Denitrifying Attached Growth Bioreactor Engineering Science Capstone Design Project

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Chicago Zoological Society — Inspiring Conservation Leadership

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Abstract

The mission of the Chicago Zoological Society (CZS) is to inspire conservation leadership by connecting people with wildlife and nature. As environmental engineering students attending Loyola University Chicago, we abide by a similar set of values devoted to achieving social justice by addressing current environmental issues to improve the community around us. The Brookfield Zoo (BZ) has been experiencing algal growth in the Great Bear Wilderness (GBW) exhibit pools. To decrease the accumulation of algae, BZ employees currently drain, scrub, and refill all three pools approximately every six weeks. To keep up with the rate of algal growth, each pool receives the manual cleaning treatment every two weeks. This current cleaning process is time consuming, labor intensive, and costly for BZ. Any efforts to increase water conservation would more closely align the maintenance process with BZ's mission of environmental sustainability. Overall, the two main problems caused by the algal growth and its subsequent cleaning process are undesirable aesthetics and poor water conservation practices.

We are proposing an attached growth biological treatment process to reduce algal growth in the GBW exhibit pools at BZ. This system will operate with the addition of a bioreactor to the current wastewater treatment system. The bioreactor will contain porous media that provides high surface area for biofilm to attach and grow on. The ratio of surface area on the media compared to the pool walls will encourage biofilm to grow within the reactor at a higher rate than in the pools and the biofilm growing inside will uptake nutrients that the algae feeds on. The accumulation of algae on the pool walls will decrease, thus reducing the frequency of pool draining, scrubbing, and refilling.

Introduction

- Original goal:
  - Eliminate the appearance of algae in BZ's GBW exhibit tanks.
  - Reduce the frequency of cleaning and draining pools.
- Steps:
  - We researched the possible processes to achieve algae removal.
    - Physical, chemical, and biological treatment processes.
    - Considering BZ's commitment to conservation and environmental justice, we decided that biological treatment was the best process.
  - The goal of biological treatment is to naturally reduce the concentration of organic and inorganic compounds.
    - In our case, biological treatment aids in reducing the concentration of nutrients that fuel algal growth, such as phosphorus and nitrogen.
  - One type of biological treatment that we found to fit the goal of our project was an attached growth bioreactor.
    - BZ's GBW exhibit water cycles through their own wastewater treatment system, including physical screens for coarse particles, sand filters for fine particles, and ozone for disinfection.
    - We have the space within their wastewater treatment system to add our bioreactor for biological treatment.
    - In attached growth biological treatment, microorganisms attach to packing material to create a biofilm which removes organic material and nutrients.
  - The media used in our design is called a bioball, which has a high surface area to volume ratio.
    - High surface area of the bioballs provide a greater space for biofilm to accumulate on which slows algal growth within the tanks.
    - The media is placed in a bioreactor within the wastewater stream to strip the water of nutrients and hinder algal growth.
  - As explained in greater detail in the methods section, we experimented with two types of biofilm, bacterial and algal.
    - Due to its ability to attach to our bioreactor better and reduce nutrients, the algal biofilm was determined superior to the bacterial biofilm.
  - We determined further specifications of our design through calculations and observations in experiments.

