters are highly sensitive to temperature and pollutants, and there is a high hazard of dangerous gases being produced. Anaerobic digesters can cost from as low as $500,000 to as high as $12 million[5]. In addition to the high cost, the wastewater also may need an aerobic polishing system after anaerobic digestion.

Land application

Land application is a treatment process where wastewater is sprayed over a section of land. This is a common practice for multiple types of food processing wastewater. Nitrogen and phosphorus within the wastewater can be used as a fertilizer, which reduces the cost of buying fertilizers[6]. Certain soil structures are preferred, and good management practices are needed to reduce biofilm production/soil fouling [7] and natural soil metal mobilization[8]. A leaching area is needed so salt buildup does not occur[7], a potential problem with winery wastewater because of its high salt content. However, there is a limit on the amount of BOD that can be applied per acre per day (50 lb. COD/ac/day in Michigan), which means that with the high amount in CBW, more land would need to be used.

Treatment Lagoons

There are four types of treatment lagoons: facultative, aerated, aerobic, and anaerobic. The main difference between lagoons and wetlands is the presence of plants. Wetlands have plants and lagoons typically do not[9].

Facultative lagoons have an aerobic zone at the top and an anaerobic zone at the bottom, and a facultative zone in the middle. The facultative zone has microorganisms that can use oxygen or not use it. The entire lagoon can treat the wastewater in the presence or absence of oxygen[10]. It is the most used type of lagoon.

Aerated lagoons are aerated. They are more costly as they use electricity to power the aeration mechanism within the lagoon. These typically require less land area and have better treatment[10].

Aerobic lagoons are shallower, which allows sunlight to get to the bottom of the lagoon. The oxygen is distributed equally through the entire lagoon. They have a larger land requirement than the other lagoons as it is shallower.

Anaerobic lagoons have no oxygen at all within the system. They are typically used for high strength, industrial wastewaters. They are deeper and have less sludge produced than aerobic lagoons[6].

Constructed wetland

There are multiple types of constructed wetlands that can be used to treat craft beverage wastewater, including free water surface wetlands, vertical flow, and horizontal flow[11]. Constructed wetlands use the soil, plants, and microbes to treat the pollutants in the water. Constructed wetlands are slightly more economical compared to a conventional treatment system and the operation costs are much lower than a conventional treatment system, depending on the wastewater's characteristics and flow [12]–[14]. All constructed wetlands require pretreatment to reduce the number of solids entering. This pretreatment step would be something as simple as a septic tank.

Free water surface wetlands are wetlands where all of the water is above media (such as soil, sand, clay, etc.) and has plants surrounding it. Aboveground wetlands treat wastewater very well and are very inexpensive, but can have problems with odors or pests, and have high detention times[6]. Aeration is not typically used in these constructed wetlands, so they are more economical than vertical or horizontal flow constructed wetlands. However, these wetlands are not as effective in winter as the water will freeze and treatment will be reduced, especially in states such as Michigan.

Vertical flow constructed wetlands are lined, filled with media (such as sand, gravel, or manufactured media), covered with few plants, and the water flows down into the cells and then pumped out through the bottom [15]. They have a smaller footprint than aboveground wetlands since the treatment is vertically into the soil and not spread out on top and is well suited for high-strength wastewater in cold temperatures[15]–[17].

Horizontal flow wetlands are similar to vertical flow, but instead of the water trickling down from the top of the cells, the water enters in at one side of the cell and collected at the other side. Horizontal flow wetlands are different than free water surface wetlands because all water is below the top of the media. In a free water surface wetland, the water is all above the media. These constructed wetlands require more surface area than vertical flow constructed wetlands. Both vertical flow and horizontal flow wetlands may have aeration, although it is not always required.

Drain Fields

Drain fields are one of the oldest methods of wastewater treatment and are typically connected to septic tanks. The septic tanks remove solids and floating fats, oil, and grease. Then the water runs through distribution pipes in the soil and slowly infiltrates into the soil. The soil quality must not be too permeable (such as sand) and not too impermeable (clays) and the water table deep compared to the location of the drain field[18]. They are simple to operate, but they do not provide the highest quality effluent and are prone to failure from too many solids, excessive biofilm growth, or clogging the drain field[19].

DECISION TOOL

The decision tool (Figure 1) was designed based on cost, size, nuisance possibilities, and marketability. This entire project assumes that centralized treatment is not possible and so decentralized methods need to be determined. The tool is then divided into space limitations, the soil conditions (poor or good), and the poor soil conditions are broken into nuisance amount (based on aesthetics and odor).

The severe space limitation has three possible treatment methods: wetlands, the greenhouse ecosystem, and anaerobic digestion. Wetlands have the lowest cost, lowest hydraulic retention time, but the highest surface area of the three choices. The greenhouse ecosystem has a cost in the middle between wetlands and anaerobic digestion and a smaller footprint than wetlands, but higher cost than wetland.

If there is no space limitation, soil conditions becomes a critical factor. If the soil is adequate for wastewater treatment, then land application or drain fields are the best options. If it is not adequate for wastewater treatment, then other methods are needed. The poor soil conditions are marked into three categories based on nuisance level. Common nuisances are poor aesthetic or, more importantly, the odor produced during treatment. Anaerobic digesters and wetlands are the highest nuisance regarding odor.

The low and moderate nuisances are then selected based on their marketability, regarding showcasing sustainability at the craft beverage facility. Aerated lagoons and other types of lagoons are less marketable. The greenhouse ecosystem is more marketable as it has decorative plants and could be used as a tourist attraction.

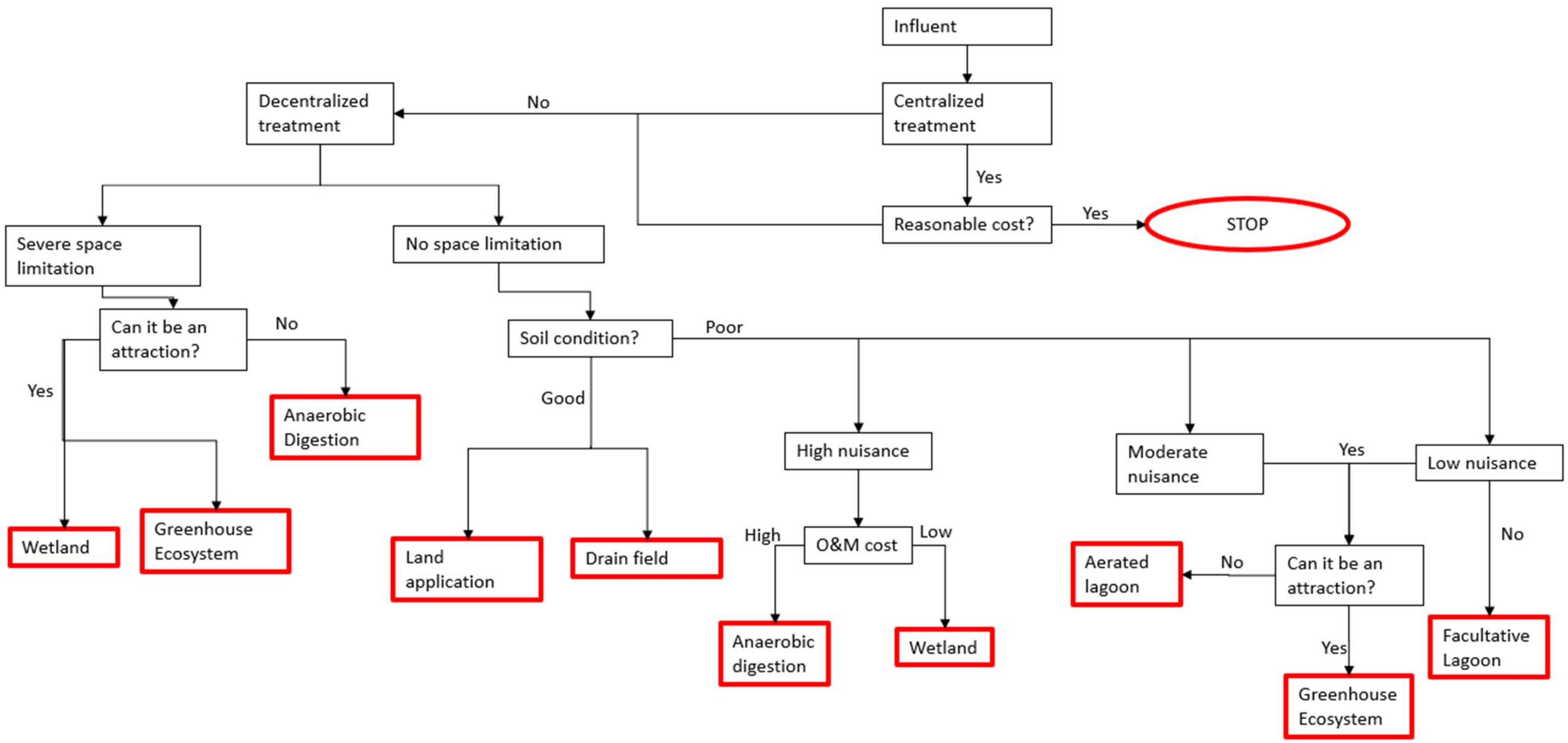

Figure 10: Decision tool for craft beverage wastewater treatment selection.

CONCLUSIONS

There are multiple treatment methods for craft beverage wastewater: wetlands, lagoons, anaerobic digestion, land application, drain fields, and greenhouse ecosystems. They have all been shown to effectively treat high strength wastewater. There are also new technologies being developed to treat wastewater biologically, such as a microbial fuel cell. To select the best, site-specific technology it is essential to discuss and tour other craft beverage facilities within the region and consult with an experienced industry professional.

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CHAPTER 4: FUTURE WORK

FIELD SCALE

In the future, a field scale demonstration is needed to determine if the greenhouse ecosystem can be run for multiple years in a greenhouse (compared to this pilot-scale project which lasted Jan-Dec in a controlled laboratory environment). The pilot-scale system was not difficult to maintain, but that is likely due to the small size and easy access. The field scale operated by craft beverage facility personnel could have maintenance issues the lack of access to tools that would be needed to troubleshoot and solve the maintenance problem.

There are also unknown impacts to running this system with this type of wastewater over the course of multiple seasons. It is unknown whether the plants will have a continuous degradation of health and treatment ability after one year or one pressing season. Related, is plant maintenance, including pruning to harvest phosphorus but as not to damage the plant. Brewery wastewater had the highest amount of sludge produced and so would require more sludge removal than the other types of wastewater.

A field scale study will also allow for optimization. This research was not optimized as it was a proof-of-concept study to determine if a greenhouse ecosystem could treat a specific type of wastewater. An optimization study would ideally examine the treatment ability of different plants, different combinations of plants, and run for at least two consecutive years. Results would be both the optimization of system size and maintenance protocols.

SAMPLE ANALYSIS

Total nitrogen values were often much higher than the other nitrogen forms put together. However, total Kjeldahl nitrogen was not analyzed, nor was nitrogen gas (N_2). Measurement of both components to determine if the difference between nitrogen forms is due to organic matter (from decaying plant leaves, algae, or components in the wastewater) or if it was fixed to become N_2. If it is N_2, then this would be good information as to how the nitrogen is removed from the system.

Phosphorus values in winery and brewery wastewater were much higher than in other wastewater types. The plant nutrient components were not measured, and neither were the nutrients within the settled sludge at the bottom of the reactors. It would be beneficial to measure the nutrient components of both to determine the main pathway of phosphorus removal. If plant uptake is the main pathway, then that is beneficial as it will more permanently remove the phosphorus from the wastewater if the plants are harvested. The plants can then be used in compost and placed next to the plants.

DECISION SUPPORT TOOL

A quantifiable, interactive decision support tool with estimated sizing and life-cycle costs to help craft beverage facilities decide on a wastewater treatment system would be of benefit. This decision support tool would require more literature to compare the treatment systems (an extension on Chapter 3), as well as considering other variables to customize the system to better fit the area. Other variables would include the groundwater level, how close are neighbors, the highest flowrate, the space available, the characteristics of the wastewater, the cost that the craft beverage facilities could afford for treatment, and others.

An economic analysis is needed for further development of the decision tool. The economic analysis will allow for better comparisons of the different treatment systems. While keeping the same wastewater, the loading and flowrate values can be used to determine the design for each type of treatment method. This will allow for determination of both the area needed for the treatment and the materials needed for building. The area needed and amount of materials will have costs associated with them and so can be included in the analysis. An effective treatment system could possibly have too big of an area needed and as such too many materials needed, and so a different treatment system should be used.

PLANT HEALTH AND MICROBIOLOGY

The plants chosen in this study were chosen mainly for their ability to treat wastewater and if they were native to Michigan. Other plants may be as effective but potentially be healthier in the long term. In addition, this study did not monitor viruses or fecal matter in comingled wastewater, but this is important to look at if the treated wastewater is discharged where exposure is possible. Constructed wetlands have been shown to remove fecal coliforms[81], but it is unknown if the plants used in this study can also remove fecal coliforms.

The plant exudates could interact with each other as well. Plant exudates are chemicals that the plant produces around the roots. The interactions between the exudates of different plants could have a negative impact on water treatment. Two plants that have been shown to treat wastewater in monocultures could have a reduced treatment ability when placed together. There have been studies on the role of plants in wastewater treatment and in wetlands, but it has been difficult to determine the interactions between plants because there is not enough literature[37].

The microbes grow around the roots of the plants and on the sides of the reactor. An option to possibly increase the removal efficiency of the greenhouse ecosystem is to add engineered media to the bottom of the drum. Adding engineered media increases the surface area available for the microbes to grow on. The increased surface area means more microbe growth, which means more organisms for treatment. These studies would need to look at what type of engineered media to use – a media to absorb the pollutants or a media that provides surface area.

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APPENDIX 1: COD DATA

Table 12: COD Raw Data

	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	2098	954	320	125	2079	1354	552	125	2034	1088	463	125
	651	817	446	125	768	1280	688	309	573	935	550	285
	1079	619	336	380	983	1013	611	496	1153	787	609	367
	861	298	125	125	978	652	488	535	570	306	125	314
Phase 2	2087	344	125	125	2111	560	294	329	1984	366	125	258
	905	323	125	125	5063	1115	285	257	876	125	125	125
	615	271	125	125	4392	1760	403	293	575	125	125	125
	763	263	125	125	5019	6128	1439	411	612	270	281	125
	3091	250	125	125	6039	4156	2628	1147	3239	394	252	125
	458	125	125	125	4395	3041	2471	921	1018	542	125	125
Phase 3	732	125	125	125	5023	710	387	125	1919	350	382	125
	1385	125	125	125	4446	1055	290	125	1624	1544	125	125
	420	125	125	125	3939	935	343	125	680	125	125	125
Phase 4	433	125	125	284	1472	1846	125	125	870	125	125	125
	1936	125	125	125	780	1017	125	125	835	125	125	125
	1646	125	125	125	25044	588	125	125	8212	4351	125	125
	125	125	125	125	8592	781	125	125	17538	14411	2688	303
	393	125	125	125	1305	592	125	125	51224	14415	2282	302
	1343	125	125	125	11642	1845	125	125	25888	12864	2028	342
	350	125	125	125	467	335	125	125	1990	15556	3953	859
Phase 5					14024	4438	766	402				
					13074	3662	706	125	1579	305	125	125
					11844	7247	1768	399	694	307	125	125

Note: If the value is 125, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 13: Train 1 Influent COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1172	651	2098	641
Std. syn. Winery WW	2	6	1320	458	3091	1044
Std. syn. Winery WW	3	3	846	420	1385	492
Std. syn. Winery WW	4	6	1017	350	1936	710
Overall SSWW		19	1118	350	3091	754

Table 14: Train 1 Reactor 1 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	672	298	954	285
Std. syn. Winery WW	2	6	264	130	344	75
Std. syn. Winery WW	3	3	145	121	171	25
Std. syn. Winery WW	4	7	85	52	116	23
Overall SSWW		20	265	52	954	252

Table 15: Train 1 Reactor 2 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	317	165	446	116
Std. syn. Winery WW	2	6	121	50	202	65
Std. syn. Winery WW	3	3	135	95	207	63
Std. syn. Winery WW	4	7	75	49	105	19
Overall SSWW		20	146	49	446	109

Table 16: Train 1 Reactor 3 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	228	151	380	107
Std. syn. Winery WW	2	6	125	37	237	75
Std. syn. Winery WW	3	3	108	84	151	37
Std. syn. Winery WW	4	7	107	59	284	79
Overall SSWW		20	137	37	380	87

Table 17: Train 2 Influent COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1202	768	2079	593
COD spike	2	6	4503	2111	6039	6039
COD+N spike	3	3	4469	3939	5023	543
Winery wastewater	4	7	7043	467	25044	9074
Brewery wastewater	5	3	12981	11844	14024	1093

Table 18: Train 2 Reactor 1 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	1075	652	1354	318
COD spike	2	6	2793	560	6128	2093
COD+N spike	3	3	900	710	1055	175
Winery wastewater	4	7	1000	335	1846	613
Brewery wastewater	5	3	5116	3662	7247	1886

Table 19: Train 2 Reactor 2 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	585	488	688	85
COD spike	2	6	1253	285	2628	1094
COD+N spike	3	3	340	290	387	49
Winery wastewater	4	7	169	136	219	32
Brewery wastewater	5	3	1080	706	1768	597

Table 20: Train 2 Reactor 3 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	393	234	535	145
COD spike	2	6	560	257	1147	378
COD+N spike	3	3	190	179	199	10
Winery wastewater	4	7	135	105	169	20
Brewery wastewater	5	3	334	202	402	115

