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Nitrification Processes and Conversion Kinetics for an Aquatic Model System

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Abstract

Aquaculture, or aqua-farming, refers to the artificial cultivation of aquatic organisms. Aquaculture could conceivably replace fisheries--which rely on natural fish populations--as a food source. This practice has the potential to alleviate the stress currently induced by over-harvesting and damming, which reduce wild fish populations. The recirculating aquaculture system we studied at the UW-Milwaukee School of Freshwater Sciences consists of three main chambers. The primary tank contains a population of yellow perch that produce ammonium as a toxic waste product. This ammonium is filtered and recycled by chemolithotrophic bacteria present in the system's two biofilters. The first filter, containing beads, is responsible for solid waste removal. Ammonium and nitrite, which can reach relative toxicity levels, leave this filter and are further neutralized into nitrate by two types of bacteria in the biofilter. Since nitrate is not toxic to fish at relatively high levels, this water is then pumped back into the initial holding tank. The mathematical model we are developing is designed to simulate the nitrogen limitations in a mature recirculating biofilter, which is the main site of nitrogen fixation. Parameters in the model were determined using a combination of roller bottle experiments and real-time spikes of the RAS.

Keywords: Recirculating Aquaculture System, Biofilter, Nitrification, Model

1. Introduction

Traditional methods of harvesting seafood disrupt natural ecosystems by threatening fish and general wildlife populations and emitting high levels of greenhouse gases[1]. Aquaculture systems provide an alternative food source with less of an impact on the natural environment[2]. Specifically, recirculating aquaculture systems (RAS) can offer an efficient, more sustainable option by which to raise fish for consumption[3]. These recycle the water flowing through the system, decreasing the required net water input. Certain purification technologies have allowed for as high as 99% water recycling[4].

At the University of Wisconsin Milwaukee's School of Freshwater Science, a large RAS has been in operation since 1999. This system pumps freshwater at an average of 757 L/min through several tanks[4]. A fluidized sand biofilter helps maintain a non-toxic environment in the main holding tank in which 5,000 Yellow Perch (*Perca flavescens*) can be grown at a time[4]. Since the water contains nutrients, such as ammonium from fish waste, biofilters are a crucial component of RASs to prevent accumulation to levels toxic to the fish.

The RAS examined consisted first of a bead biofilter (2,260 L) for solid waste removal, and second of a fluidized sand biofilter (5,350 L) in which sand serves as the media for nitrifying bacterial growth (Figure 1). These bacteria are generally considered responsible for the stepwise conversion of ammonium into nitrite (Nitrosomonas spp.) and

nitrite into nitrate (Nitrobacter spp.), among other minor species[5]. Nitrate is relatively non-toxic to the fish[6], and effluent from the biofilter can safely be pumped back into the main holding tank (15,000 L).

Fish from RASs are periodically harvested for consumption. The system's ammonium content is greatly reduced by the lack of fish waste, which serves as the primary food source for the biofilter. In the present study, perch from the RAS had been harvested leaving an empty tank. This allowed for a closer look at the biogeochemical processes – specifically N-cycle processes – occurring in the system. Experimentation on both the large RAS and benchtop roller bottles provided for a closer analysis of these processes and the recovery ability of the biofilter's community. How quickly a biofilter is able to respond to increased levels of ammonium has practical relevance. Microbial dynamics change upon fish harvest. Subsequent bacterial starvation will impact how and when new fish stocks can be established without the threat of poisoning them due to lack of nitrification activity. Understanding just how starvation period length effects recovery time will provide important, operational insight. The goal was to apply experimental data to parameterize a mathematical model of nitrification kinetics within the fluidized sand biofilter (Figure 1, site 3) of the RAS, which can ultimately serve as a reference for RAS developers and operators.

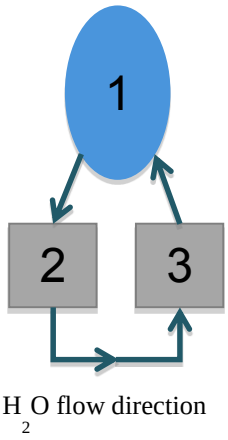

Figure 1. Simplified diagram of the RAS. Site 1 is the fish holding tank, Site 2 is the bead biofilter, and Site 3 represents the fluidized sand biofilter. Arrows convey water flow direction. Substations between major sites are not depicted.