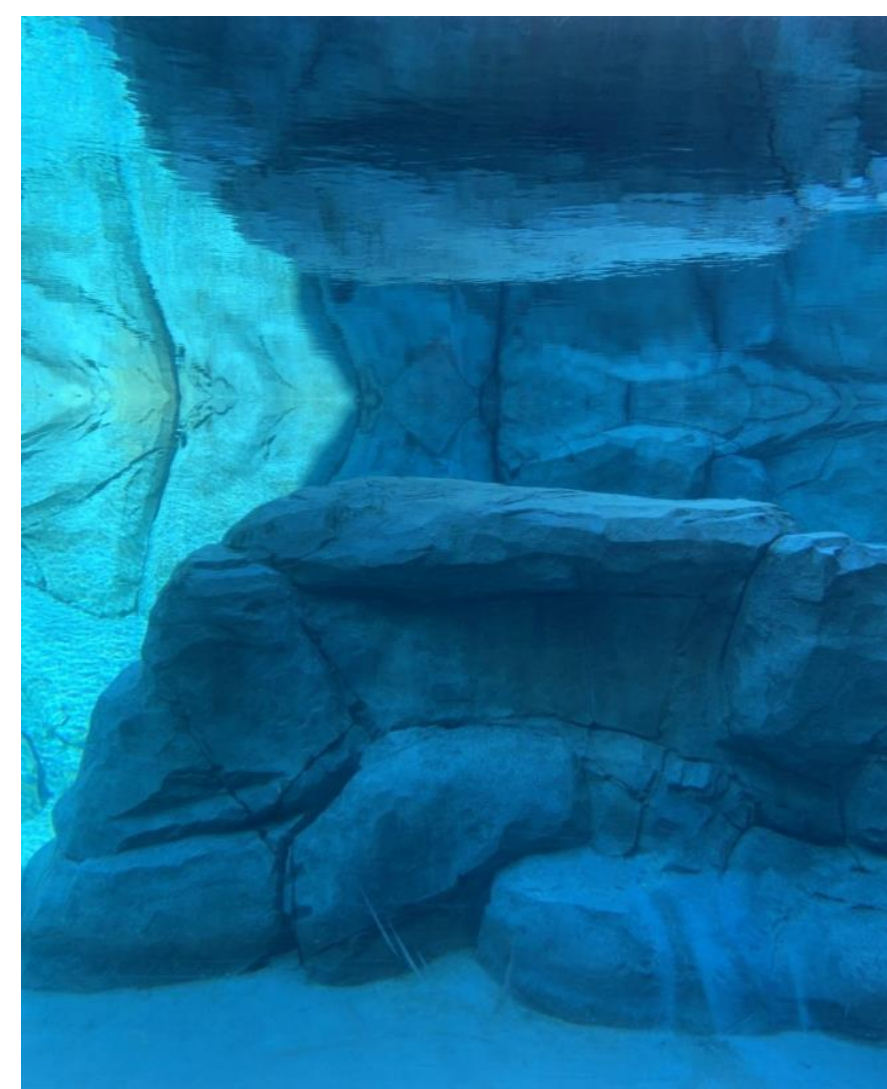Figure 1: This is a figure showing one of the GBW Exhibit tanks after several weeks without a cleaning.

Figure 2: This is a figure showing one of the GBW Exhibit tanks a few days after a physical cleaning.