Table 21: Train 3 Influent COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1082	570	2034	691
Nutrient Spike	2	6	1384	575	3239	1043
Salt spike	3	3	1408	680	1919	647
Cidery wastewater	4	7	15222	835	51224	18495
Recovery	5	2	1137	694	1579	626

Table 22: Train 3 Reactor 1 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	779	306	1088	338
Nutrient Spike	2	6	330	182	542	132
Salt spike	3	3	673	125	1544	763
Cidery wastewater	4	7	8840	137	15556	7011
Recovery	5	2	306	305	307	1

Table 23: Train 3 Reactor 2 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	464	234	609	165
Nutrient Spike	2	6	184	58	281	87
Salt spike	3	3	205	110	382	153
Cidery wastewater	4	7	1609	93	3953	1532
Recovery	5	2	129	112	146	24

Table 24: Train 3 Reactor 3 COD

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	284	172	367	82
Nutrient Spike	2	6	148	70	258	69
Salt spike	3	3	138	124	164	23
Cidery wastewater	4	7	303	94	859	267
Recovery	5	2	129	120	137	12

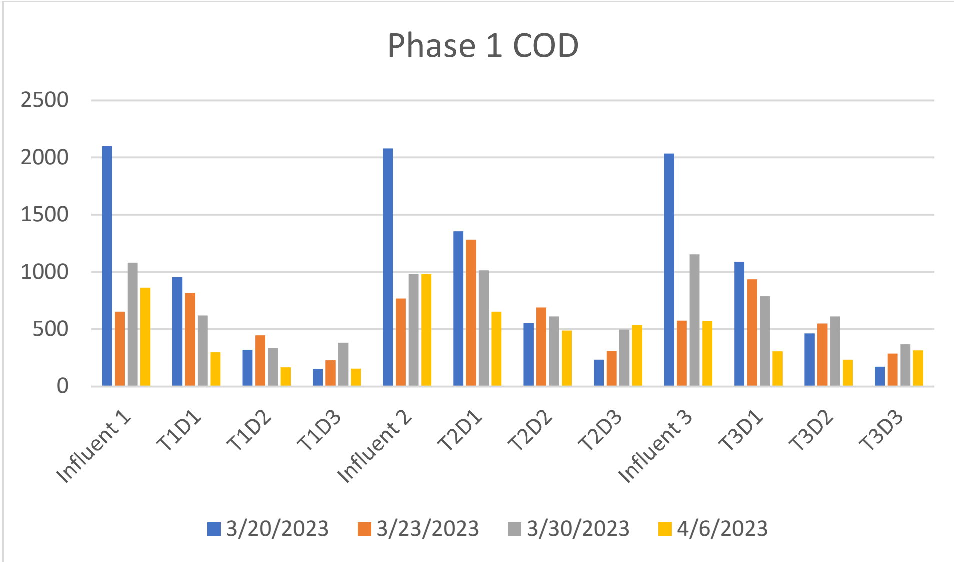

Figure 11: Phase 1 COD for all 3 trains

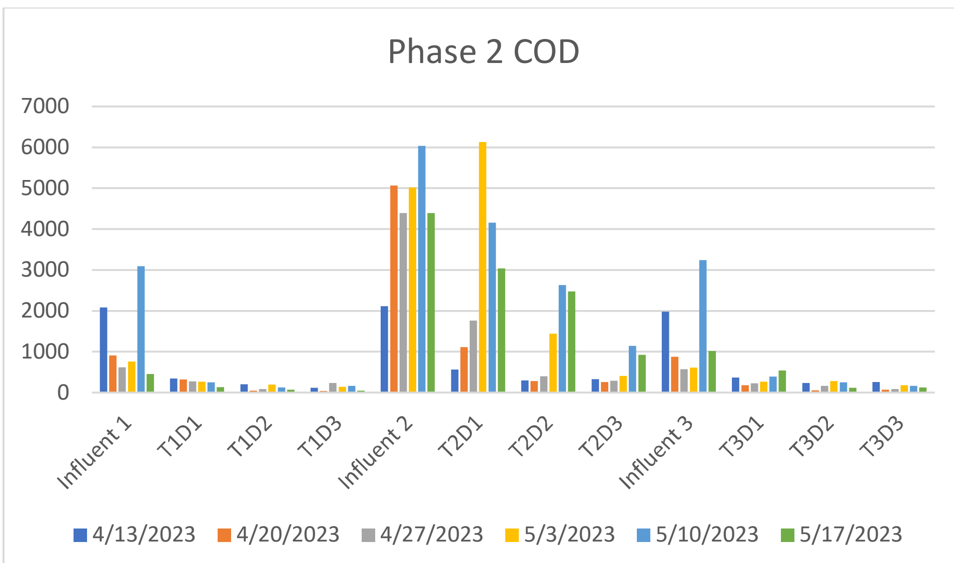

Figure 12: Phase 2 COD for all 3 trains

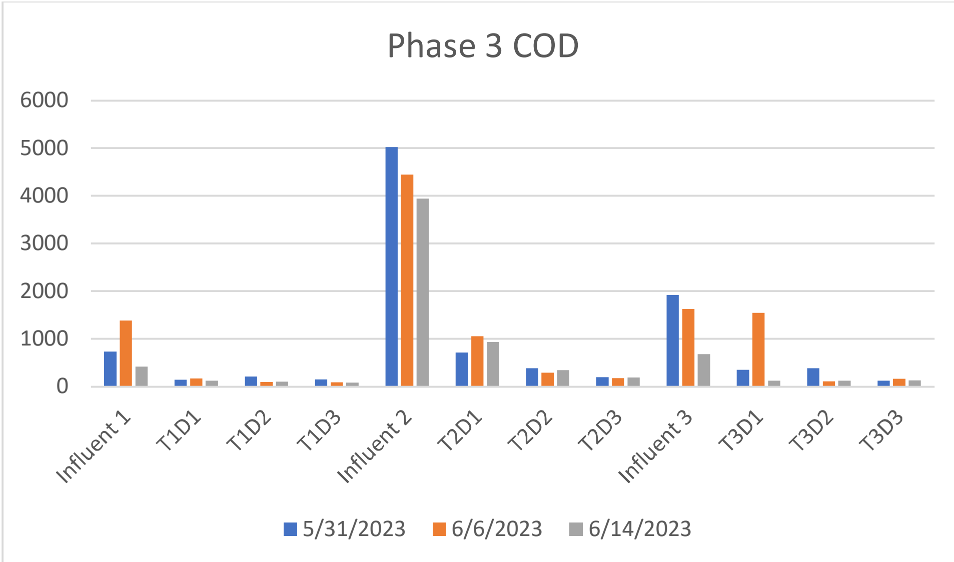

Figure 13: Phase 3 COD for all 3 trains

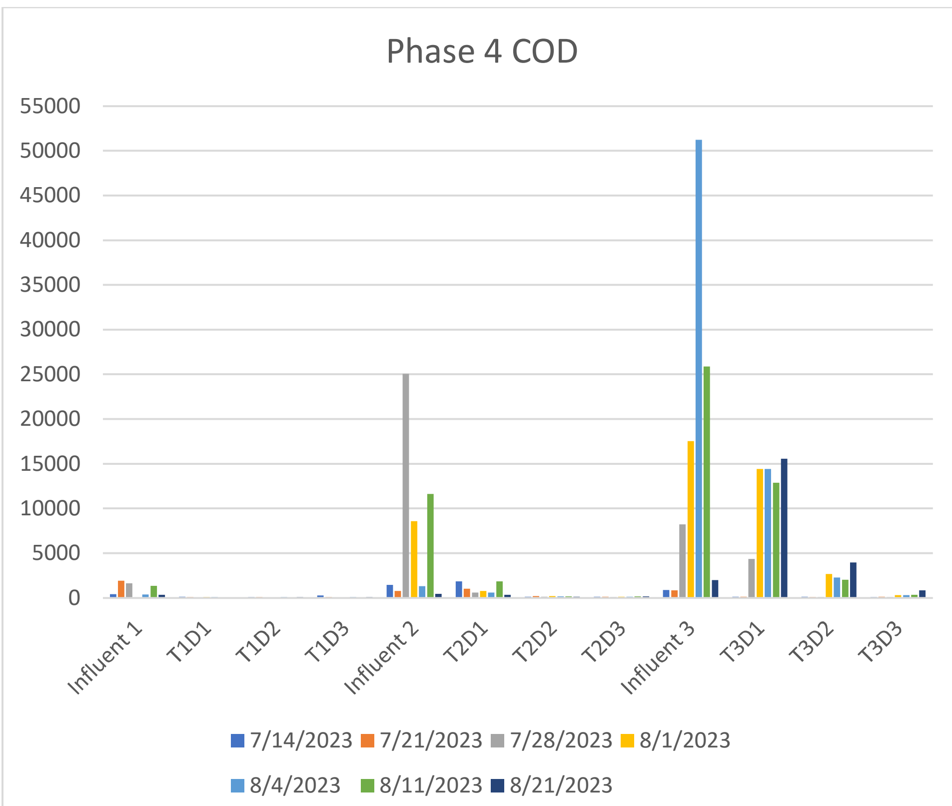

Figure 14: Phase 4 COD for all 3 trains

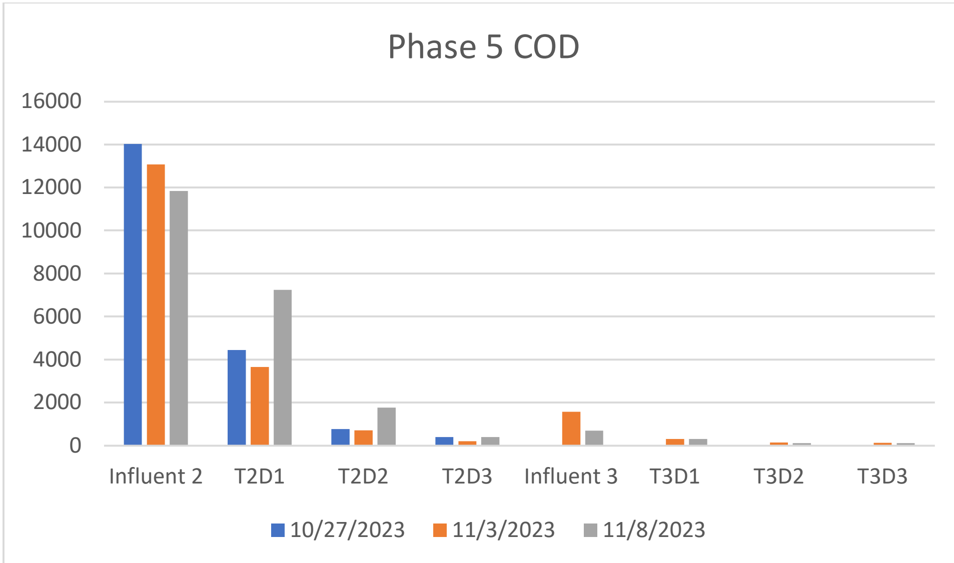

Figure 15: Phase 5 COD for all 3 trains

APPENDIX 2: TOTAL PHOSPHORUS DATA

Table 25: Total Phosphorous Raw Data

Total P	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	2.25	0.25	0.25	0.25	2.32	1.57	0.25	0.25	2.33	0.98	0.25	0.25
	2.17	0.64	0.25	0.25	2.28	0.25	0.25	0.25	2.19	0.66	0.25	0.55
	2.20	0.25	0.25	0.25	2.33	0.25	0.25	0.25	2.65	0.25	0.25	0.25
	2.51	1.50	0.25	0.25	2.28	0.85	0.25	0.25	2.10	0.93	0.25	0.25
Phase 2	2.22	2.30	0.52	0.25	2.19	2.08	0.25	0.25	2.38	2.25	0.25	0.25
	2.43	2.25	0.91	0.25	3.16	1.59	0.25	0.25	3.04	2.46	0.66	0.60
	2.19	1.95	1.17	0.25	3.40	2.07	1.09	0.55	4.35	4.31	1.61	0.25
	2.77	2.06	1.37	0.65	3.44	3.48	1.98	1.01	7.40	5.02	2.47	0.82
	2.59	1.42	1.45	0.80	3.80	2.03	1.69	0.51	5.46	6.39	3.59	1.90
	2.15	1.98	1.26	0.77	3.53	4.40	3.75	1.57	7.01	6.66	3.66	2.53
Phase 3	2.18	2.42	1.34	0.66	7.83	5.65	3.67	1.87	6.66	4.59	2.86	2.55
	2.27	2.04	0.88	0.68	7.76	6.49	3.33	1.84	7.54	4.28	1.59	2.40
	1.56	1.50	0.99	0.64	4.39	3.62	2.13	1.43	4.34	2.67	1.65	1.74
	1.70	1.85	0.68	0.80	5.66	5.90	2.32	1.37	6.72	4.10	1.18	1.82
Phase 4: Winery and Cidery	2.43	2.39	0.92	0.25	3.50	6.16	1.99	0.25	8.95	5.01	1.63	1.43
	2.51	2.07	0.82	0.53	5.91	8.38	3.29	1.09	8.70	5.13	1.49	1.55
	0.97	1.59	0.63	0.25	16.50	7.35	2.02	0.90	3.85	7.44	1.46	1.56
	0.25	1.79	0.82	0.25	2.03	7.88	2.34	1.11	36.24	18.36	9.75	3.02
	2.60	1.58	0.92	0.25	15.70	8.96	2.51	1.13	50.30	29.75	11.49	3.29
	2.20	1.45	1.06	0.25	19.50	13.48	2.84	1.41	58.52	38.92	15.48	0.25
	3.03	1.82	3.67	0.25	2.69	8.43	2.67	1.63	0.78	39.78	18.30	7.07
Phase 5	0.25	0.25	0.25	0.25	19.60	14.75	8.73	6.08	0.25	0.25	0.25	0.25
	0.25	0.25	0.25	0.25	117.2	63.36	15.51	4.80	2.46	5.41	1.98	2.94
	0.25	0.25	0.25	0.25	132.4	107.2	42.63	12.60	2.42	5.47	3.34	2.80

Note: If the value is 0.25, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 26: Train 1 Influent Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	2.28	2.17	2.51	0.16
Std. syn. Winery WW	2	6	2.39	2.15	2.77	0.25
Std. syn. Winery WW	3	4	1.93	1.56	2.27	0.35
Std. syn. Winery WW	4	6	2.29	0.97	3.03	0.70
Overall SSWW		20	2.25	0.97	3.03	0.45

Table 27: Train 1 Reactor 1 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.66	0.11	1.50	0.60
Std. syn. Winery WW	2	6	1.99	1.42	2.30	0.31
Std. syn. Winery WW	3	4	1.95	1.50	2.42	0.38
Std. syn. Winery WW	4	7	1.81	1.45	2.39	0.33
Overall SSWW		21	1.67	0.11	2.42	0.62

Table 28: Train 1 Reactor 2 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.12	0.02	0.30	0.13
Std. syn. Winery WW	2	6	1.11	0.52	1.45	0.35
Std. syn. Winery WW	3	4	0.97	0.68	1.34	0.28
Std. syn. Winery WW	4	7	1.26	0.63	3.67	1.07
Overall SSWW		21	0.95	0.02	3.67	0.75

Table 29: Train 1 Reactor 3 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.23	0.08	0.47	0.18
Std. syn. Winery WW	2	6	0.50	0.09	0.80	0.29
Std. syn. Winery WW	3	4	0.70	0.64	0.80	0.07
Std. syn. Winery WW	4	7	0.44	0.33	0.53	0.06
Overall SSWW		21	0.46	0.08	0.80	0.22

Table 30: Train 2 Influent Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	2.30	2.28	2.33	0.03
COD spike	2	6	3.25	2.19	3.80	3.80
COD+N spike	3	4	6.41	4.39	7.83	1.68
Winery wastewater	4	7	9.40	2.03	19.50	7.51
Brewery wastewater	5	3	90	20	132	61

Table 31: Train 2 Reactor 1 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.82	0.39	1.57	0.54
COD spike	2	6	2.61	1.59	4.40	1.09
COD+N spike	3	4	5.42	3.62	6.49	1.25
Winery wastewater	4	7	8.66	6.16	13.48	2.31
Brewery wastewater	5	3	62	15	107	46

Table 32: Train 2 Reactor 2 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.25	0.25	0.25	0.03
COD spike	2	6	1.55	0.25	3.75	1.26
COD+N spike	3	4	2.86	2.13	3.67	0.75
Winery wastewater	4	7	2.52	1.99	3.29	0.25
Brewery wastewater	5	3	22	9	43	18

Table 33: Train 2 Reactor 3 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.25	0.25	0.25	0.16
COD spike	2	6	0.69	0.17	1.57	0.51
COD+N spike	3	4	1.63	1.37	1.87	0.26
Winery wastewater	4	7	1.09	0.40	1.63	0.39
Brewery wastewater	5	3	8	5	13	4

Table 34: Train 3 Influent Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	2.32	2.10	2.65	0.24
Nutrient Spike	2	6	4.94	2.38	7.40	2.05
Salt spike	3	4	6.31	4.34	7.54	1.38
Cidery wastewater	4	7	23.90	0.78	58.52	23.94
Recovery	5.0	2	2.4	2.4	2.5	0.0

Table 35: Train 3 Reactor 1 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.77	0.50	0.98	0.23
Nutrient Spike	2	6	4.51	2.25	6.66	1.88
Salt spike	3	4	3.91	2.67	4.59	0.85
Cidery wastewater	4	7	20.63	5.01	39.78	15.53
Recovery	5	2	5.44	5.41	5.47	0.04