2. Methods

Spiking the RAS with various concentrations of ammonium and nitrite and collecting subsequent samples to measure decay rates would have been impractical and costly. A smaller scaled approach using benchtop roller bottles was developed to mimic the nitrogen cycling processes that occur in the actual biofilter. The most extensive experimentation was performed using these roller bottle simulations. However, the RAS was empty during the time of experimentation, which allowed for direct sampling of the system and a verification of transformation rates as measured in the roller bottles. These transformation rates were used to parameterize a mathematical model of the RAS's biofilter.

2.1 Model Development

A general model was developed to describe the removal of ammonium and nitrite by two respective bacteria. Two model parameters—maximal growth/transformation rate (r_{max}) and half-saturation constant (k_N) of removal rate—were determined over the course of the experiment. We hypothesize that parameters estimated from roller bottle simulations would be similar to those measured in situ (i.e. in the RAS biofilter).

The following were considered during model development. Water is pumped upwards, against gravity through the biofilter. As a result, it becomes and remains well-mixed with the sand. The sand serves as a substrate for bacterial growth, which is thus limited by (i.e. reaches a carrying capacity) surface area availability. Bottles with water from the RAS but no sand were spiked separately with ammonium and nitrite as a control, and no net change in ammonium or nitrite was measured; as a result, nitrification in the fluid phase is considered negligible. Two types of bacteria—

ammonium oxidizing (AOB) and nitrite oxidizing (NOB)—are assumed to be primarily responsible for transformation of ammonium into nitrite and nitrite into nitrate, respectively. AOB and NOB compete for space, but do not have to compete for nutrients. The model consists of five equations to address the three forms of nitrogen present (i.e. ammonium, nitrite, and nitrate) and the nitrogen contained in bacterial biomass.

2.2 Kinetic Experiments In Bottles

Roller bottles were assembled using sand from the biofilter and water from the RAS. Sand was collected in June of 2015. Water was collected from the RAS after flowing through the biofilter (between sites 3 and 1 in Figure 1) once in June and once in early July of 2015.

The perch in the system had been harvested several months prior to experimentation, but the previously inhabited water remained to circulate through the system. The RAS was considered to be in "starvation" since no continuous ammonium addition from fish waste occurred to 'feed' the bacteria in the biofilter. After collection, the sand was rinsed with distilled water to remove any particulates, then aliquoted out in predetermined weights—10, 25, 50, 75, and 100 g—and placed in separate 1-liter bottles. Estimating the amount of sediment relative to water in the functioning recirculating system proved impractical, but the importance of that factor on the system's operational ability is unclear. These various treatments were thus selected to test the effect of different amounts of sediment to water on nutrient uptake. These bottles were filled with water from the RAS to a total volume of 1 liter. Sand to water ratios established in bottles were not considered relative to that of the large scale biofilter. When placed on benchtop rollers, the movement of water through the sand in the bottles simulated water's movement through the biofilter of the RAS. In order to observe the maximum uptake rates of individual bottles for each experiment, sample times varied based on expected rates from preliminary trial experiments. For example, a bottle with 250 g of sand and 25 µM ammonium was sampled every 10 minutes for six hours, then every four hours for 12 hours, In contrast, a bottle with the same amount of sand spiked with 250 µM ammonium was found to have a longer uptake time and steady rate of conversion, so samples were taken every 20 minutes for six hours, then every four hours for 12 hours. This allowed us to minimize amount of samples while still being able to observe conversion rates.

For the first experiment (varying sand), 500 µM ammonium were injected into bottles, each with a different amount of sand. The bottles were placed on rollers. During sample collection, bottles were removed from the rollers and approximately 30 mL of water was withdrawn using sterile syringes and filtered through 0.2 micron filters to remove particulates and inhibit further nitrification. This procedure was repeated using 1,000 µM nitrite and fresh sand and water. Time points were collected approximately every 15 minutes over two and a half hours.

For a second experiment (varying nutrient concentration), the entire experimental design was performed on bottles containing equal amounts of sand (250 g). Individual bottles were spiked with different concentrations of ammonia or nitrite—2, 5, 10, 25, 50, 100, 250, 500, 1,000, and 2,000 µM. Removal of ammonium or nitrite was monitored by measuring ammonium and nitrite concentrations using spectrophotometric methods[7].