It's Algae...Naturally!
That green growth in the bears' underwater pool is algae, a natural part of an underwater habitat's "biofilm" (a thin layer of organisms that coat a surface). By not using chemicals to clean this pool, we are being environmentally friendly and offering the bears a space that better simulates their habitat in the wild.
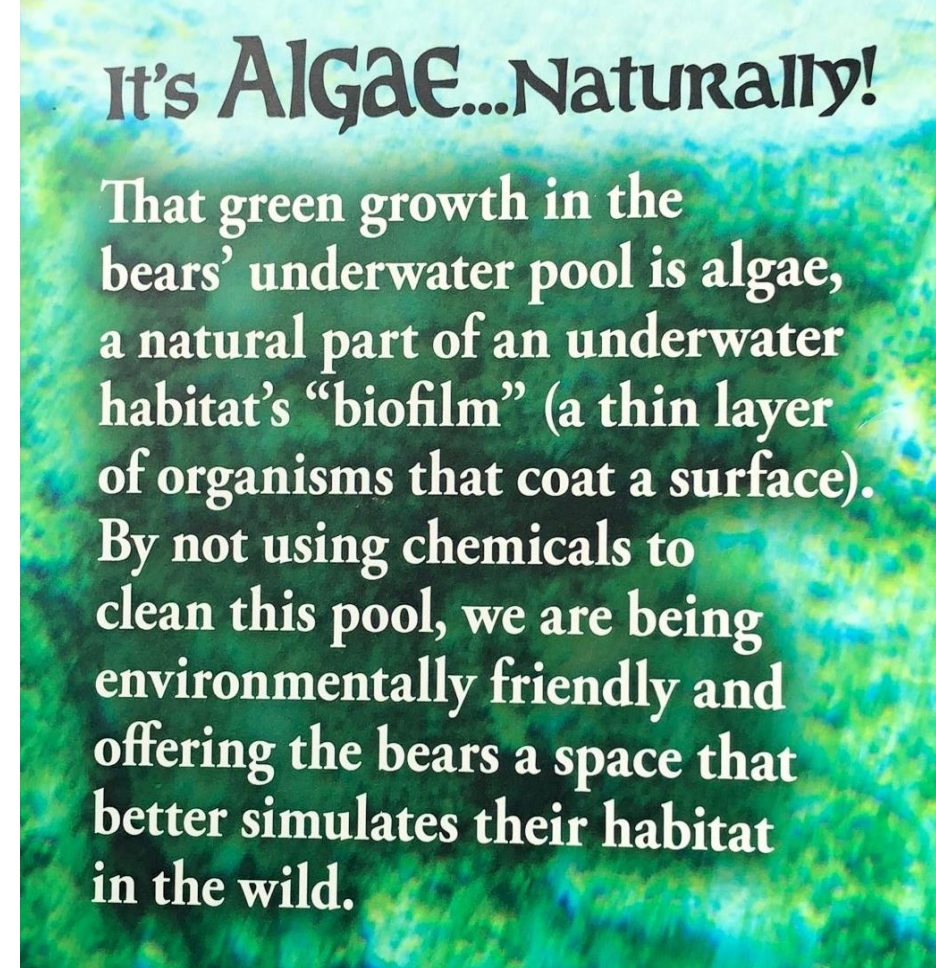
Figure 3: This is an example of a sign BZ put up in the GBW Exhibit to inform visitors of the natural occurrence of algae.
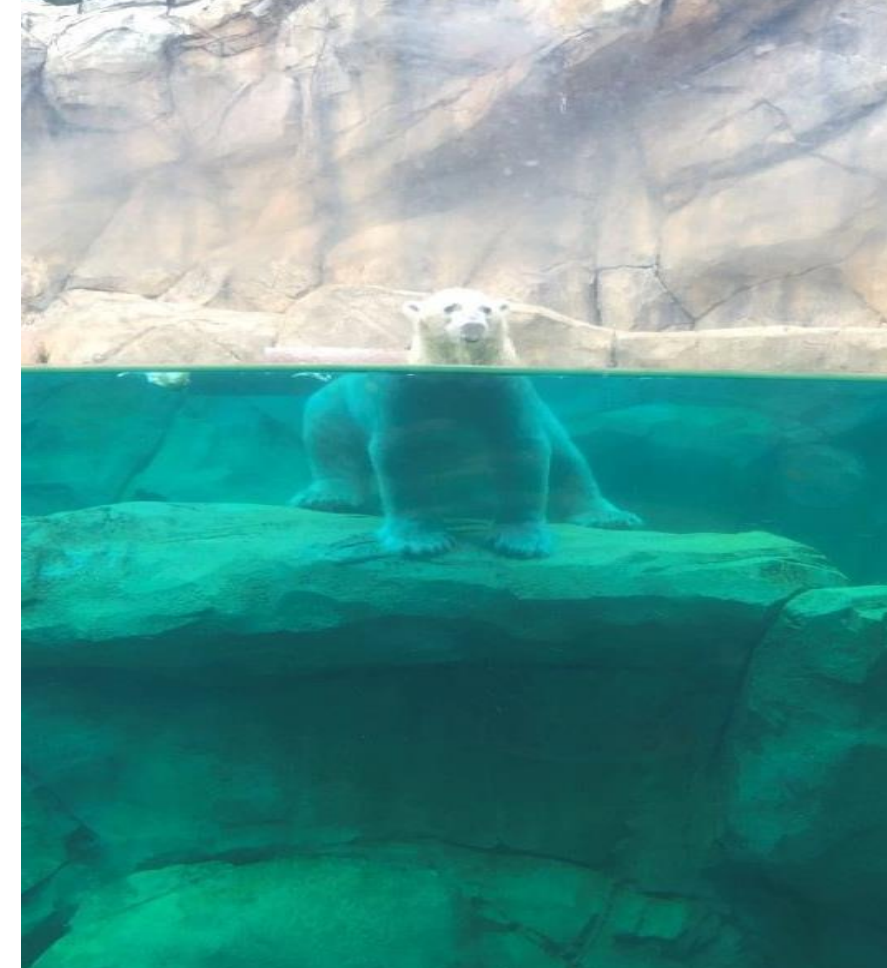