Table 36: Train 3 Reactor 2 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.25	0.25	0.25	0.11
Nutrient Spike	2	6	2.06	0.35	3.66	1.43
Salt spike	3	4	1.82	1.18	2.86	0.72
Cidery wastewater	4	7	8.51	1.46	18.30	7.08
Recovery	5	2	2.7	2.0	3.3	1.0

Table 37: Train 3 Reactor 3 Total Phosphorus

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.25	0.25	0.55	0.23
Nutrient Spike	2	6	1.12	0.25	2.53	0.89
Salt spike	3	4	2.13	1.74	2.55	0.40
Cidery wastewater	4	6	2.99	1.43	7.07	2.16
Recovery	5	2	2.87	2.80	2.94	0.10

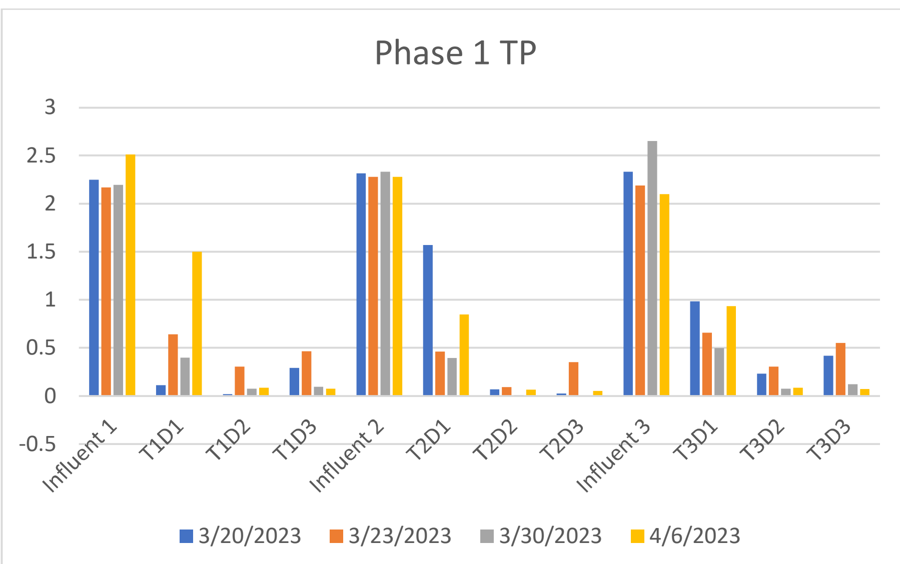

Figure 16: Phase 1 TP for all 3 trains

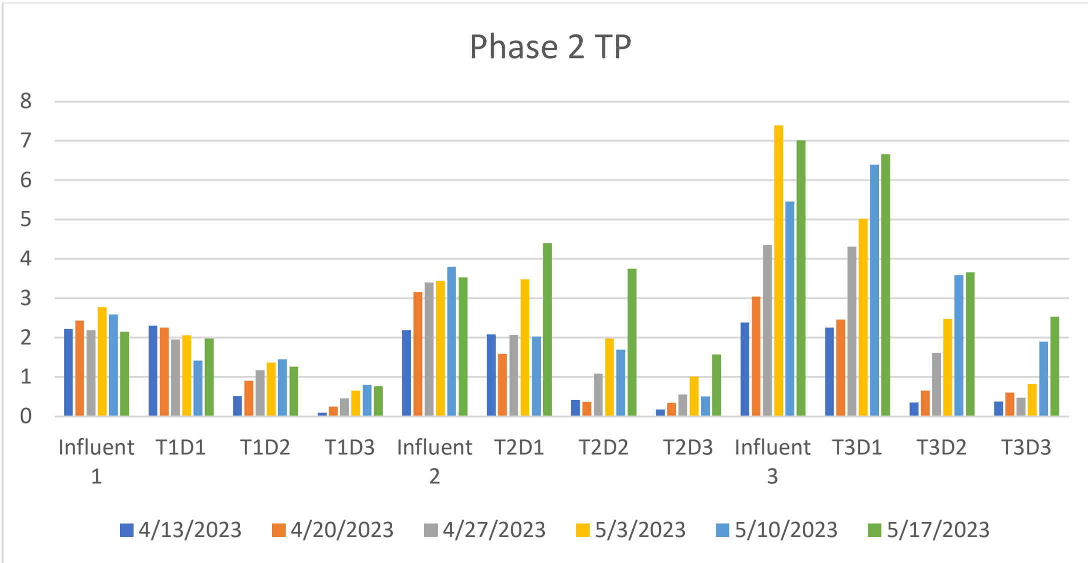

Figure 17: Phase 2 TP for all 3 trains

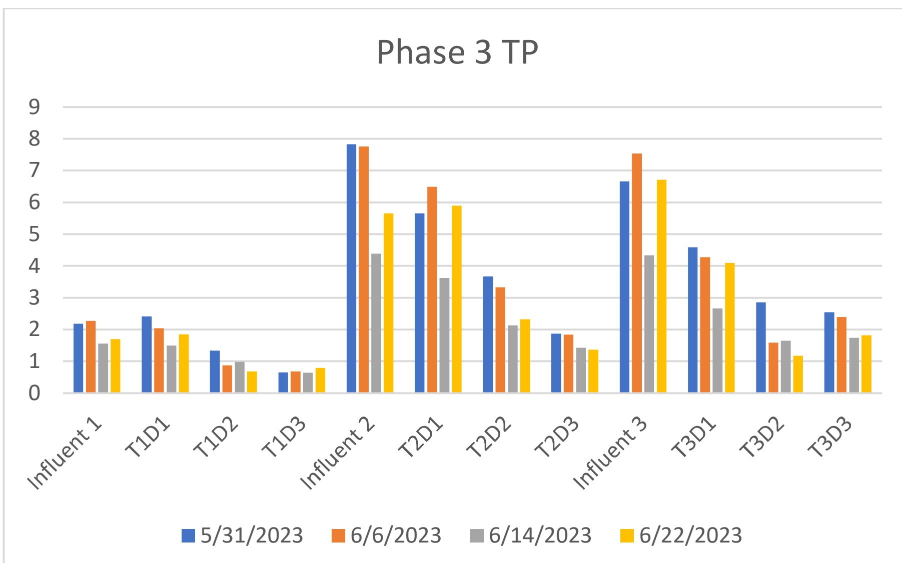

Figure 18: Phase 3 TP for all 3 trains

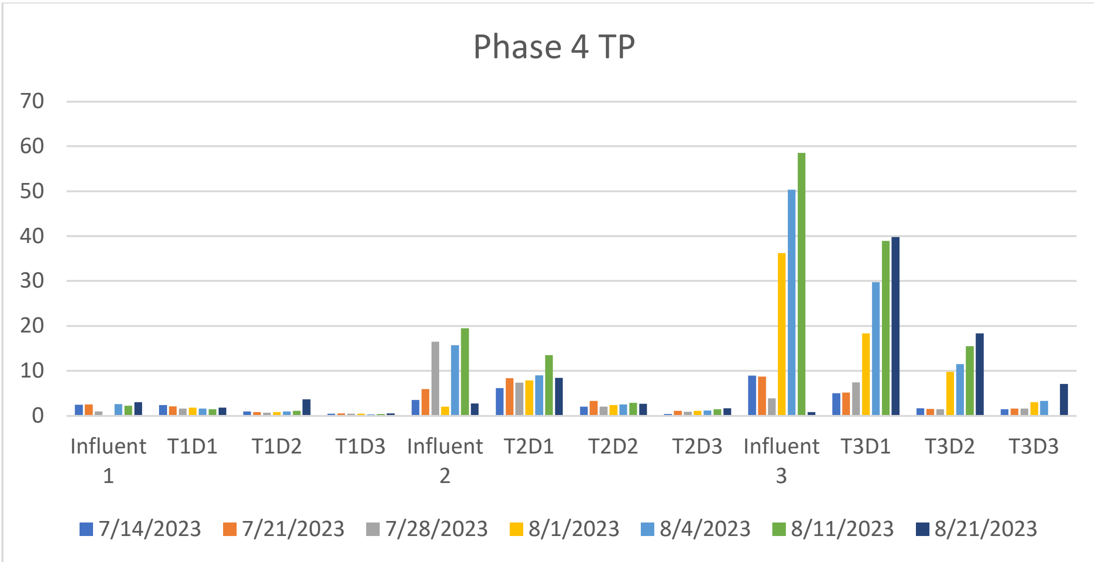

Figure 19: Phase 4 TP for all 3 trains

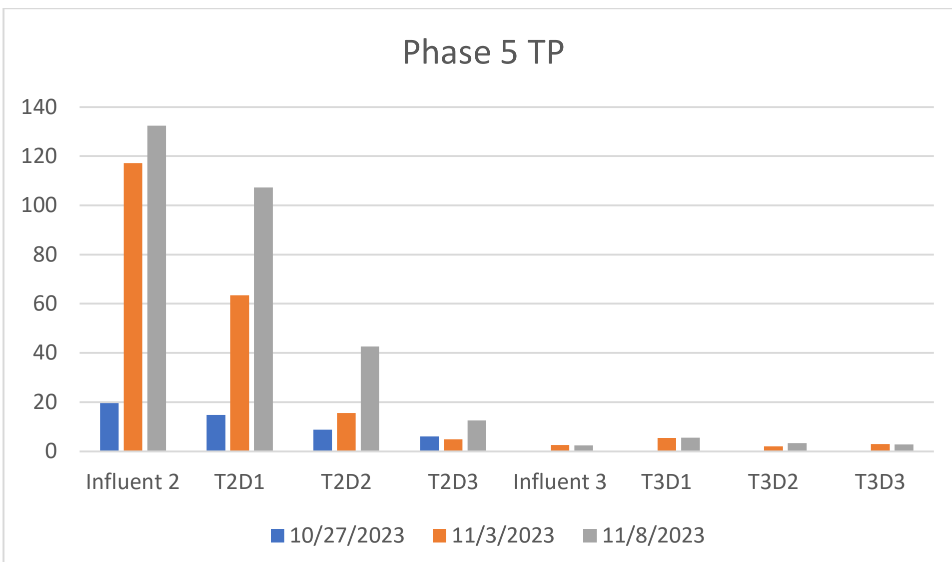

Figure 20: Phase 5 TP for all 3 trains

APPENDIX 3: TOTAL NITROGEN DATA

Table 38: Total Nitrogen Raw Data

	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	0.60	1.58	1.35	1.29	0.65	2.24	1.06	1.33	0.70	2.19	1.85	0.5
	0.83	4.41	1.44	1.69	0.65	2.53	0.74	1.15	0.74	2.12	1.71	2.44
	5.87	1.83	1.66	2.23	6.26	1.57	1.15	2.02	5.60	3.60	1.52	1.94
	5.07	5.28	1.29	1.12	7.06	3.37	1.97	1.50	4.88	5.66	1.76	1.70
Phase 2	7.43	9.37	5.76	2.08	6.60	9.79	3.26	4.38	7.49	6.61	5.61	2.59
	4.64	7.69	3.51	1.87	6.30	18.80	10.02	6.26	4.91	6.10	4.21	2.13
	6.10	7.53	3.99	1.61	22.30	13.80	11.60	7.50	22.15	5.50	3.48	2.19
	2.17	8.28	4.43	3.25	47.10	4.78	12.05	26.20	4.92	13.10	5.67	2.85
	4.94	6.66	5.14	1.42	1.42	11.10	14.50	13.10	14.40	11.15	5.28	3.23
Phase 3	4.82	6.20	4.51	2.60	25.80	12.80	21.80	15.30	9.27	13.10	7.76	3.38
	4.91	9.44	5.70	2.20	14.80	28.90	27.70	15.30	12.40	11.90	9.09	4.04
	6.58	7.41	5.46	2.43	10.58	59.70	18.80	14.55	17.90	2.90	9.24	8.48
	5.81	4.07	3.68	1.08	15.40	35.10	16.70	14.00	18.80	2.97	5.35	5.23
Phase 4	5.87	1.56	2.56	1.91	20.10	12.30	7.72	12.45	13.50	4.76	3.33	3.79
	5.42	2.67	2.41	1.83	21.10	57.20	11.60	10.80	12.50	3.46	2.58	3.01
	1.37	5.13	2.00	1.39	0.5	47.00	13.60	10.50	56.40	3.70	2.11	2.78
	0.5	3.68	1.76	0.49	0.5	32.00	9.69	9.45	0.5	0.5	5.55	2.85
	9.95	1.56	1.83	7.72	22.00	36.61	9.16	9.40	160.60	35.97	8.68	3.91
	16.30	9.31	2.35	1.24	22.33	41.35	8.00	64.20	23.54	43.23	22.05	6.02
	9.50	2.15	2.41	1.55	25.63	27.20	7.88	8.28	5.41	35.53	32.92	14.50
Phase 5	0.5	0.5	0.5	0.5	0.5	18.00	7.57	21.50	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	23.70	8.36	3.59	5.24	6.70	5.92	12.30
	0.5	0.5	0.5	0.5	0.5	25.00	24.50	6.44	8.80	8.49	9.16	9.09

Note: If the value is 0.5, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 39: Train 1 Influent Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	3.09	0.60	5.87	2.77
Std. syn. Winery WW	2	5	5.06	2.17	7.43	1.95
Std. syn. Winery WW	3	4	5.53	4.82	6.58	0.83
Std. syn. Winery WW	4	6	8.07	1.37	16.30	5.10
Overall SSWW		19	5.69	0.60	16.30	3.60

Table 40: Train 1 Reactor 1 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	3.28	1.58	5.28	1.85
Std. syn. Winery WW	2	5	7.91	6.66	9.37	1.00
Std. syn. Winery WW	3	4	6.78	4.07	9.44	2.25
Std. syn. Winery WW	4	7	3.72	1.56	9.31	2.77
Overall SSWW		20	5.29	1.56	9.44	2.83

Table 41: Train 1 Reactor 2 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1.43	1.29	1.66	0.16
Std. syn. Winery WW	2	5	4.57	3.51	5.76	0.90
Std. syn. Winery WW	3	4	4.84	3.68	5.70	0.93
Std. syn. Winery WW	4	7	2.19	1.76	2.56	0.32
Overall SSWW		20	3.16	1.29	5.76	1.56

Table 42: Train 1 Reactor 3 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1.58	1.12	2.23	0.49
Std. syn. Winery WW	2	5	2.05	1.42	3.25	0.72
Std. syn. Winery WW	3	4	2.08	1.08	2.60	0.68
Std. syn. Winery WW	4	7	2.30	0.5	7.72	2.43
Overall SSWW		20	2.05	0.5	7.72	1.47

Table 43: Train 2 Influent Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	3.65	0.5	7.06	3.48
COD spike	2	5	16.74	1.42	47.10	18.70
COD+N spike	3	4	16.65	10.58	25.80	6.47
Winery wastewater	4	5	22.23	20.10	25.63	2.09
Brewery wastewater	5	0	N/A	0	0	N/A

Note: The N/A values mean that the measurements were too high to use data and so an average and STD. could not be calculated.