This second experiment, including separate sand and water collection from the RAS, was repeated in January of 2016. Sand and water were extracted from the system as previously described. Fish had not been restocked; the RAS remained empty. To more accurately observe initial transformation rates, we hoped to decrease those initial rates by using less sand (100 g) per bottle, instead of the original 250 g.

Concentration data from both the varying sand and varying nutrient concentration experiments were used to determine rates of uptake and conversion of ammonium or nitrate. These data were then used to determine half-saturation constants (k_N) and maximum uptake rates (r_{max}) using non-linear fitting methods[8].

2.3 Whole System Experiment

A third experiment was performed in August of 2015. The system—void of fish—was spiked with two different concentrations of nitrite in order to compare the rate of nitrification of the true biofilter with the nitrification rates determined from roller bottle simulations. Nitrite was added to the system at the inflow to site 2 (Figure 1) in three separate instances: twice to reach a concentration of 20 µM in the RAS as a whole and once to reach 500 µM. Water samples were collected at multiple sites simultaneously. Initial concentration of nitrite at each of these sites were measured as T=0 prior to any nitrite addition. Time between collection points increased as nitrite levels plateaued or decreased to initial concentrations. Time intervals were determined in part by how long these concentrations were transformed in the roller bottle simulations. The addition of sulfanilamide to occasional time points and the resulting degree of color change—indicating the presence of nitrite—was also used to designate sample collection times.

3. Results

3.1 Developing The Model

The model is comprised of a system of ordinary differential equations. Parameters considered include volume (V), maximal growth/transformation rates (r_{max}), half-saturation constants of nitrogen transformations (k_N), carrying capacity of the sand substrate for bacterial growth (M), spatial competition characteristics (c), decay rates (d), proportion of nitrogen used by bacteria for growth (α). The first two–r_{max} and k_N–were determined by simulations and in situ experiments. The different stages of nitrogen are represented by N_1 (ammonium), N_2 (nitrite), and N_3 (nitrate). B_1 and B_2 symbolize the nitrogen content in the *Nitrosomonas spp*. and the *Nitrobacter spp*. respectively. The model also has a loading function (L(t)), which is the average loading rate of ammonium under normal operation. It is proportional to the exogenous input and fish-feeding rate. When applied to the roller bottle simulations, it is determined by the nutrient addition schedule.

$$N_1 = N_1(t), N_2 = N_2(t),\ N_3 = N_3(t),\ B_1 = B_1(t),\ B_2 = B_2(t),\ t \geq 0.$$

$$\frac{dN_1}{dt} = -r_{max}^1\, B_1\ \frac{N_1/V}{K_{N_1} + N_1/V} + L(t)$$

$$\frac{dN_2}{dt} = -r_{max}^2\, B_2\ \frac{N_2/V}{K_{N_2} + N_2/V} + \alpha_1 r_{max}^1\, B_1\ \frac{N_1/V}{K_{N_1} + N_1/V}$$

$$\frac{dN_3}{dt} = \alpha_2\, r_{max}^2\, B_2\ \frac{N_2/V}{K_{N_2} + N_2/V}$$

$$\frac{dB_1}{dt} = (1-\alpha_1)\, r_{max}^1\ \left(1 - \frac{c_1B_1 + c_2B_2}{M}\right) B_1\ \frac{N_1/V}{K_{N_1} + N_1/V} - d_1B_1$$

$$\frac{dB_2}{dt} = (1-\alpha_2)\, r_{max}^2\ \left(1 - \frac{c_1B_1 + c_2B_2}{M}\right) B_2\ \frac{N_2/V}{K_{N_2} + N_2/V} - d_2B_2$$

Initial conditions: $N_1(0) = N_1^0,\ N_2(0) = N_2^0,\ N_3(0) = N_3^0,\ B_1(0) = B_1^0,\ B_2(0) = B_2^0,$

Parameters: $r_{max}^1, r_{max}^2, \alpha_1, \alpha_2, K_{N_1}, K_{N_2}, d_1, d_2, c_1, c_2, M$

Forcing function: L(t)