Figure 4: Above is a picture showing a polar bear swimming in a GBW Exhibit pool which hasn't been cleaned in a few weeks.
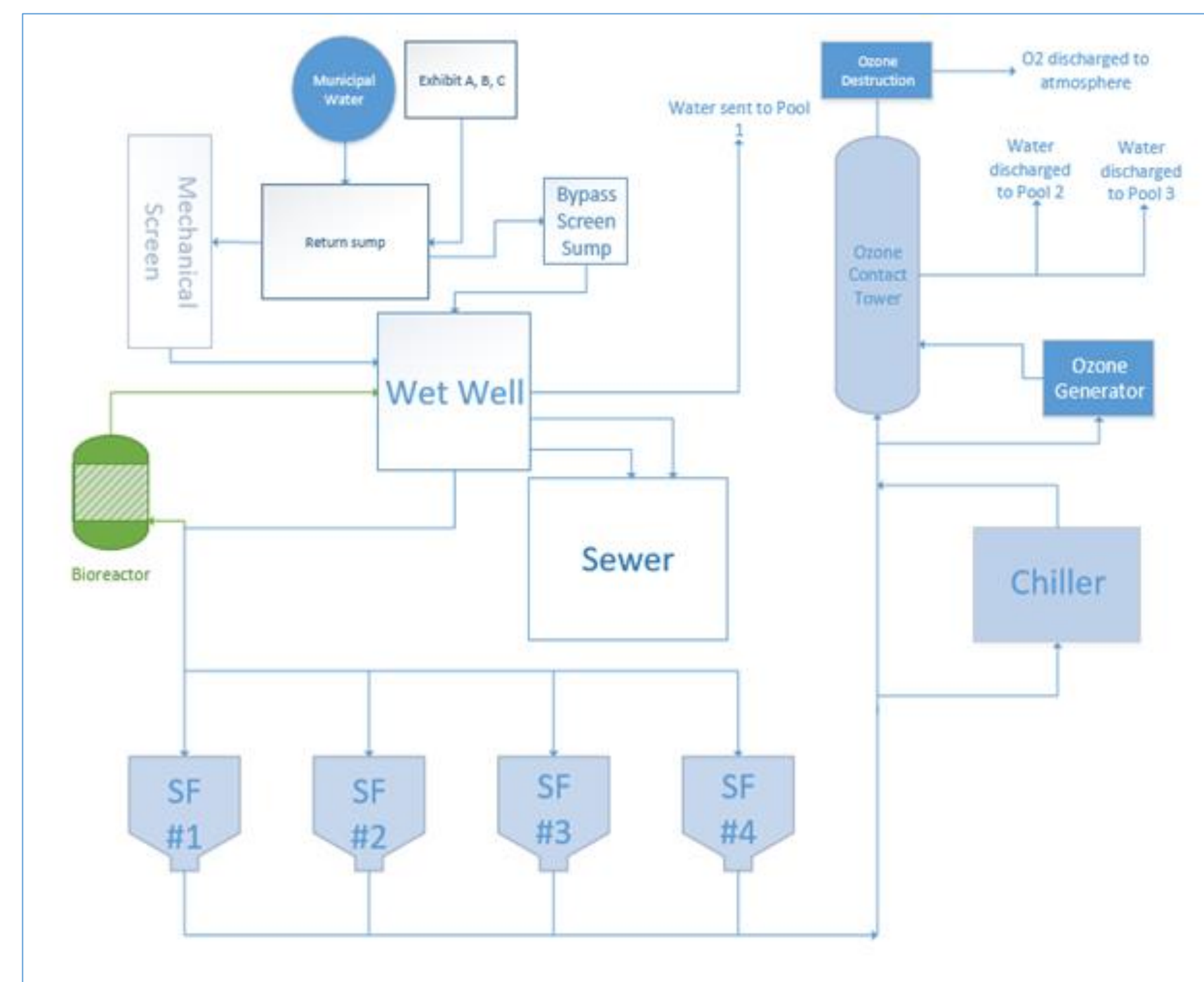
(Center figures)

Figure 5: This is a system diagram highlighting the placement of the bioreactor in the GBW wastewater treatment system. Some of the water will enter the bioreactor from the wet well, while the rest will go straight to the sand filters (SF) for physical treatment.
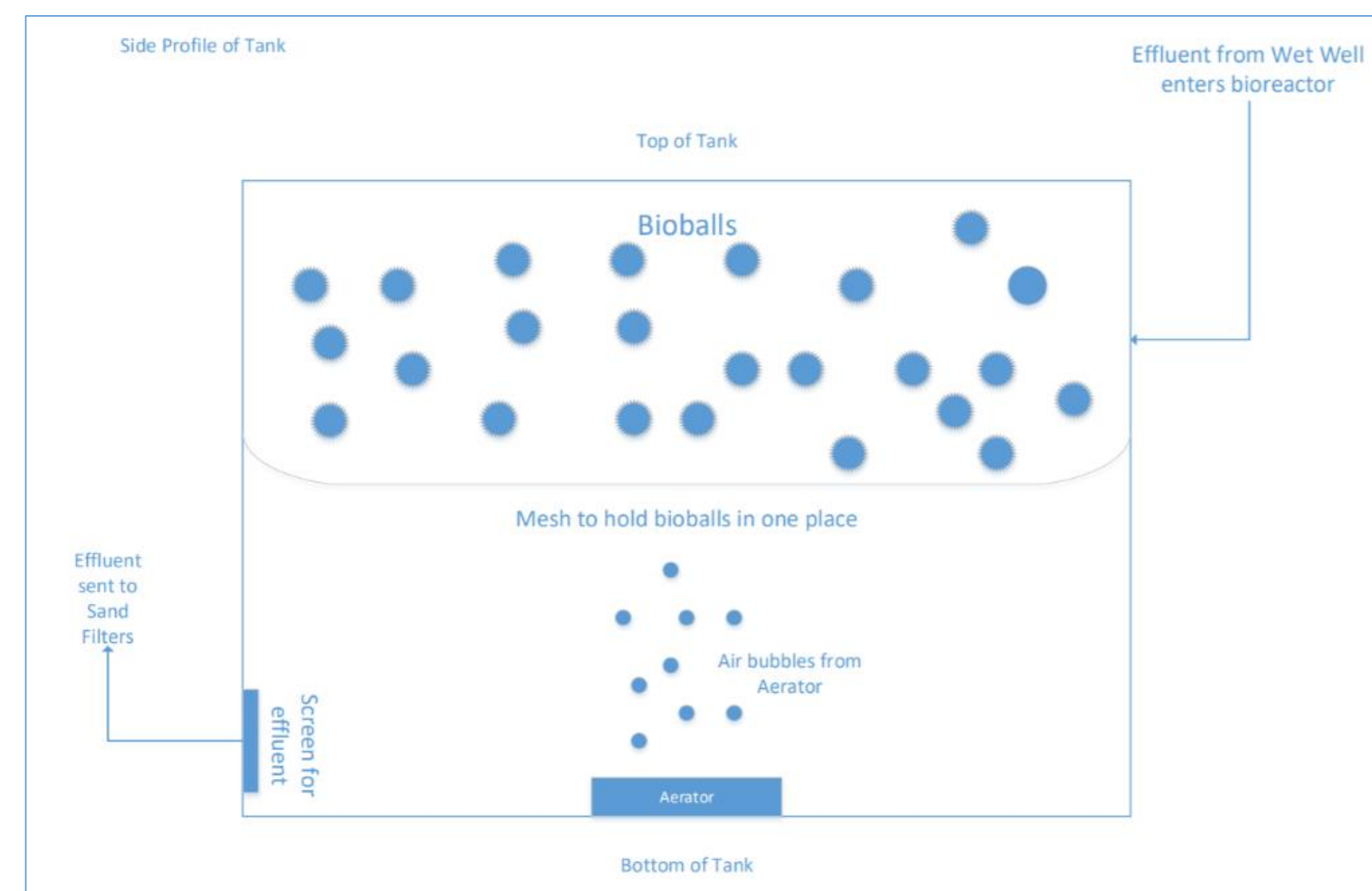