Table 44: Train 2 Reactor 1 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	2.43	1.57	3.37	0.75
COD spike	2	5	11.65	4.78	18.80	5.16
COD+N spike	3	4	34.13	12.80	59.70	19.47
Winery wastewater	4	7	36.24	12.30	57.20	14.46
Brewery wastewater	5	3	22	18	25	4

Table 45: Train 2 Reactor 2 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	1.23	0.74	1.97	0.52
COD spike	2	5	10.29	3.26	14.50	4.24
COD+N spike	3	4	21.25	16.70	27.70	4.78
Winery wastewater	4	7	9.66	7.72	13.60	2.21
Brewery wastewater	5	3	13	8	25	10

Table 46: Train 2 Reactor 3 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	1.50	1.15	2.02	0.38
COD spike	2	5	11.49	4.38	26.20	8.84
COD+N spike	3	4	14.79	14.00	15.30	0.63
Winery wastewater	4	7	17.87	8.28	64.20	20.47
Brewery wastewater	5	3	11	4	22	10

Table 47: Train 3 influent Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	2.98	0.70	5.60	2.62
Nutrient Spike	2	5	10.77	4.91	22.15	7.45
Salt spike	3	4	14.59	9.27	18.80	4.54
Cidery wastewater	4	6	45.33	5.41	160.60	59.28
Recovery	5	2	7	5	9	3

Table 48: Train 3 Reactor 1 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	3.39	2.12	5.66	1.66
Nutrient Spike	2	5	8.49	5.50	13.10	3.41
Salt spike	3	4	7.72	2.90	13.10	5.55
Cidery wastewater	4	6	21.11	3.46	43.23	18.97
Recovery	5	2	8	7	8	1

Table 49: Train 3 Reactor 2 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	1.71	1.52	1.85	0.14
Nutrient Spike	2	5	4.85	3.48	5.67	0.96
Salt spike	3	4	7.86	5.35	9.24	1.80
Cidery wastewater	4	7	11.03	2.11	32.92	11.88
Recovery	5	2	8	6	9	2

Table 50: Train 3 Reactor 3 Total Nitrogen

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	3	2.03	1.70	2.44	0.38
Nutrient Spike	2	5	2.60	2.13	3.23	0.46
Salt spike	3	4	5.28	3.38	8.48	2.26
Cidery wastewater	4	7	5.27	2.78	14.50	4.22
Recovery	5	2	11	9	12	2

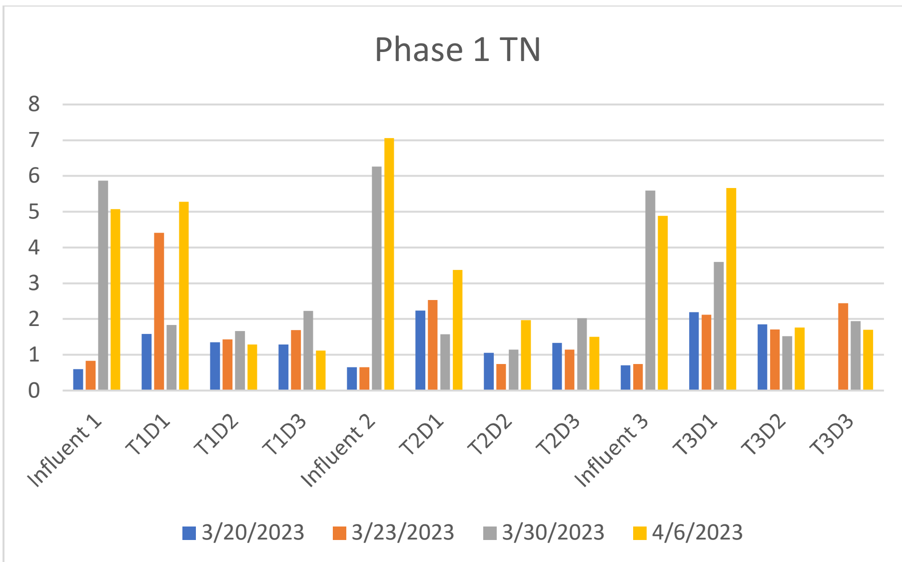

Figure 21: Phase 1 TN for all 3 trains

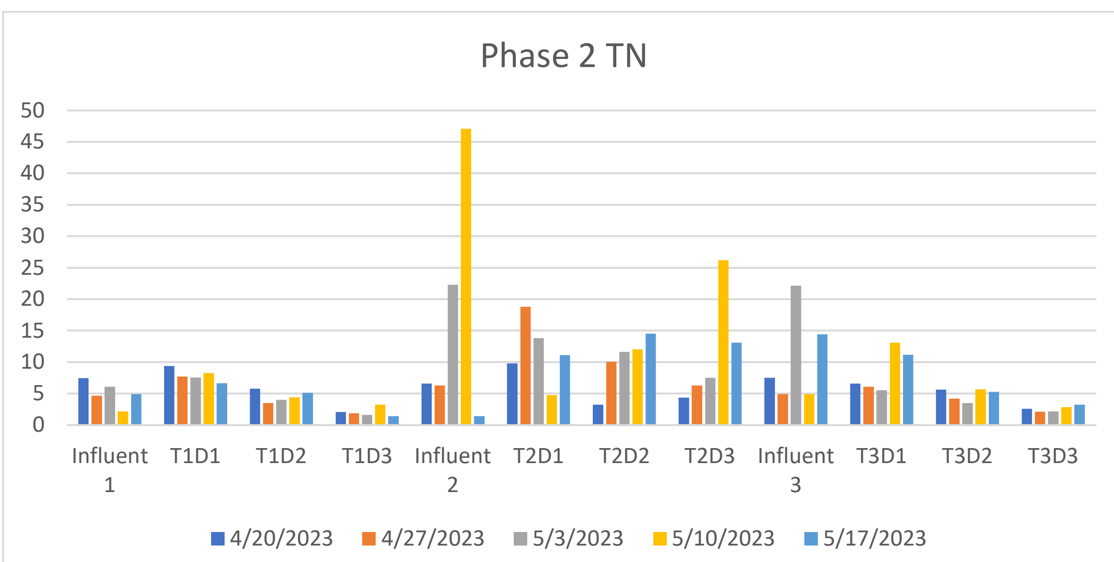

Figure 22: Phase 2 TN for all 3 trains

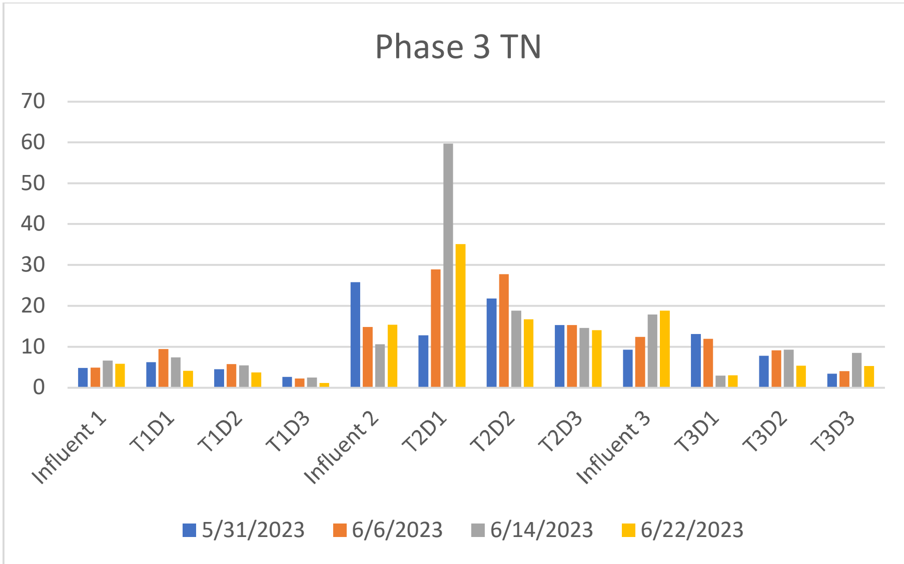

Figure 23: Phase 3 TN for all 3 trains

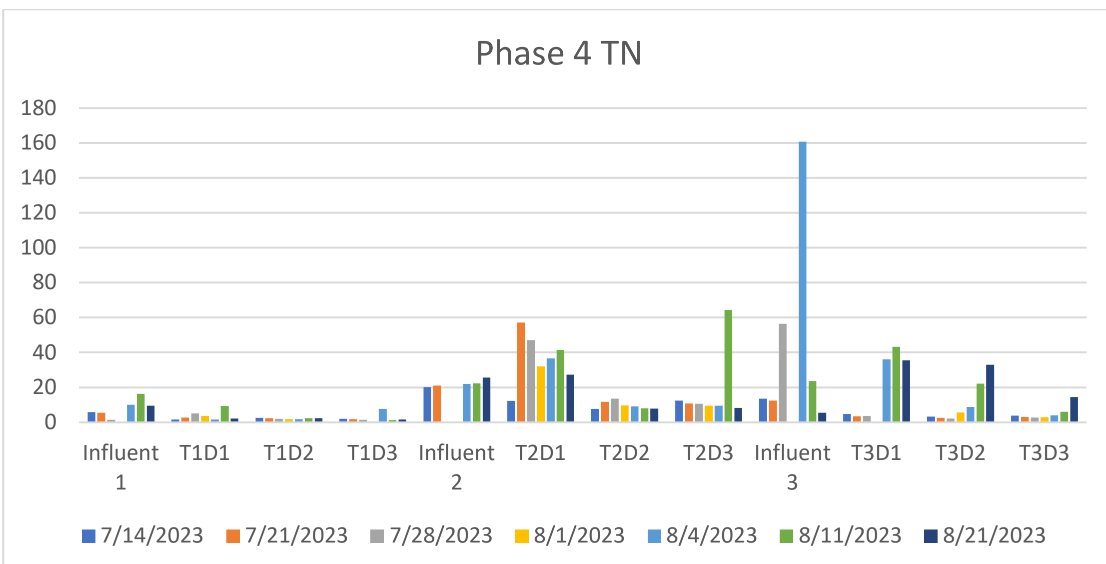

Figure 24: Phase 4 TN for all 3 trains

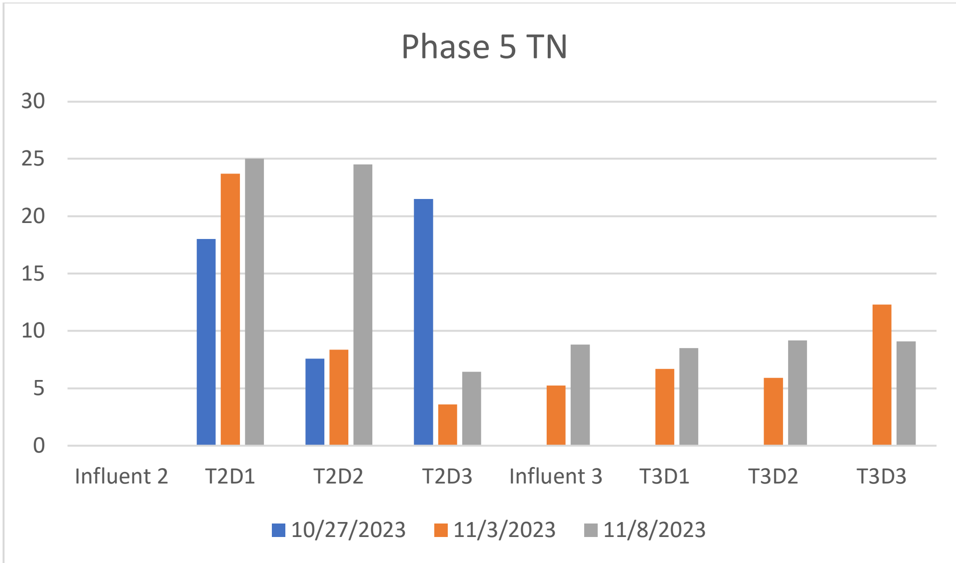

Figure 25: Phase 5 TN for all 3 trains

APPENDIX 4: NITRATE DATA

Table 50: Nitrate Raw Data

	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	0.91	0.115	0.115	0.115	1.02	0.115	0.115	0.115	0.68	0.115	0.115	0.115
	1.25	0.115	0.25	0.115	1.31	0.115	0.115	0.115	1.18	0.115	0.115	0.115
	1.10	0.115	0.115	0.115	1.11	0.115	0.115	0.115	1.33	0.115	0.115	0.115
	0.41	0.115	0.115	0.115	0.74	0.115	0.115	0.115	0.78	0.115	0.115	0.115
Phase 2	1.60	0.27	0.27	0.115	1.62	0.26	0.27	0.24	1.91	0.25	0.23	0.24
	1.09	0.115	1.25	0.38	6.89	0.115	0.115	0.115	0.91	0.115	0.40	0.115
	0.70	0.115	3.58	1.17	4.81	0.63	0.35	0.115	0.52	0.115	2.73	1.29
	1.20	0.115	4.32	0.98	8.25	0.55	0.48	0.38	1.42	0.25	2.57	1.92
Phase 3	1.09	0.115	4.16	0.84	7.44	0.64	0.65	0.32	1.30	0.28	6.72	2.62
	1.15	1.73	5.05	1.58	8.41	0.48	0.43	2.94	1.21	0.32	7.32	2.89
	0.42	0.72	3.83	1.62	2.42	0.43	1.88	4.13	0.42	0.115	5.78	4.45
	0.115	1.52	3.32	0.69	0.65	0.44	9.99	12.00	0.115	0.83	4.88	4.30
Phase 4	1.24	0.115	1.61	0.53	4.67	0.96	4.38	9.77	0.99	0.59	1.71	2.34
	1.85	0.36	1.11	0.34	0.74	0.92	4.62	7.77	0.97	0.52	1.42	1.95
	1.46	3.94	1.30	0.30	13.20	0.76	9.12	7.52	4.00	0.93	1.25	1.63
	0.115	2.64	1.25	0.33	3.72	0.98	5.67	7.43	7.28	2.13	0.83	0.36
	0.25	0.46	1.19	0.23	0.67	0.94	5.82	7.22	7.64	2.27	0.86	0.36
	0.115	2.32	1.42	0.29	1.10	1.22	4.07	6.70	11.40	3.60	1.28	0.47
	0.115	0.23	1.61	0.76	0.50	0.59	17.20	9.64	5.11	3.93	1.46	0.68
Phase 5	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115
	0.115	0.115	0.115	0.115	10.70	2.83	0.54	0.31	1.84	0.36	2.19	8.71

Note: If the value is 0.115, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 51: Train 1 Influent Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.92	0.41	1.25	0.36
Std. syn. Winery WW	2	4	1.15	0.70	1.60	0.37
Std. syn. Winery WW	3	4	0.69	0.12	1.15	0.51
Std. syn. Winery WW	4	6	0.83	0.115	1.85	0.78
Overall SSWW		18	0.89	0.115	1.85	0.54

Table 52: Train 1 Reactor 1 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.115	0.115	0.115	0.02
Std. syn. Winery WW	2	4	0.115	0.115	0.27	0.08
Std. syn. Winery WW	3	4	1.04	0.21	1.73	0.71
Std. syn. Winery WW	4	7	1.45	0.21	3.94	1.50
Overall SSWW		19	0.83	0.07	3.94	1.09

Table 53: Train 1 Reactor 2 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.115	0.115	0.25	0.05
Std. syn. Winery WW	2	4	2.35	0.27	4.32	1.91
Std. syn. Winery WW	3	4	4.09	3.32	5.05	0.73
Std. syn. Winery WW	4	7	1.35	1.11	1.61	0.20
Overall SSWW		19	1.90	0.14	5.05	1.61

Table 54: Train 1 Reactor 3 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.115	0.115	0.115	0.03
Std. syn. Winery WW	2	4	0.68	0.115	1.17	0.46
Std. syn. Winery WW	3	4	1.18	0.69	1.62	0.49
Std. syn. Winery WW	4	7	0.40	0.23	0.76	0.18
Overall SSWW		19	0.58	0.15	1.62	0.47

Table 55: Train 2 Influent Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	1.04	0.74	1.31	0.24
COD spike	2	4	5.39	1.62	8.25	2.89
COD+N spike	3	4	4.73	0.65	8.41	3.78
Winery wastewater	4	7	3.51	0.50	13.20	4.58
Brewery wastewater	5	2	12	11	12	1

Table 56: Train 2 Reactor 1 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.115	0.115	0.115	0.02
COD spike	2	4	0.41	0.115	0.63	0.21
COD+N spike	3	4	0.50	0.43	0.64	0.10
Winery wastewater	4	7	0.91	0.59	1.22	0.19
Brewery wastewater	5	2	4	3	4	1

Table 57: Train 2 Reactor 2 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.115	0.115	0.115	0.02
COD spike	2	4	0.31	0.115	0.48	0.14
COD+N spike	3	4	3.24	0.43	9.99	4.55
Winery wastewater	4	7	7.27	4.07	17.20	4.70
Brewery wastewater	5	2	1	1	1	0

Table 58: Train 2 Reactor 3 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.115	0.115	0.115	0.02
COD spike	2	4	0.23	0.115	0.38	0.11
COD+N spike	3	4	4.85	0.32	12.00	5.03
Winery wastewater	4	7	8.01	6.70	9.77	1.21
Brewery wastewater	5	2	0.38	0.31	0.45	0.10

Table 59: Train 3 Influent Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.99	0.68	1.33	0.31
Nutrient Spike	2	4	1.19	0.52	1.91	0.60
Salt spike	3	4	0.79	0.22	1.30	0.55
Cidery wastewater	4	7	5.34	0.97	11.40	3.78
Recovery	5	2	2	2	2	0

Table 60: Train 3 Reactor 1 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.115	0.115	0.115	0.01
Nutrient Spike	2	4	0.115	0.115	0.25	0.12
Salt spike	3	4	0.40	0.115	0.83	0.29
Cidery wastewater	4	7	2.00	0.52	3.93	1.40
Recovery	5	2	0.29	0.115	0.36	0.10

Table 61: Train 3 Reactor 2 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	3	0.115	0.115	0.115	0.02
Nutrient Spike	2	4	1.48	0.23	2.73	1.35
Salt spike	3	4	6.18	4.88	7.32	1.07
Cidery wastewater	4	7	1.26	0.83	1.71	0.32
Recovery	5	2	2.10	2.01	2.19	0.13

Table 62: Train 3 Reactor 3 Nitrate

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.115	0.115	0.115	0.02
Nutrient Spike	2	4	0.89	0.115	1.92	0.86
Salt spike	3	4	3.56	2.62	4.45	0.94
Cidery wastewater	4	7	1.11	0.36	2.34	0.84
Recovery	5	2	7.69	6.68	8.71	1.44