3.2 Various Volumes Of Sand

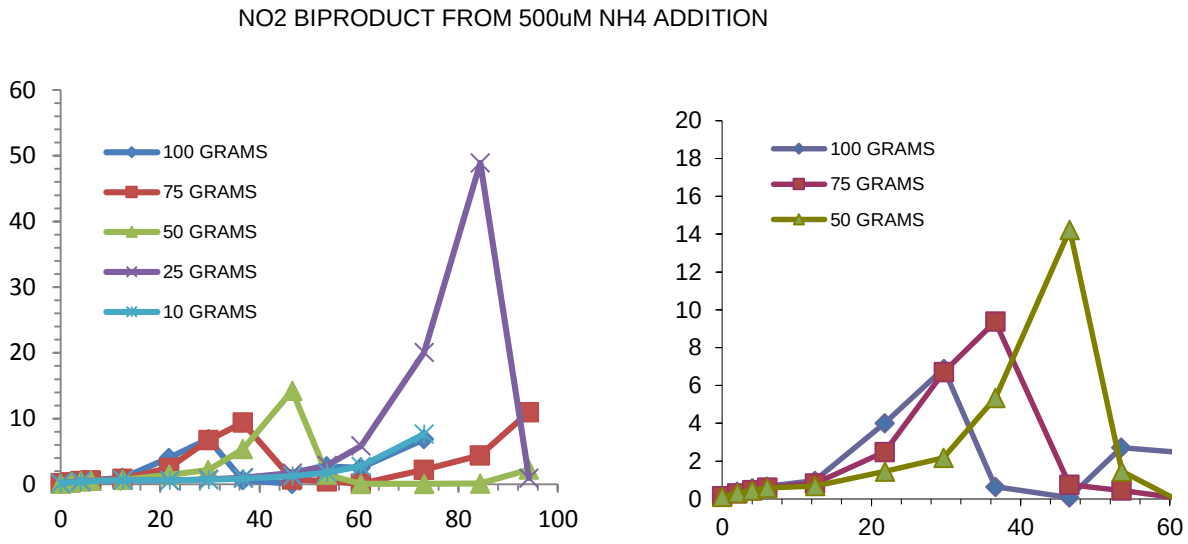

Figure 2 (above): Results of different masses of sand and corresponding ammonium transformation rates. The results from the bottles containing 50, 75, and 100 grams are pictured at right. The graph at left shows all sand masses, including 50, 75, and 100 grams for comparison.

One way the measure ammonium conversion by AOB is to measure the nitrite byproduct. Results of the ammonium additions to various amounts of sand are shown this way in Figure 2. In general, as the amount of substrate increases, the total amount of ammonium conversion decreases, as does the time it takes for initial conversion to occur. Most peaks, representing the maximum amount of nitrite reached in the system before NOB activity re-established, are observable in Figure 2; however, sampling of the bottle containing 10g of sand was stopped before a peak of nitrogen conversion was reached. It likely would have exceeded the maximum nitrite concentration seen in the bottle with 25 g of sand.

3.3 Results Of Kinetics Experiments

Injecting greater concentrations of ammonium and nitrite into the roller bottles resulted in increased rates of ammonium and nitrite transformation to a point (Figures 3 and 4). Maximum rates (r_{max}) of ammonium and nitrite transformation of 18.57 µM/h and 60.22 µM/h were reached at concentrations of about 500 µM and 200 µM, respectively. Transformation of ammonium tended to be slower than that of nitrite. Half-saturation constants, or concentrations of ammonium and nitrite that lead to transformation rates half of what can be reached given the surrounding environment, were found at 20.41 µM and 14.49 µM, respectively.

The second time spiking roller bottles with ammonium and nitrite separately resulted in no observed AOB or NOB activity (i.e. no transformation of either ammonium or nitrite). At the time of the January sampling, the system had been void of fish for eight to nine months meaning the biofilter was in starvation four times longer than when summer sampling occurred. As expected, there was no measurable nitrification processes transpiring from this ammonium addition.

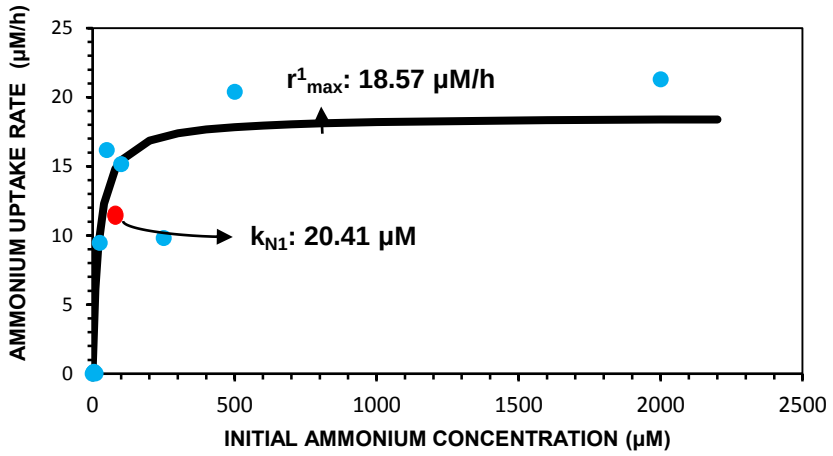

Figure 3 (left): Comparison of initial ammonium concentrations (each done in separate 1L bottles) and their corresponding uptake rates of ammonium. Using Berges's non-linear data fitting methods, k_{N1} and r^1_{max} were determined.