Figure 6: This is a system diagram of the bioreactor. It shows the placement of media in the mesh bag and the direction of flow of the wastewater to be treated.

Methods

- While designing our system, we had to keep in mind some environmental, engineering, regulatory, and financial constraints.
  - Our design needed to fit within the existing wastewater treatment system at BZ.
  - The water quality was subject to any weather conditions surrounding BZ, including sunlight, temperature, precipitation and humidity.
  - When BZ refills the pools, they utilize the City of Chicago's municipal water, so our system is subject to its nutrient concentrations and water quality.
- To select the design, our team filled out a morph matrix.
  - We first outlined several functions our design must accomplish.
  - For each function, we drew upon our research to determine the best methods.
  - Once we figured out the options for each function, we chose to create and focus on three designs.
    - The designs included the current cleaning process, an attached biological growth treatment process, and a chemical flocculant process.
- Next, we began creating a Pugh matrix.
  - After deliberating what is important to both our sponsors at BZ and our team, we chose twelve criteria our design must meet.
    - These criteria include aesthetics, frequency of cleaning pools, water conservation, animal risks, operational cost, maintenance of the system, longevity, capital cost, seasonal adaptability, footprint, training cost, and resource recovery.
  - Each team member then individually weighted how important these criteria are to the design.
  - The highest scoring design was the attached growth bioreactor.
- Once we determined the attached growth bioreactor was the best option, we created an experimentation protocol for our first round of experiments.
  - After researching types of biofilms, we found that Pseudomonas fluorescens was a good option for denitrification.
  - In a 1000 mL Erlenmeyer flask, we seeded the water with a sample of the bacteria along with a nutrient broth.
  - We observed the growth over the next several weeks.
- Around the same time, we used a Chicago native algae sample along with Bristol medium solution to grow an algal biofilm to compare the bacterial biofilm with.
- Once the biofilms grew, we moved each to two different 10-gallon tanks with the bioballs and aeration stones.
  - In only the algal biofilm tanks, we placed grow lights overhead to aid growth and wrapped tin foil around the sides of the tanks to help distribute the light evenly throughout.
  - Every two days, we sampled each of the four 10-gallon tanks to determine any nutrient reduction.
  - We used a spectrophotometer to measure absorbances of nitrates and phosphates to determine a concentration curve for our samples.
  - From this data and other observational analyses, we decided that the algal biofilm was most efficient for our design purposes.
- For nine weeks, we placed a mesh bag filled with bioballs into the GBW wastewater treatment stream.
  - We transported this makeshift bioreactor to a 10-gallon tank filled with water from BZ.
  - We took a couple measurements of nutrient concentrations before our experimentation was unexpectedly halted by the closing of our university and of BZ.
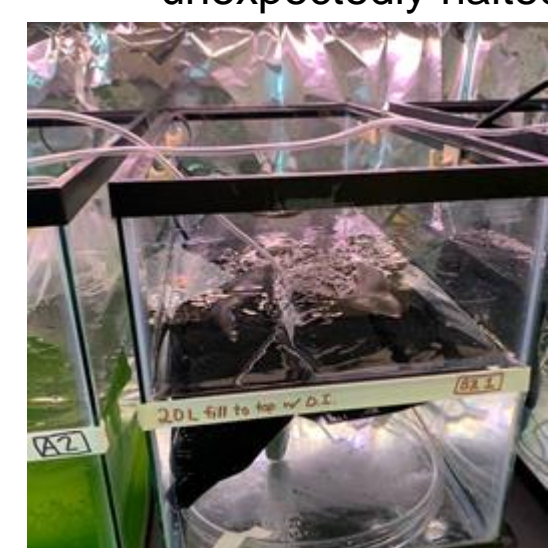