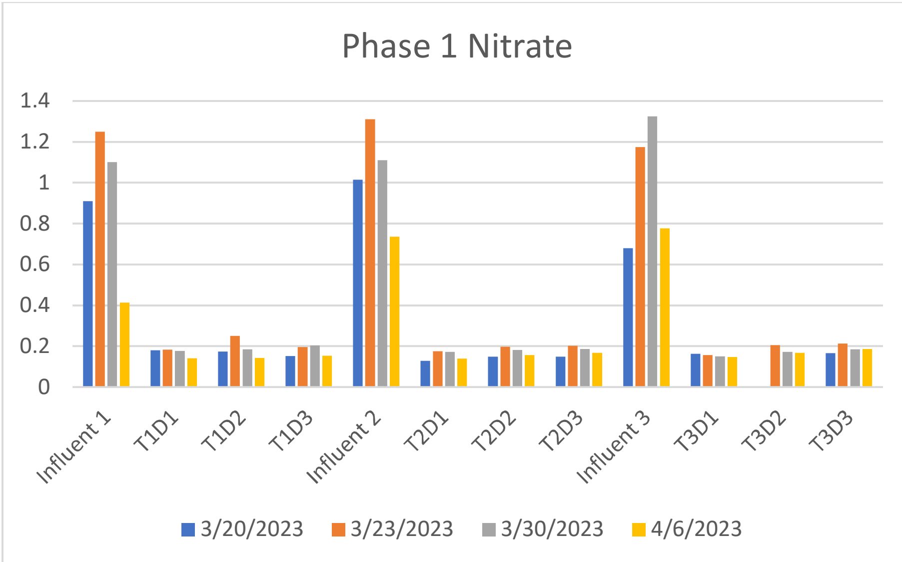

Figure 26: Phase 1 Nitrate for all 3 trains

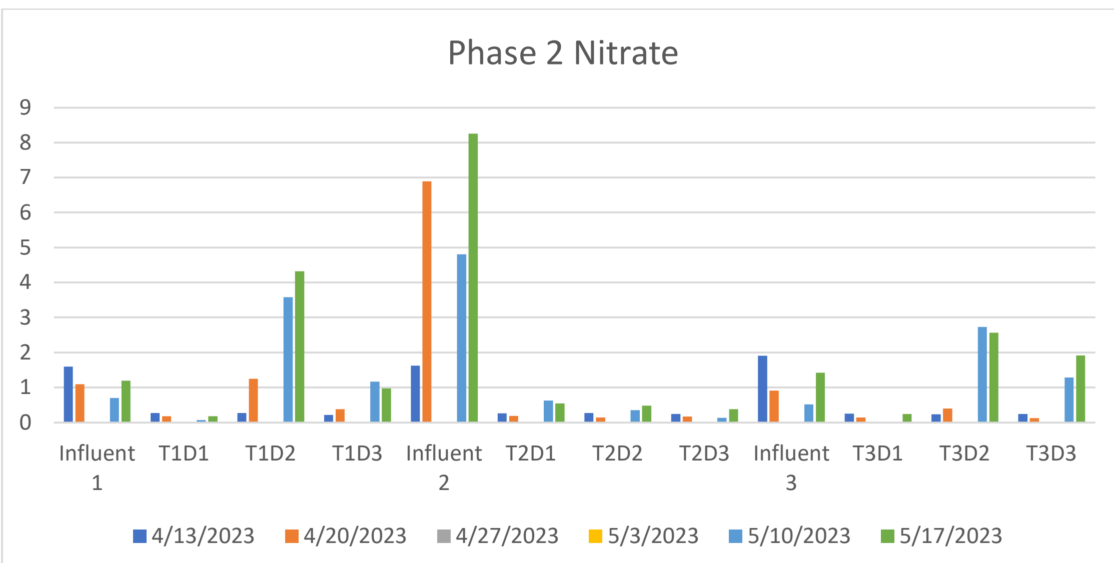

Figure 27: Phase 2 Nitrate for all 3 trains

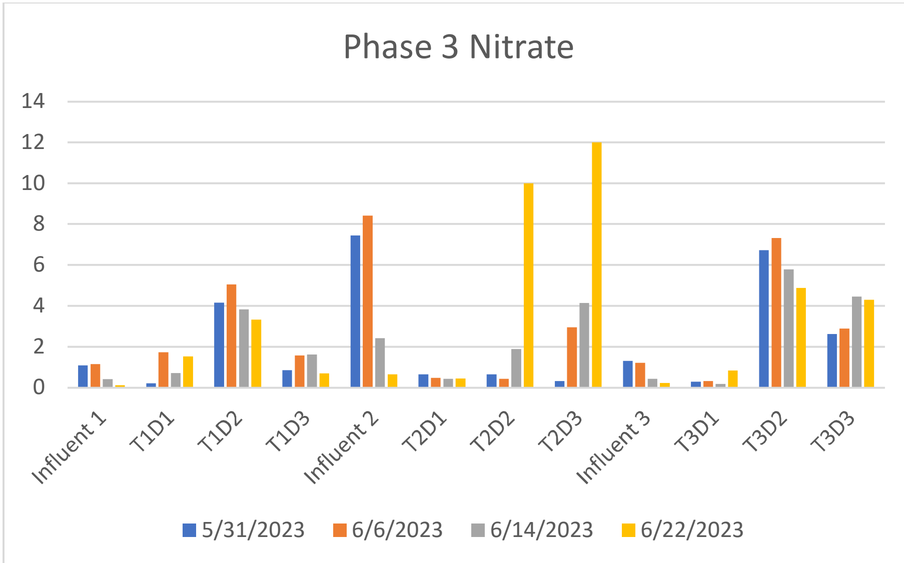

Figure 28: Phase 3 Nitrate for all 3 trains

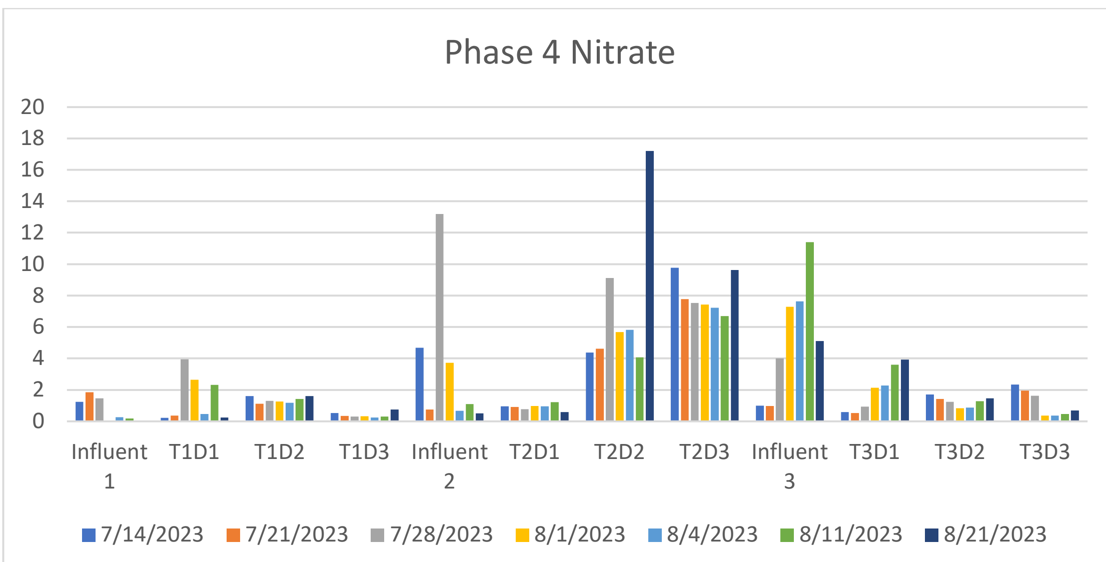

Figure 29: Phase 4 Nitrate for all 3 trains

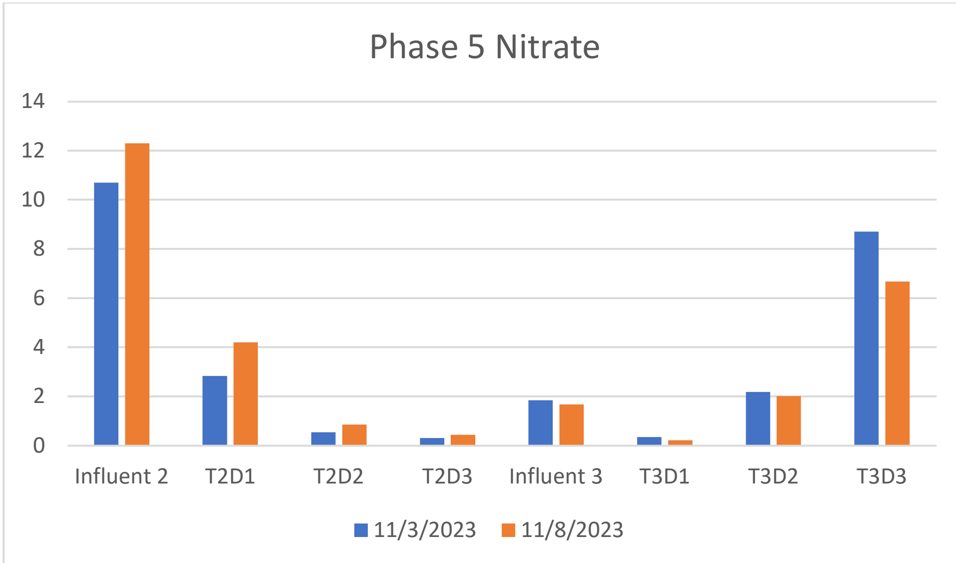

Figure 30: Phase 5 Nitrate for all 3 trains

APPENDIX 5: NITRITE DATA

Table 63: Nitrite Raw Data

	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075
	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075
	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075
	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.025	0.025	0.0075
Phase 2	0.0075	0.053	0.0075	0.48	0.0075	0.045	0.0075	0.0075	0.0075	0.0075	0.788	0.0075
	0.0075	0.061	0.944	0.3295	0.0075	0.107	0.029	0.0075	0.0075	0.015	1.05	0.642
	0.0075	0.03	0.181	0.0075	0.0075	0.94	0.122	0.028	0.0075	0.0075	0.131	0.158
	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.054	0.0075	0.0075	0.0075	0.102	0.0075
Phase 3	0.0075	0.0075	0.047	0.0075	0.0075	0.103	0.05	0.0075	0.0075	0.0075	0.199	0.0075
	0.0075	0.0725	0.018	0.0075	0.0075	0.09	0.03	1.24	0.0075	0.509	0.045	0.0075
	0.0075	1.49	0.0075	0.0075	0.0075	0.123	0.629	0.93	0.0075	0.0075	0.0075	0.0075
	0.0075	1.36	0.0075	0.0075	0.0725	0.05	0.594	0.024	0.0075	1.155	0.0075	0.0075
Phase 4	0.0075	0.0075	0.0075	0.0075	0.148	0.117	0.099	0.0075	0.062	0.153	0.0075	0.0075
	0.0075	0.069	0.0075	0.0075	0.031	0.197	0.03	0.0075	0.063	0.049	0.0075	0.0075
	0.0075	0.231	0.0075	0.0075	0.147	0.1	0.049	0.0075	0.134	0.0075	0.0075	0.0075
	0.0075	0.207	0.0075	0.0075	0.025	0.146	0.05	0.0075	0.133	0.124	0.037	0.0075
	0.0075	0.016	0.0075	0.0075	0.06	0.189	0.0325	0.0075	0.136	0.087	0.041	0.0075
	0.0075	0.041	0.0075	0.0075	0.086	0.214	0.044	0.0075	0.12	0.2995	0.22	0.219
Phase 5	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075
	0.0075	0.0075	0.0075	0.0075	0.3745	0.229	0.0075	0.0075	0.0075	0.045	0.067	0.0075
	0.0075	0.0075	0.0075	0.0075	1.32	0.355	0.016	0.0075	0.0075	0.036	0.259	0.016

Note: If the value is 0.0075, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 64: Train 1 Influent Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.05
Std. syn. Winery WW	2	4	0.0075	0.0075	0.0075	0.02
Std. syn. Winery WW	3	4	0.0075	0.0075	0.0075	0.01
Std. syn. Winery WW	4	5	0.0075	0.0075	0.0075	0.01
Overall SSWW		18	0.0075	0.0075	0.0075	0.03

Table 65: Train 1 Reactor 1 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.04
Std. syn. Winery WW	2	4	0.03	0.0075	0.06	0.04
Std. syn. Winery WW	3	4	0.73	0.0075	1.49	0.80
Std. syn. Winery WW	4	6	0.09	0.0075	0.23	0.10
Overall SSWW		18	0.20	0.0075	1.49	0.45

Table 66: Train 1 Reactor 2 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.04
Std. syn. Winery WW	2	3	0.36	0.0075	0.94	0.52
Std. syn. Winery WW	3	4	0.02	0.0075	0.05	0.02
Std. syn. Winery WW	4	6	0.0075	0.0075	0.0075	0.00
Overall SSWW		17	0.06	0.0075	0.94	0.23

Table 67: Train 1 Reactor 3 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.04
Std. syn. Winery WW	2	4	0.20	0.0075	0.48	0.25
Std. syn. Winery WW	3	4	0.0075	0.0075	0.0075	0.00
Std. syn. Winery WW	4	6	0.0075	0.0075	0.0075	0.00
Overall SSWW		18	0.04	0.0075	0.0075	0.14

Table 68: Train 2 Influent Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.04
COD spike	2	4	0.0075	0.0075	0.0075	0.09
COD+N spike	3	4	0.02	0.0075	0.07	0.04
Winery wastewater	4	6	0.08	0.03	0.15	0.05
Brewery wastewater	5	2	1	0	1	1

Table 69: Train 2 Reactor 1 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.0075	0.0075	0.0075	0.04
COD spike	2	4	0.27	-0.03	0.94	0.45
COD+N spike	3	4	0.09	0.05	0.12	0.03
Winery wastewater	4	6	0.16	0.10	0.21	0.05
Brewery wastewater	5	2	0.29	0.23	0.36	0.09

Table 70: Train 2 Reactor 2 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.0075	0.0075	0.0075	0.00
COD spike	2	4	0.05	0.0075	0.12	0.05
COD+N spike	3	4	0.33	0.03	0.63	0.33
Winery wastewater	4	6	0.05	0.03	0.10	0.03
Brewery wastewater	5	2	0.0075	0.0075	0.02	0.01

Table 71: Train 2 Reactor 3 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.0075	0.0075	0.0075	0.04
COD spike	2	4	0.01	0.0075	0.03	0.02
COD+N spike	3	4	0.55	0.01	1.24	0.63
Winery wastewater	4	6	0.0075	0.0075	0.01	0.00
Brewery wastewater	5	2	0.0075	0.0075	0.0075	0.001

Table 72: Train 3 Influent Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.0075	0.0075	0.0075	0.04
Nutrient Spike	2	4	0.0075	0.0075	0.0075	0.02
Salt spike	3	4	0.0075	0.0075	0.01	0.01
Cidery wastewater	4	6	0.11	0.06	0.14	0.04
Recovery	5	2	0.0075	0.0075	0.0075	0.001

Table 73: Train 3 Reactor 1 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.01	0.0075	0.03	0.02
Nutrient Spike	2	4	0.01	0.0075	0.02	0.01
Salt spike	3	4	0.42	0.0075	1.16	0.54
Cidery wastewater	4	6	0.12	0.01	0.30	0.10
Recovery	5	2	0.041	0.036	0.045	0.006

Table 74: Train 3 Reactor 2 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.0075	-0.08	0.03	0.04
Nutrient Spike	2	4	0.52	0.10	1.05	0.48
Salt spike	3	4	0.06	0.0075	0.20	0.09
Cidery wastewater	4	6	0.05	0.0075	0.22	0.09
Recovery	5	2	0.163	0.067	0.259	0.136

Table 75: Train 3 Reactor 3 Nitrite

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.0075	0.0075	0.01	0.05
Nutrient Spike	2	4	0.20	0.0075	0.64	0.31
Salt spike	3	4	0.0075	0.0075	0.01	0.01
Cidery wastewater	4	6	0.04	0.0075	0.22	0.09
Recovery	5	2	0.010	0.0075	0.016	0.009