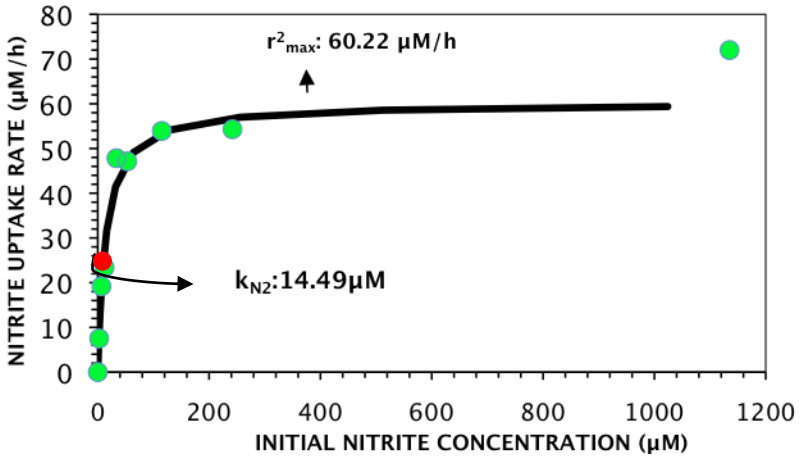

Figure 4 (right): Comparison of different initial nitrite concentrations (each in separate 1L bottles) and corresponding uptake rates of nitrite. Using Berges's non-linear data fitting methods, k_{N2} and r^2_{max} were determined.

3.4 Results Of 3 Large System Spikes

The RAS was empty during the time of experimentation, so the system was spiked with 20 µM and 500 µM nitrite. The results from the 20 µM addition are shown in Figure 5. It takes 20 to 25 minutes for water to make a complete cycle through the system. Relative to the starting concentration, the peak 20 µM concentration is reached around that time after injection. It took longer for complete mixing to occur after the 500 µM addition (data not shown). It took noticeably longer before nitrite transformation was measured during the larger spike, as well. The data from the 20 µM spike are shown, since they are more representative of concentrations that may actually be seen in a re-circulating aquaculture system. A maximum rate of transformation of ammonia of 17.3 µM/h was observed.

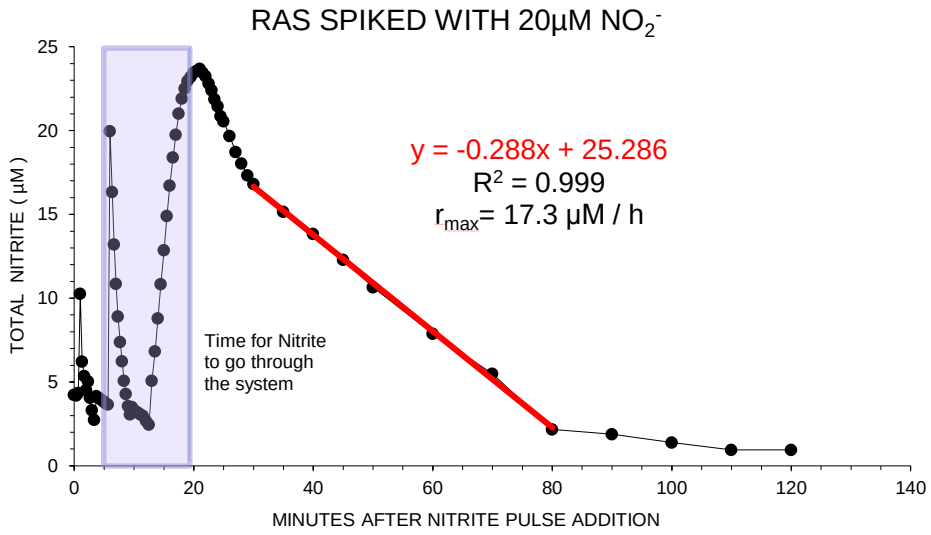

Figure 5 (left): Total nitrite after 20µM of nitrite was added to the system at the inflow to site 2 (Figure 1). Measurements were taken at longer time intervals once the system was well mixed (shown by the shaded section). The r_{max} defines the average rate of nitrification throughout as determined by the time points between 30 to 60 minutes.