Figure 7: This is an image of an algae experiment tank containing a mesh bag of bioballs and aeration stones before the algae was introduced.
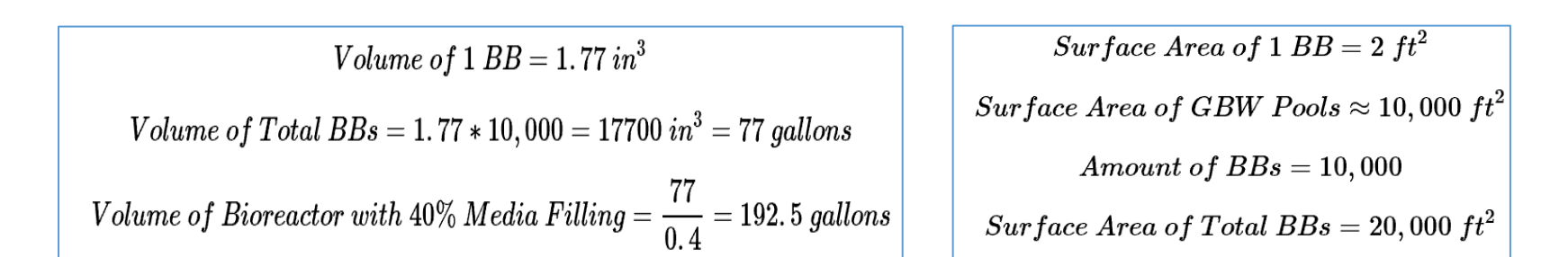

Figure 8: This is an image of a bioball with attached growth of algae after the experiment ran.


Figure 9: This is an image of an algae tank during the experiment. You can see that algae is growing on the bioballs and mesh bag they are contained in.

Results

- For our design to work, our bioreactor needs to have a higher surface area than the GBW exhibits. The surface area of the exhibit pools reaches about 10,000 sq. ft. Since each bioball (BB) is 2 sq. ft. we recommend using 10,000 BBs to double the surface area available.
- Each BB has a volume of 1.77 square inches. We were able to calculate the volume the BB would take up by multiplying it by the surface area of the exhibits. Since we want the BBs to fill 40% of the reactor, dividing the volume by that percentage gives us a reactor that needs a volume of almost 200 gallons.
- After collecting the data from our experiments, we observed that nitrate and phosphate concentrations went down in our algae experiments. Notice, however; that the phosphate fluctuates periodically. This can be attributed to the limited surface area in our experiments, as algae grew considerably, which could have left some to die and decompose. Decomposition produces phosphate, but this will be avoided in a larger scale experiment as there will be more surface area in the bioreactor for algae to grow.
- The same observations can be made for the bacteria biofilm. The nitrates significantly went down as well, whereas the phosphates stayed relatively the same. We believe the bacteria was able to efficiently decrease nutrient uptake, but the environment is not suited to sustain bacterial growth.
- Since algae did a good job at decreasing the nutrients, as well as had observable attached growth, we decided an algal biofilm would be best suited for our bioreactor.
- To calculate the concentrations of the nutrients in our experiments, a calibration curve was needed. The curve was made using nitrate and phosphate stock solutions of 4.8e-4 M and 2.6e-5 M respectively. Prior to data collection, we were able to determine the concentration of these solutions (29.76 mg/L of NO3 and 2.47 mg/L of PO4). This calculation is necessary as it gives us the high-end value to our calibration curve.
- Once the absorbances were measured, they were graphed in relation to the amount of stock solution that was in the dilutions. The higher the concentration of stock solution, the higher the absorbance.
- After the calibration curve is made, a best fit line gives us an equation that we can use to plug in any absorbance and measure the concentration of the solution. We calculated the concentration by rearranging the equation given. The y in our equation will be the absorbance readings from our samples and the x will be the concentration. So, for example, if we got a reading of 1.25 absorbance for phosphate, the equation would look like x = (1.25)/0.5614 which gives us a concentration of 2.22 mg/L.