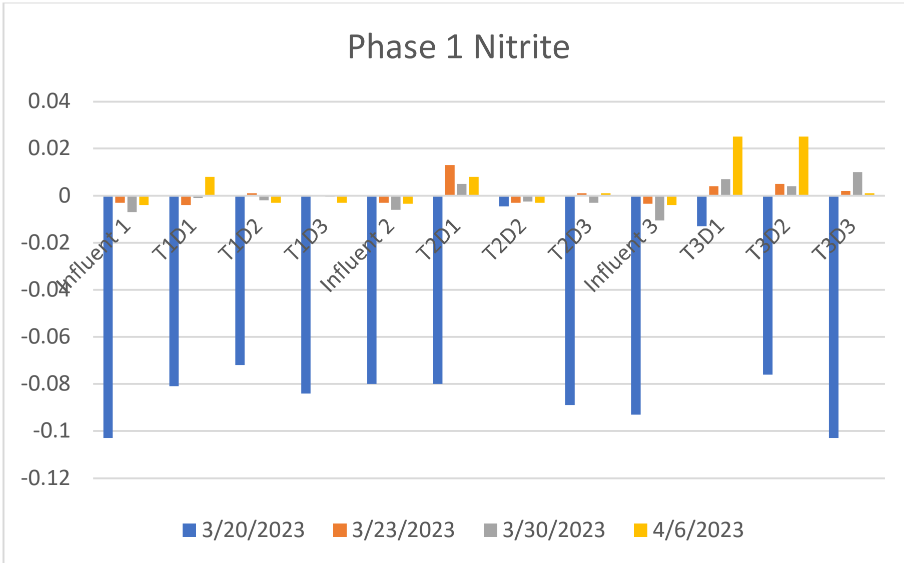

Figure 31: Phase 1 Nitrite for all 3 trains

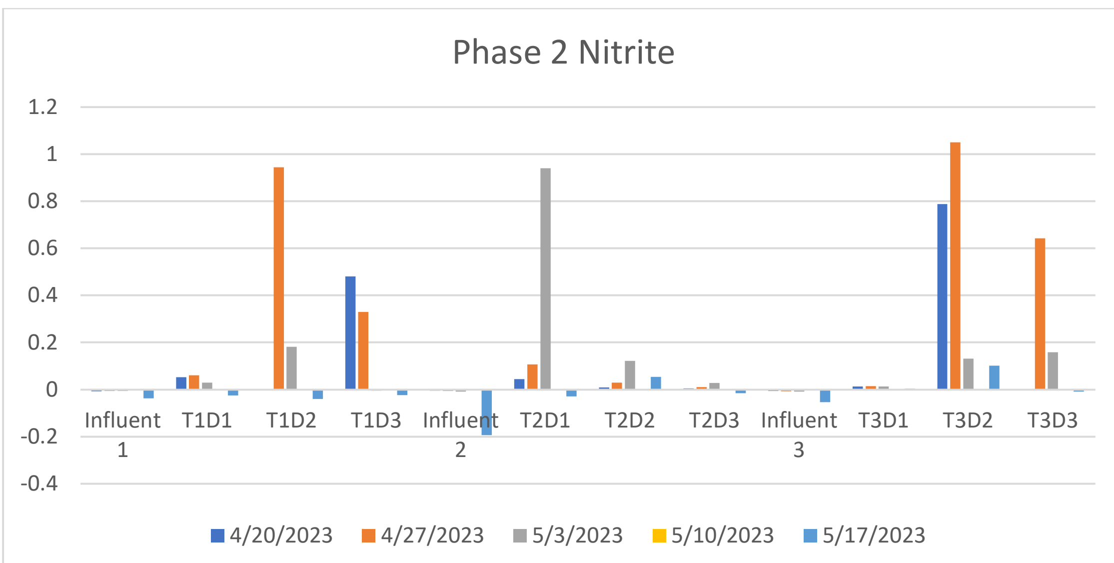

Figure 32: Phase 2 Nitrite for all 3 trains

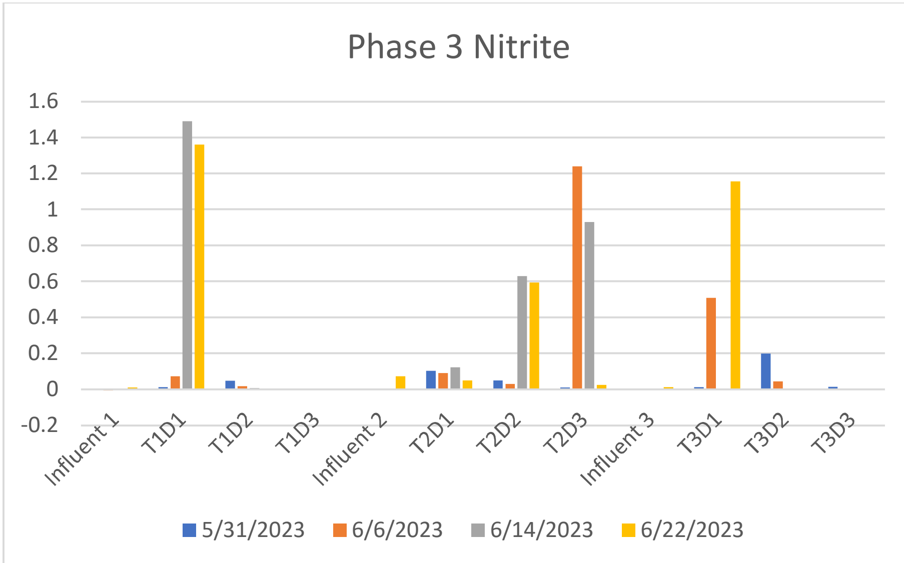

Figure 33: Phase 3 Nitrite for all 3 trains

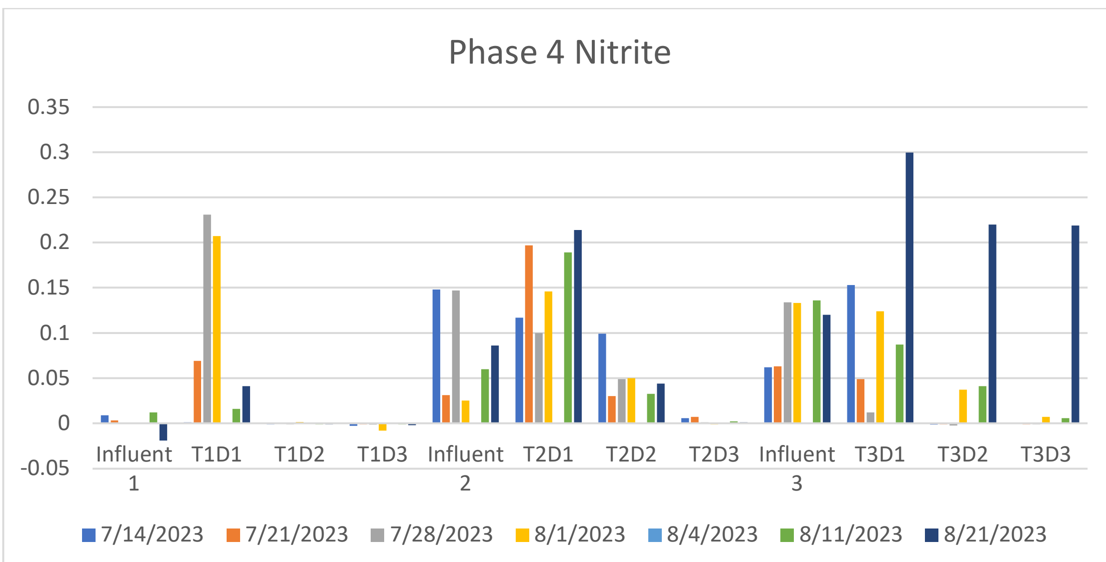

Figure 34: Phase 4 Nitrite for all 3 trains

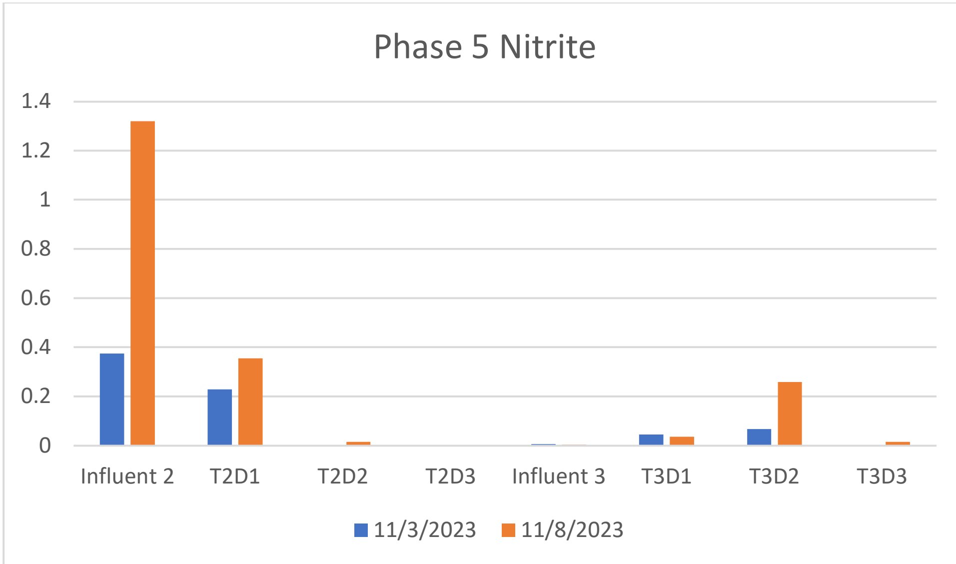

Figure 35: Phase 5 Nitrite for all 3 trains

APPENDIX 6: AMMONIA DATA

Table 76: Ammonia Raw Data

	Inf. 1	T1D1	T1D2	T1D3	Inf. 2	T2D1	T2D2	T2D3	Inf. 3	T3D1	T3D2	T3D3
Phase 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phase 2	0.5	1.6	1.94	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.09	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.01	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	3.23	0.5	0.5	0.5	0.5	2.80	2.99	0.5	3.03	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phase 3	0.5	3.35	0.5	0.5	1.33	0.5	6.18	11.7	0.5	11	0.5	0.5
	0.5	1.88	0.5	0.5	1.74	8.3	17.8	0.5	0.5	11.6	0.5	0.5
	0.5	0.5	0.5	0.5	1.43	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2.25	0.5	0.5	0.5	8.00	19.2	0.5	0.5	13.05	0.5	0.5	0.5
Phase 4	0.5	0.5	0.5	0.5	4.37	0.5	0.5	0.5	2.69	1.91	0.5	0.5
	0.5	0.5	0.5	0.5	12.9	4.76	1.12	0.5	1.23	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	20.4	0.5	0.5	8.89	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	5.33	1.26	0.5	0.5	0.5	0.5	0.5	0.5
	2.25	0.5	0.5	0.5	8.15	0.5	0.5	0.5	0.5	1.01	0.5	1.71
	2.28	0.5	0.5	0.5	13.9	10.05	0.5	0.5	0.5	0.5	5.55	0.5
Phase 5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	17.8	13.1	4.42	0.5	0.5	0.5	0.5	0.5
	0.5	0.5	0.5	0.5	28.1	0.5	17.9	2.75	0.5	0.5	0.5	0.5

Note: If the value is 0.5, then it is equivalent to BDL/2 to get the midpoint between the BDL and 0.

Table 77: Train 1 Influent Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.12
Std. syn. Winery WW	2	6	0.5	0.5	0.5	0.13
Std. syn. Winery WW	3	4	0.5	0.5	2.25	1.05
Std. syn. Winery WW	4	6	0.5	0.5	2.28	1.06
Overall SSWW		20	0.5	0.5	2.28	0.77

Table 78: Train 1 Reactor 1 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.33
Std. syn. Winery WW	2	6	0.5	0.5	3.23	1.32
Std. syn. Winery WW	3	4	1.46	0.5	3.35	1.46
Std. syn. Winery WW	4	7	0.5	0.5	0.5	0.18
Overall SSWW		21	0.5	0.5	3.35	1.04

Table 79: Train 1 Reactor 2 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.02
Std. syn. Winery WW	2	6	0.5	0.5	1.94	0.81
Std. syn. Winery WW	3	4	0.5	0.5	0.5	0.01
Std. syn. Winery WW	4	7	0.5	0.5	0.5	0.01
Overall SSWW		21	0.5	0.5	1.94	0.43

Table 80: Train 1 Reactor 3 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.02
Std. syn. Winery WW	2	6	0.5	0.5	0.5	0.06
Std. syn. Winery WW	3	4	0.5	0.5	0.5	0.01
Std. syn. Winery WW	4	7	0.5	0.5	0.5	0.01
Overall SSWW		21	0.5	0.5	0.5	0.03

Table 81: Train 2 Influent Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.14
COD spike	2	6	0.5	0.5	0.5	0.26
COD+N spike	3	4	3.12	1.33	8.00	3.25
Winery wastewater	4	7	6.48	0.5	13.90	5.48
Brewery wastewater	5	2.000	22.950	17.800	28.100	7.283

Table 82: Train 2 Reactor 1 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.09
COD spike	2	6	0.5	0.5	0.5	0.24
COD+N spike	3	4	7.00	0.5	19.20	8.97
Winery wastewater	4	7	5.36	0.5	20.40	7.53
Brewery wastewater	5	1	13.100	13.100	13.100	N/A

Note: The STD could not be calculated as only 1 value was available.

Table 83: Train 2 Reactor 2 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.05
COD spike	2	6	0.5	0.5	2.80	1.14
COD+N spike	3	4	6.20	0.5	17.80	8.20
Winery wastewater	4	7	0.5	0.5	1.12	0.40
Brewery wastewater	5	2	11.158	4.415	17.900	9.535

Table 84: Train 2 Reactor 3 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.02
COD spike	2	6	0.5	0.5	2.99	1.21
COD+N spike	3	4	2.95	0.5	11.70	5.83
Winery wastewater	4	7	0.5	0.5	0.5	0.01
Brewery wastewater	5	2	1.391	0.5	2.750	1.923

Table 85: Train 3 Influent Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Std. syn. Winery WW	1	4	0.5	0.5	0.5	0.10
Nutrient Spike	2	6	0.5	0.5	0.5	0.32
Salt spike	3	4	3.76	0.5	13.05	6.19
Cidery wastewater	4	7	1.75	0.5	8.89	3.35
Recovery	5	2	0.5	0.5	0.5	0.021

Table 86: Train 3 Reactor 1 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.07
Nutrient Spike	2	6	0.5	0.5	3.03	1.19
Salt spike	3	4	5.76	0.5	11.60	6.40
Cidery wastewater	4	7	0.5	0.5	1.91	0.62
Recovery	5	2	0.5	0.5	0.5	0.078

Table 87: Train 3 Reactor 2 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.10
Nutrient Spike	2	6	0.5	0.5	3.01	1.16
Salt spike	3	4	0.5	0.5	0.5	0.01
Cidery wastewater	4	7	0.5	0.5	5.55	2.07
Recovery	5	2	0.5	0.5	0.5	0.069

Table 88: Train 3 Reactor 3 Ammonia

Wastewater Type	Phase	n	Average	Min value	Max value	Standard deviation
Equilibrium stage	1	4	0.5	0.5	0.5	0.17
Nutrient Spike	2	6	0.5	0.5	0.5	0.19
Salt spike	3	4	0.5	0.5	0.5	0.02
Cidery wastewater	4	7	0.5	0.5	1.71	0.64
Recovery	5	2	0.5	0.5	0.5	0.009