4. Discussion

The initial goal of this model was to quantify the nitrogen limitations of recirculating aquaculture systems in a general manner, providing a resource for potential RAS developers. Not all parameters of the model were quantified by these experiments. Many of the variables in the RAS model such as volumes, flow rates, nutrient concentration can be estimated during operation by those applying the model, but the kinetic uptake properties (e.g. r_{max} and k_N) cannot, nor are they easily obtained from the literature. Thus, experiments such as those of the present study are essential in order to provide working estimates. Additional projects to aid in the finalization of model coding include: quantifying the B_1 and B_2 values using the biofilter sand from the RAS at the School of Freshwater Science, directly spiking the system with multiple concentrations of ammonium to verify roller bottle kinetics of that nutrient, and adjusting the model to include the presence and coexistence of the complete nitrifiers, or bacteria that fully oxidize ammonium to nitrate, of the genus *Nitrospira*[9].

Denitrifying bacteria, or those responsible for the reduction of NO_3^- to nitrogen oxide intermediates and ultimately to dinitrogen, were not a focus of this model. Although NO_3^- is considered relatively nontoxic to aquatic organisms, denitrifcation will likely be an important consideration for those maintaining or looking to establish an RAS, as recent findings suggest its accumulation in closed systems can frequently approach hazardous levels[10].

4.1 Various Amounts Of Sand

In general, larger amounts of sand corresponded to lower peak concentrations of the nitrite byproduct of conversion in response to spikes of ammonium. The peaks of nitrite (Figure 3) are likely preceded in time by a growth of AOB. When these bacterial populations increase, greater conversion can occur. Once this nitrite peak is achieved, NOB respond in a similar fashion, and subsequently metabolize the nitrite, oxidizing it to nitrate. Since both AOB and NOB are then present, they compete for space and oxygen availability. Oxygen is limited in both the roller bottles and the fluidized biofilter. The lower levels of conversion of ammonium into nitrite in bottle with greater substrate likely reflect this competition. This supports the idea and the inclusion of a term for carrying capacity in this model.

4.2 First Kinetics Experiments

When compared to ammonium kinetics done in similar recirculating systems, the k_{N1} and r^1_{max} values achieved with the roller bottles fall within an order of magnitude of these previous studies[11,12]. This study predicted a k_{N1} value of

20.41μM compared to real system values of 89.28μM and 311.8μM as well as having a predicted r^1_{max} of 18.57μM/h with comparison of 10.8μM/h and 138.8μM/h respectively. These values obviously differ, but are within enough reason of each other to suggest that the roller bottles may in fact be a reasonable model system for a fluidized sand biofilter.

4.3 Second Kinetics Experiments

There are a multitude of reasons why the sand biofilter may not have been undergoing nitrification during the second sampling time. Since it was about eight months from the time the system had any substantial amount of ammonium influx, the AOB and NOB may have died or become dormant due to lack of nutrient availability. Another factor suggesting this was the case was the high level of biofilm covering the bottom of the holding tank. The concentrations of nitrite was observed for several days after the additions to determine if there was just a delay in the nutrient uptake since it could quickly determine the presence of both AOB and NOB. Since nitrite concentration stayed constant after approximately a week of observations, it was assumed there would be no ammonium or nitrate transformation at all.

4.4 Large System Spike

The actual k_{N1}, k_{N2}, and r^1_{max} of the large system were not determined due to limited resources and restrictions placed on the use of the system. Although there were three values missing from the large system to compare the kinetics experiment to, as previously mentioned, large system spikes in similar systems suggest the attained values in this study fall within reasonable amounts[11,12]. Only one of the results from the system spikes performed in this study is depicted, but the other spikes performed resulted in similar nitrite transformation rates. The r^2_{max} predicted by the roller bottles was 60.22 μM/h whereas the rate in the system came out to be 17.3 μM/h. The value from the system may have been different if the system was freshly emptied of fish, but when compared to the roller bottles it is still a reasonable value to use in the model.

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