$$Volume\ of\ 1\ BB = 1.77\ in^2$$
$$Volume\ of\ Total\ BBs = 1.77*10,000 = 17700\ in^3 = 77\ gallons$$
$$Volume\ of\ Bioreactor\ with\ 40\%\ Media\ Filling = \frac{77}{0.4} = 192.5\ gallons$$

$$Surface\ Area\ of\ 1\ BB = 2\ ft^2$$
$$Surface\ Area\ of\ GBW\ Pools \approx 10,000\ ft^2$$
$$Amount\ of\ BBs = 10,000$$
$$Surface\ Area\ of\ Total\ BBs = 20,000\ ft^2$$

Figures 10 and 11: Surface area and volume calculations for bioballs and bioreactor
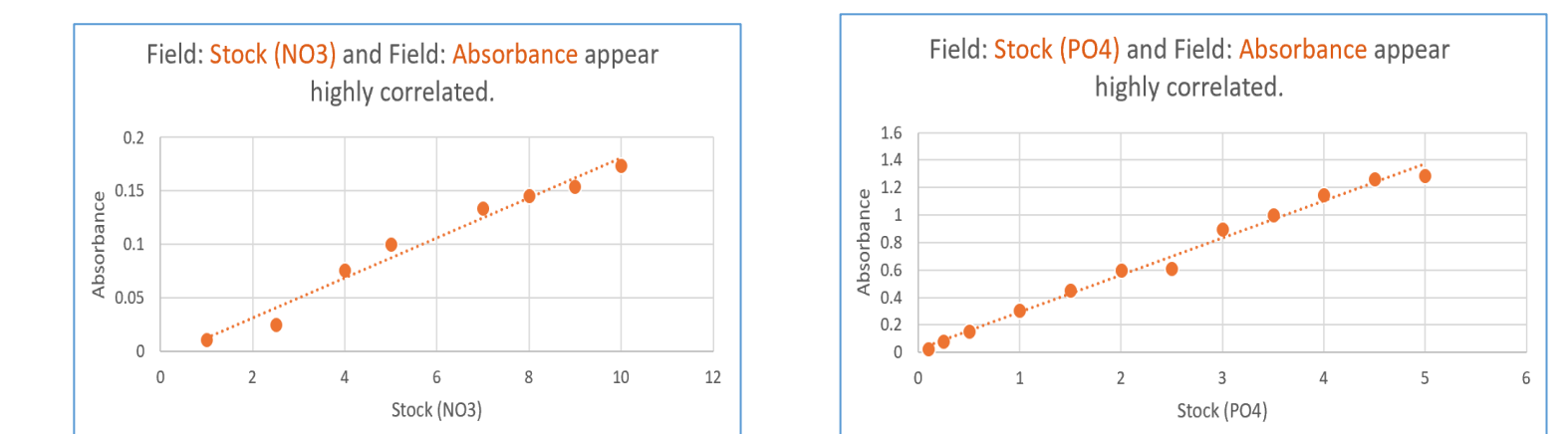
Figures 12 and 13: Both graphs depict the relationship between absorbance and mL of stock solution mixed with the reagent powder. As the equation for the trendline is made, we can calculate concentration based on absorbance.
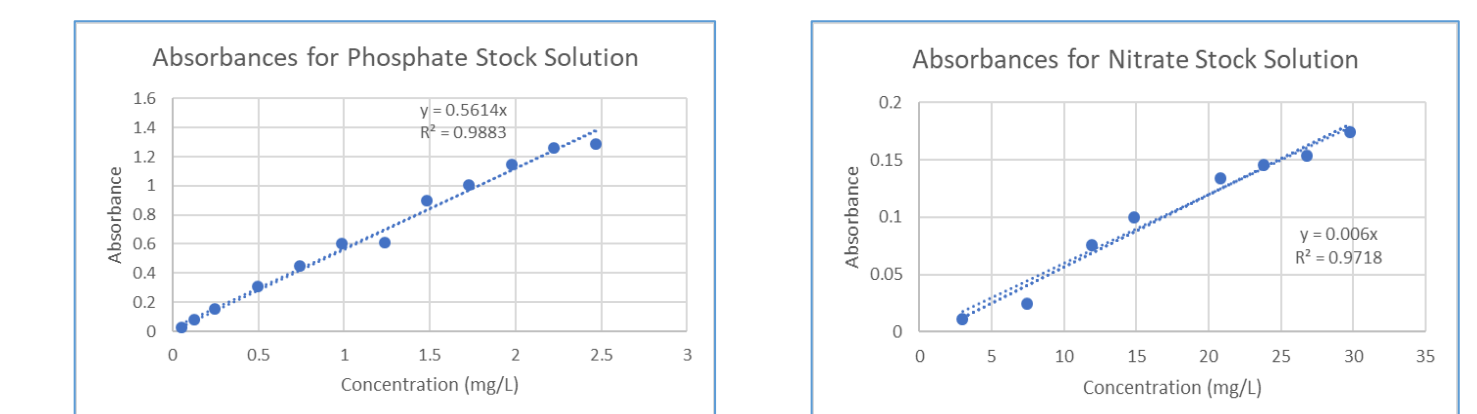
Figures 14 and 15: These lines depict the correlation between absorbance of the nutrients and the concentration it's associated with. This line can only be made because of the stock solution concentration calculations.
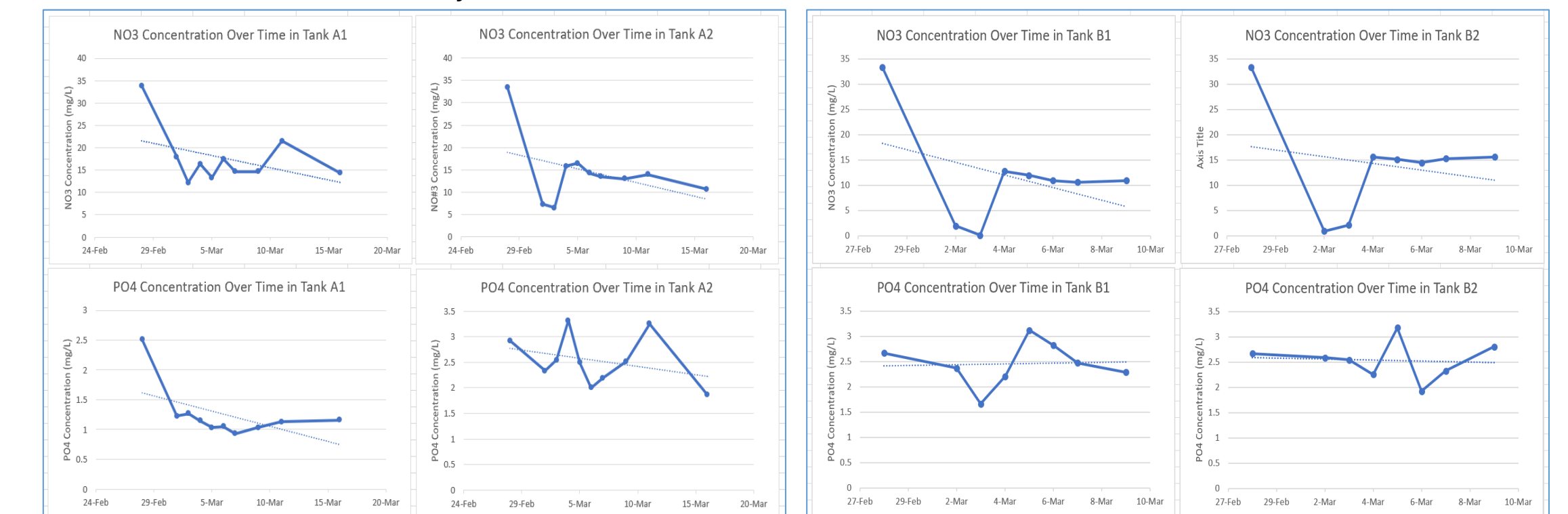
Figures 16 and 17: Depicted above is the trend over time of nutrients in the experiment tanks, algae and bacteria, respectively. The data shows that over time, both algae and bacteria were efficient in lowering nitrate counts.

Discussion

- Even though our experimentation was interrupted by closures of BZ and our university due to the COVID-19 pandemic, we still believe that our recommended design is effective in reducing algal accumulation and frequency of cleaning and draining pools according to prior data collected.
- We suggest that on the pilot round of testing, BZ continue our experimentation on a larger scale by placing bioballs in the wet well to observe reduction of algal growth and nutrient removal in GBW tanks.
- Maintenance of the bioreactor will be required as needed to ensure efficiency of nutrient removal.
  - Our design is an extractable bioreactor in which maintenance staff can remove the media from the bioreactor to clean and replace it easily.
  - Over time, biofilm will accumulate and eventually cover the surface area of the media.
  - As a result, periodic cleaning will be required to unclog the media by rinsing it with water to remove excess biofilm.
  - The entire biofilm should not be removed when cleaning, as leftover microbes from the biofilm will promote regrowth on the media to sustain the biological treatment process.

References & Acknowledgements

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