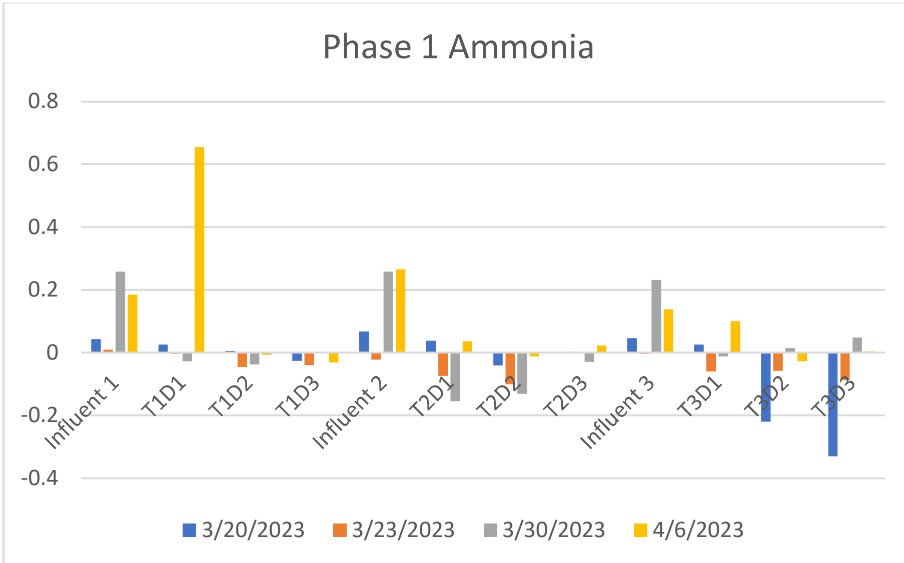

Figure 36: Phase 1 Ammonia for all 3 trains

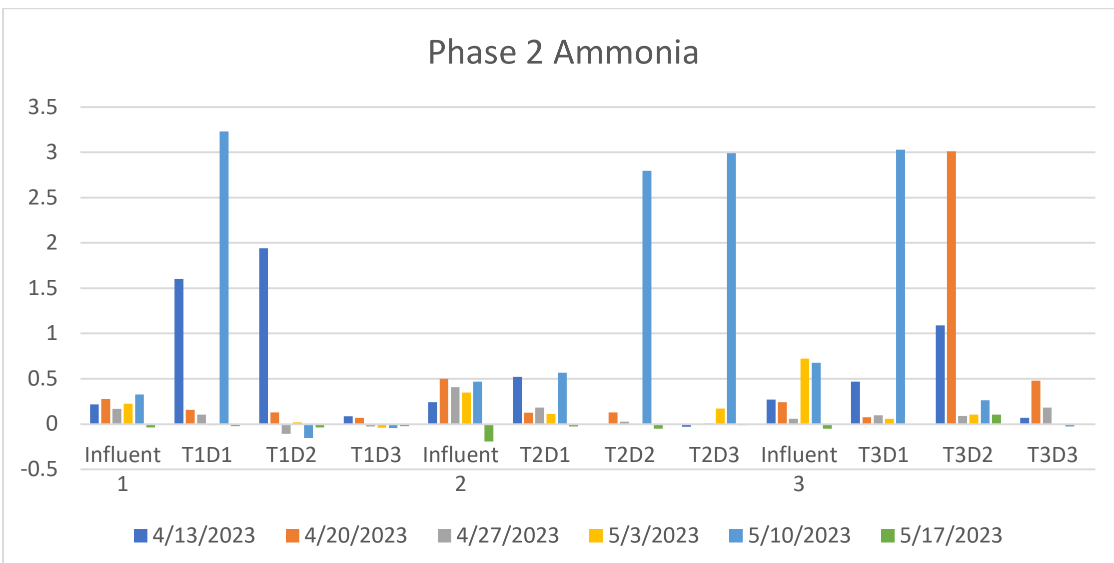

Figure 37: Phase 2 Ammonia for all 3 trains

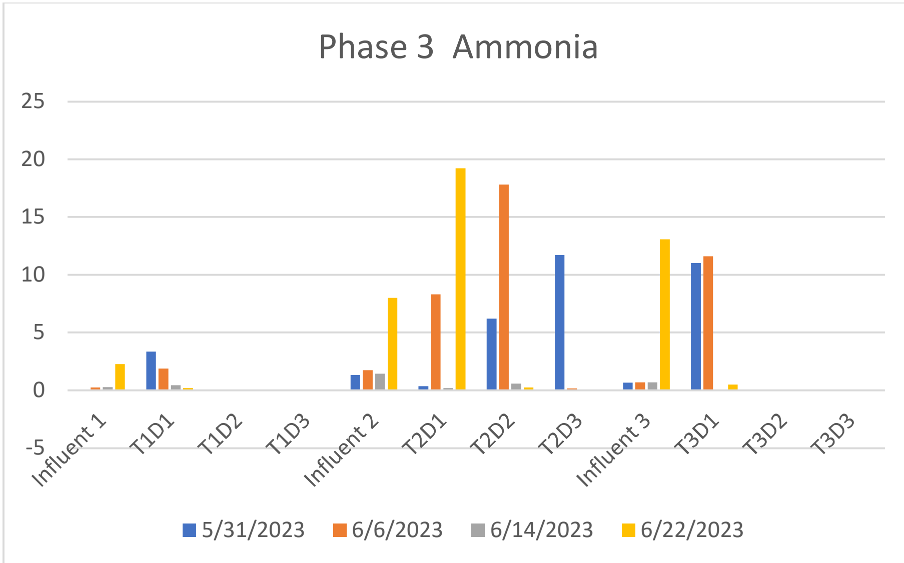

Figure 38: Phase 3 Ammonia for all 3 trains

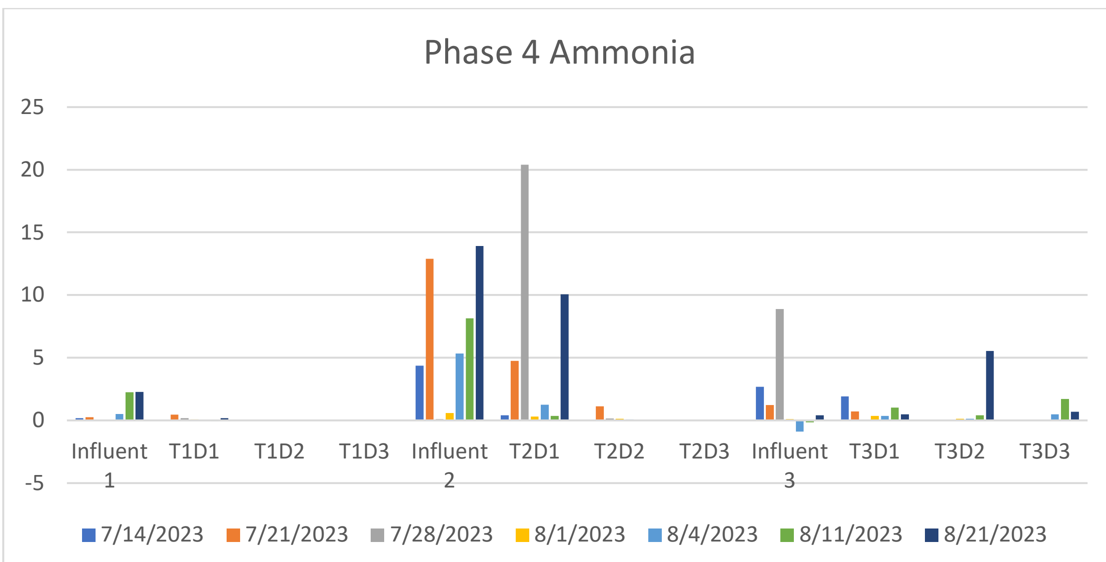

Figure 39: Phase 4 Ammonia for all 3 trains

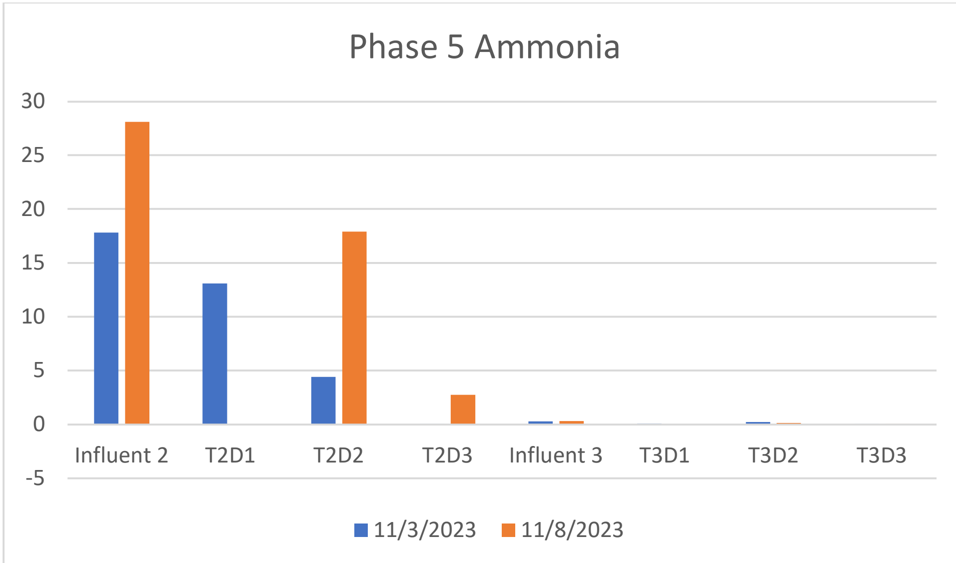

Figure 40: Phase 5 Ammonia for all 3 trains

APPENDIX 7: CODE USED FOR STATISTICS

```
---
title: "Carley_with_phase5"
author: "Rabin KC"
date: "`r Sys.Date()`"
output: html_document
---

```{r}
#Carley data analysis------
#Rabin KC
#SCC



rm(list = ls())

getwd()

#set working directory---

setwd("C:/Users/rabin/OneDrive - Michigan State
University/MSU/STAT_CONSULTING/SCC_2.0/10182023_Carley/")

#load libraries------

library(tidyverse)
library(readxl)
library(lme4)
library(ggResidpanel)
```
```

```
library(car)
library(MASS)
library(emmeans)

dt <- read_excel("Carley_long_format_data_with_phase_5_V2.xlsx")
dim(dt)
str(dt)
head(dt)

dt[,1:7] <- lapply(dt[,1:7], factor)



####################################################################################
###

#For COD----



dt_cod <- subset(dt, response == "COD")
head(dt_cod)
xtabs(~ phase + train_drum, dt_cod)
xtabs(~phase+ sample_name, dt_cod) #samples nested within phases.  (No data for train_drum, rank deficient issues)

head(dt_cod)

#model-------

m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_cod)
```

```
summary(m1)

joint_tests(m1)

outlierTest(m1)


m1.cleaned <- lmer(value ~ phase * train_drum + (1|sample_name), dt_cod[-
c(213,185,177,165),])

resid_panel(m1.cleaned)


#logging the response---


m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_cod)

joint_tests(m1.log)

resid_panel(m1.log)

outlierTest(m1.log)


m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_cod[-
c(193,16,178,166),])

resid_panel(m1.log.clean)

outlierTest(m1.log.clean)


joint_tests(m1.log.clean)# Anova----



cod.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")

cod.emm


plot(cod.emm, comparisons = T)
```

```
##########################################################################################
#######

#total P---------

dt_Total_P <- subset(dt, response == "Total_P")
head(dt_Total_P)
xtabs(~ phase + train_drum, dt_Total_P)
xtabs(~phase+ sample_name, dt_Total_P) #samples nested within phases.

head(dt_Total_P)

#model-------

m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Total_P)
summary(m1)
joint_tests(m1)
outlierTest(m1)
resid_panel(m1)

m1.cleaned <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Total_P[-
c(237,225,249,250,201),])
resid_panel(m1.cleaned)

#logging the response---

m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Total_P)

joint_tests(m1.log)
```

```
resid_panel(m1.log)

outlierTest(m1.log)


m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Total_P[-c(249,240,201),])

resid_panel(m1.log.clean)

outlierTest(m1.log.clean)


joint_tests(m1.log.clean)# Anova----




Total_P.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")

Total_P.emm


plot(Total_P.emm, comparisons = T)


########################################################################################


#total N---------


dt_Total_N <- subset(dt, response == "Total_N")

head(dt_Total_N)

xtabs(~ phase + train_drum, dt_Total_N)

xtabs(~phase+ sample_name, dt_Total_N) #samples nested within phases.


head(dt_Total_N)


#model-------
```

```
m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Total_N)
summary(m1)
joint_tests(m1)
outlierTest(m1)
resid_panel(m1)

m1.cleaned <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Total_N[-c(213,224),])
resid_panel(m1.cleaned)

#logging the response---

m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Total_N)

joint_tests(m1.log)
resid_panel(m1.log)
outlierTest(m1.log)

m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Total_N[-
c(201),])
resid_panel(m1.log.clean)
outlierTest(m1.log.clean)

joint_tests(m1.log.clean)# Anova----

Total_N.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")
Total_N.emm
```

```
plot(Total_N.emm, comparisons = T)

##########################################################################################

#Nitrate---------

dt_Nitrate <- subset(dt, response == "Nitrate")
head(dt_Nitrate)
xtabs(~ phase + train_drum, dt_Nitrate)
xtabs(~phase+ sample_name, dt_Nitrate) #samples nested within phases.

head(dt_Nitrate)

#model-------

m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Nitrate)
summary(m1)
joint_tests(m1)
outlierTest(m1)
resid_panel(m1)

m1.cleaned <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Nitrate[-c(223,173,140,139),])
resid_panel(m1.cleaned)

#logging the response---
```

```
m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Nitrate)

joint_tests(m1.log)
resid_panel(m1.log)
outlierTest(m1.log)

m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Nitrate[-
c(173,139),])
resid_panel(m1.log.clean)
outlierTest(m1.log.clean)

joint_tests(m1.log.clean)# Anova----


Nitrate.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")
Nitrate.emm

plot(Nitrate.emm, comparisons = T)

##################################################################################
######
##################################################################################
########


#Nitrite---------

dt_Nitrite <- subset(dt, response == "Nitrite")
head(dt_Nitrite)
```

```
xtabs(~ phase + train_drum, dt_Nitrite)

xtabs(~phase+ sample_name, dt_Nitrite) #samples nested within phases.


head(dt_Nitrite)


#model-------


m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Nitrite)

summary(m1)

joint_tests(m1)

outlierTest(m1)

resid_panel(m1)



#logging the response---


m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Nitrite)


joint_tests(m1.log)

resid_panel(m1.log)

outlierTest(m1.log)


m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Nitrite[-
c(78,142,116),])

resid_panel(m1.log.clean)

outlierTest(m1.log.clean)


joint_tests(m1.log.clean)# Anova----
```

```
Nitrite.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")
Nitrite.emm

plot(Nitrite.emm, comparisons = T)

########################################################################################
################

########################################################################################
#########

#---------

dt_Ammonia <- subset(dt, response == "Ammonia")
head(dt_Ammonia)
xtabs(~ phase + train_drum, dt_Ammonia)
xtabs(~phase+ sample_name, dt_Ammonia) #samples nested within phases.

head(dt_Ammonia)

#model-------

m1 <- lmer(value ~ phase * train_drum + (1|sample_name), dt_Ammonia)
summary(m1)
joint_tests(m1)
outlierTest(m1)
resid_panel(m1)
```

```
#logging the response---

m1.log <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Ammonia)

joint_tests(m1.log)
resid_panel(m1.log)
outlierTest(m1.log)

m1.log.clean <- lmer(log1p(value) ~ phase * train_drum + (1|sample_name), dt_Ammonia[-
c(225),])
resid_panel(m1.log.clean)
outlierTest(m1.log.clean)

joint_tests(m1.log.clean)# Anova----

Ammonia.emm<- emmeans(m1.log.clean, ~train_drum|phase, type = "response")
Ammonia.emm

plot(Ammonia.emm, comparisons = T)
```

\`\`\`

APPENDIX 8: VISUAL REPRESENTATIONS OF STATISTICAL DATA

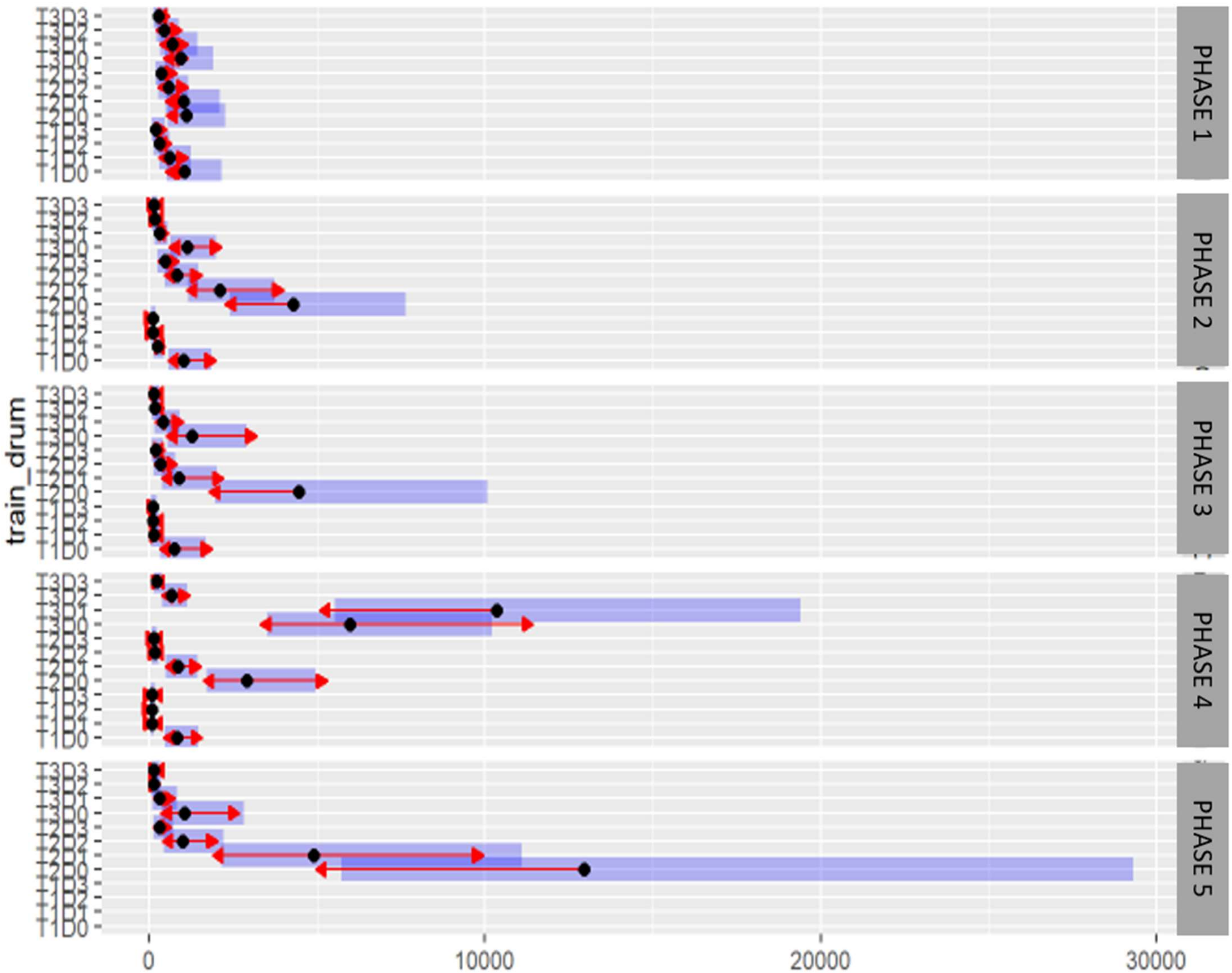

Figure 41: COD Visual Results. x-axis is concentration, y-axis is the location of the samples.

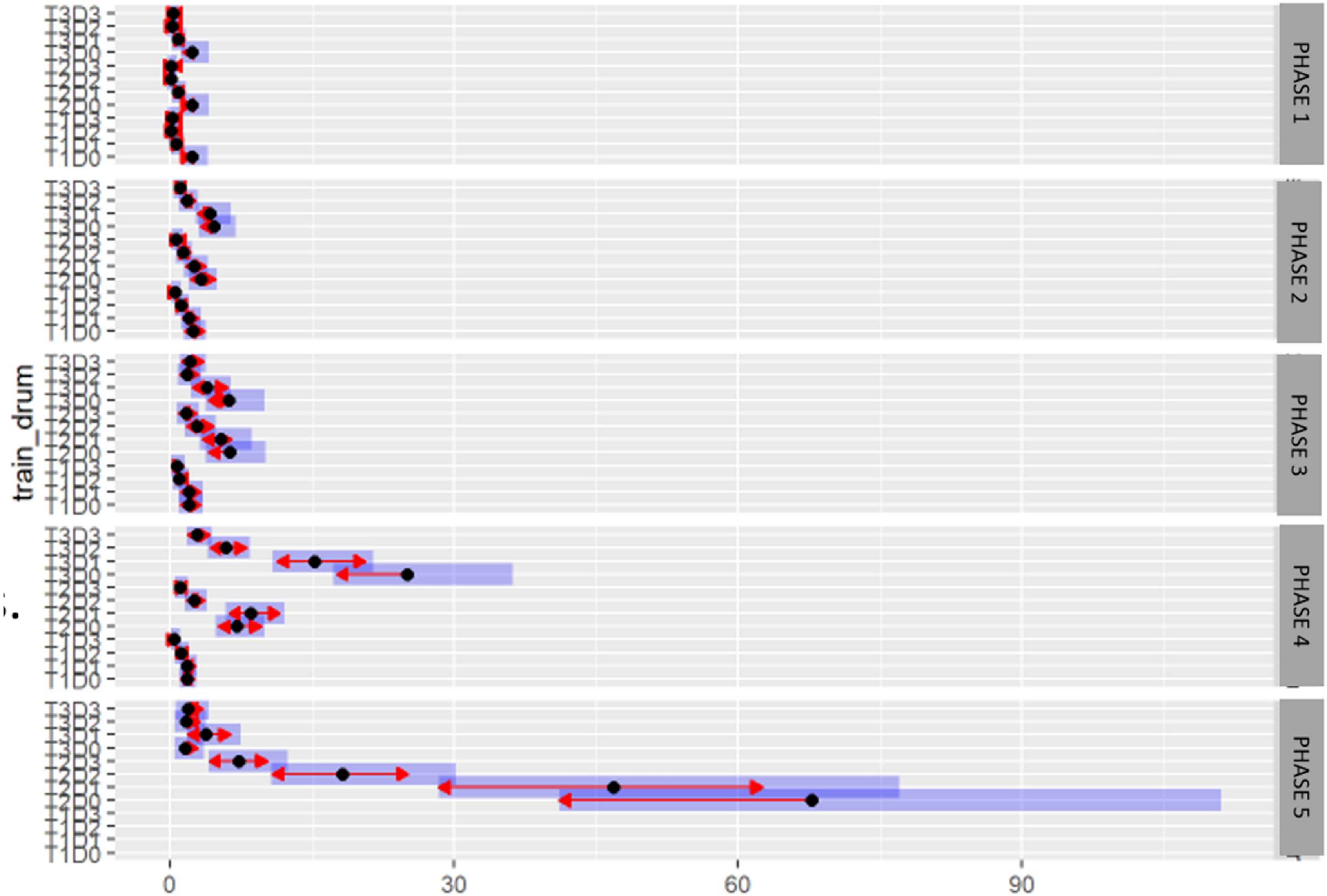

Figure 42 Total Phosphorus Visual Results. x-axis is concentration, y-axis is the location of the samples.

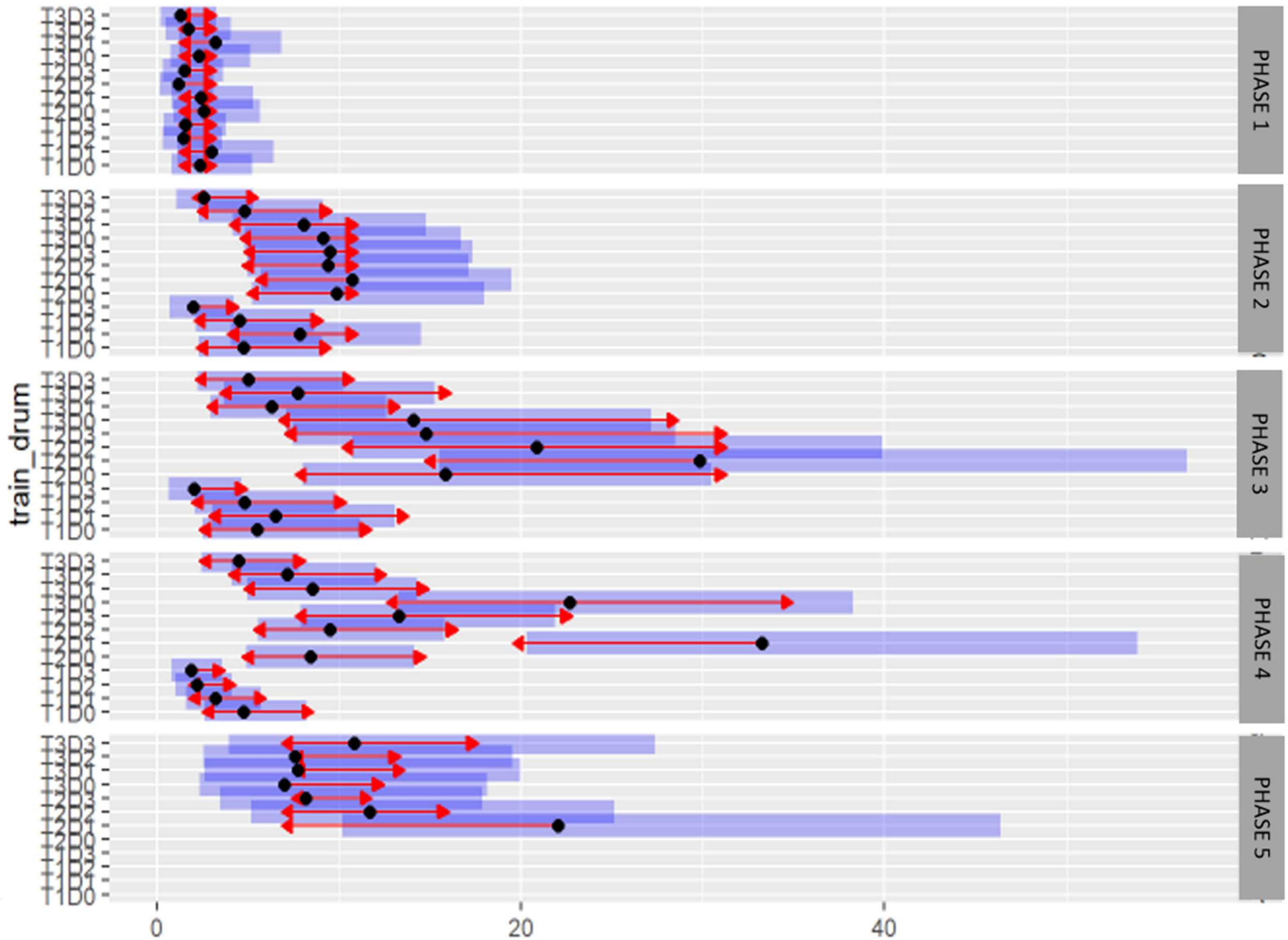

Figure 43 Total Nitrogen Visual Results. x-axis is concentration, y-axis is the location of the samples.

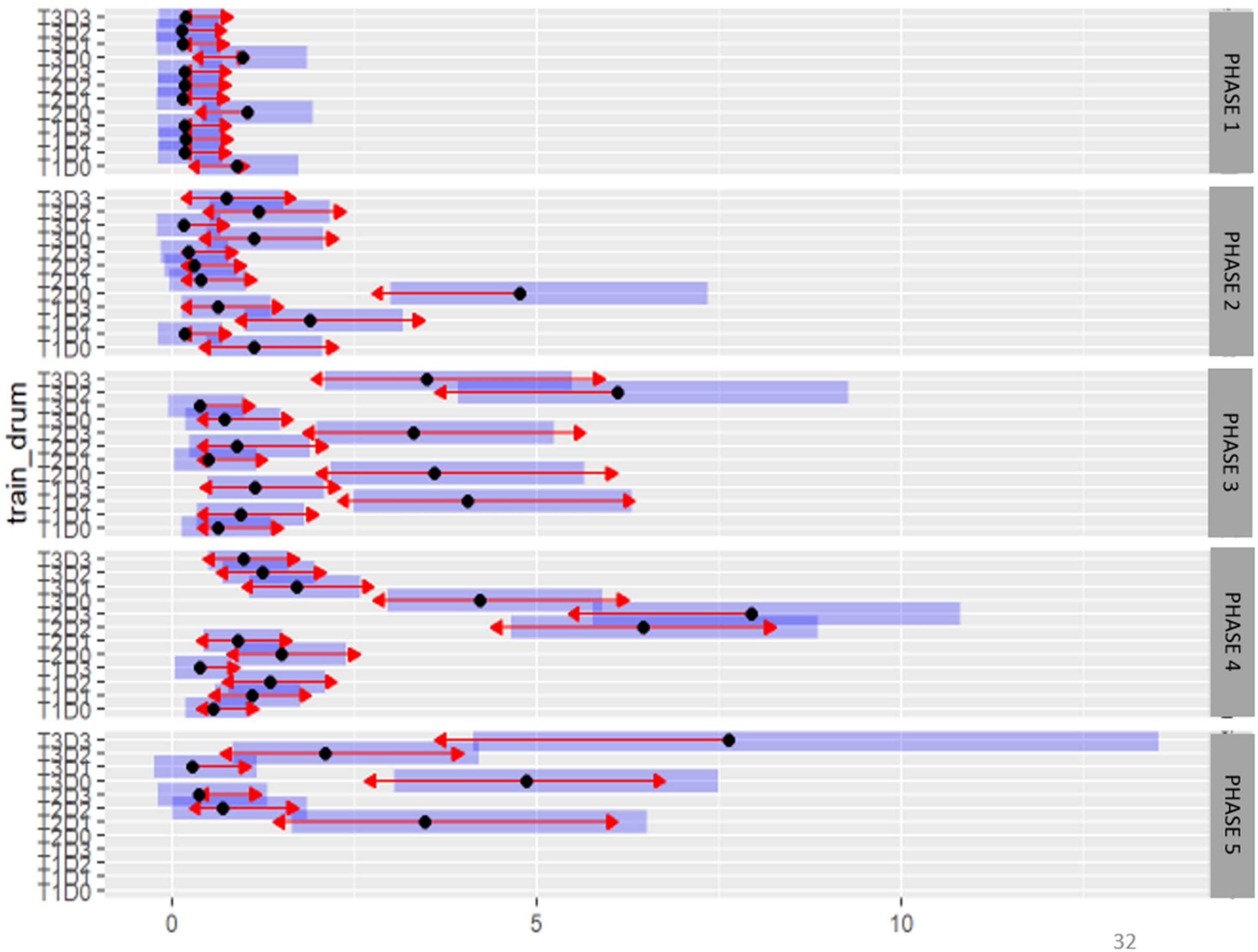

Figure 44: Nitrate Visual Results. x-axis is concentration, y-axis is the location of the samples.

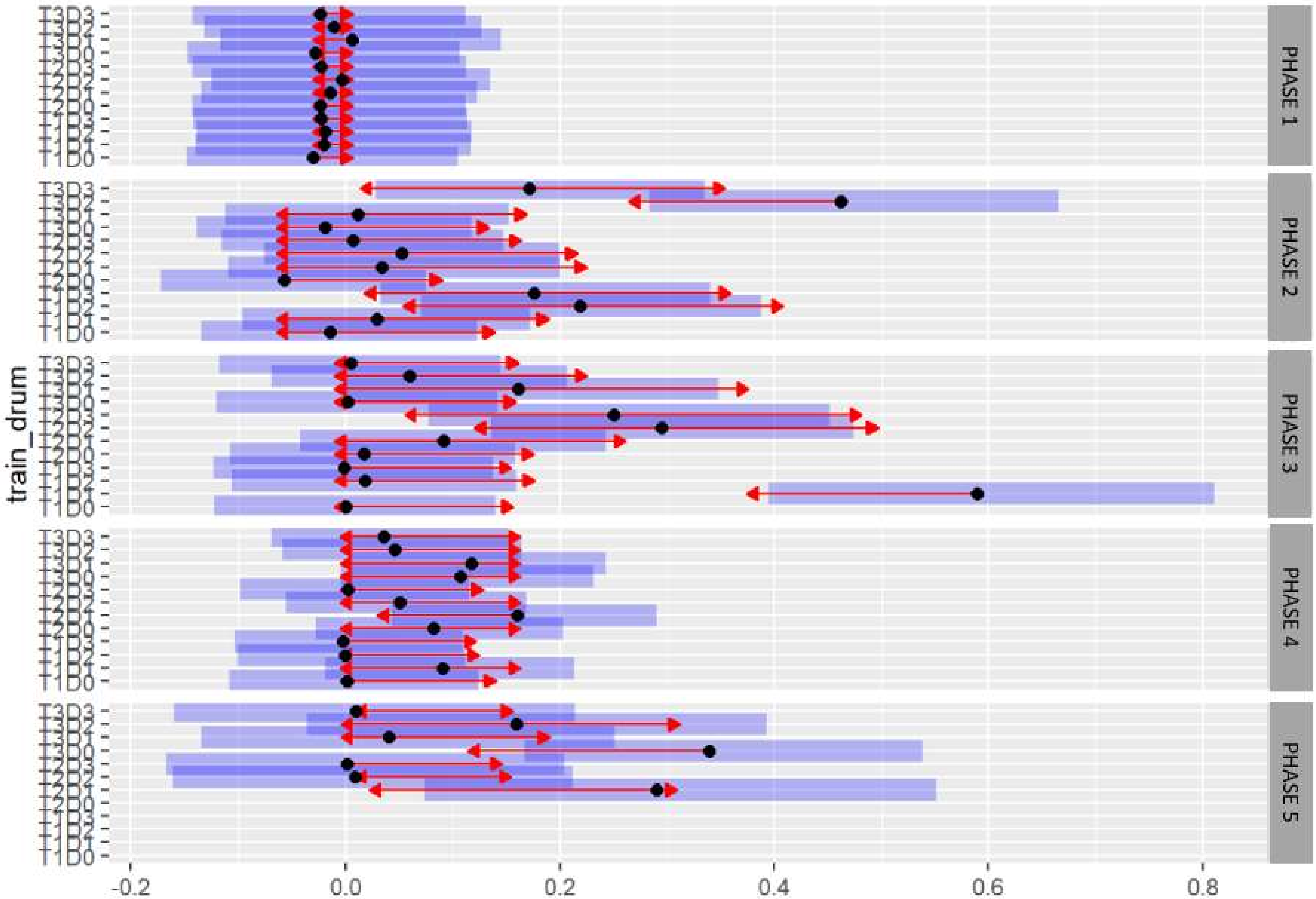

Figure 45: Nitrite Visual Results. x-axis is concentration, y-axis is the location of the samples.

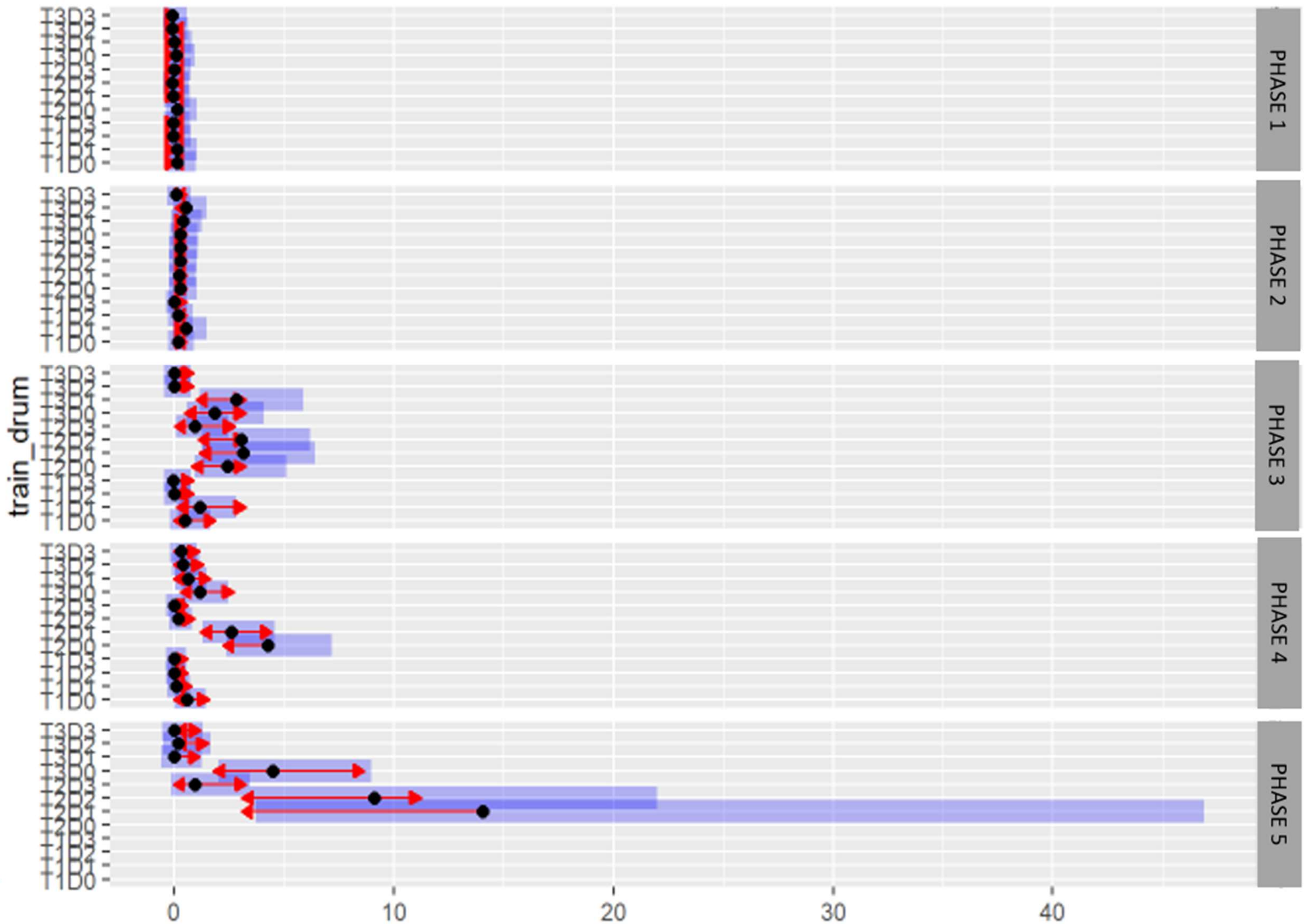

Figure 46: Ammonia Visual Results. x-axis is concentration, y-axis is the location of the samples.