Model of a Sulfur-based Cyclic Denitrification Filter for Marine Recirculating Aquaculture Systems

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Model of a Sulfur-based Cyclic Denitrification Filter for Marine Recirculating Aquaculture Systems

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
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DEDICATION

I would like to dedicate this work to my family; mom, dad, uncle, aunt and, especially, my grandpa.
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I would like to thank my committee, Dr. Sarina Ergas, Dr. Mahmood Nachabe and Dr. Qiong Zhang who provided significant support for my research. I would also like to thank Karl Payne and other members of our research group who provided help and advice for my model development. I would thank Qiaochong He, my co-worker, who guided me into this new research environment and provided necessary experimental data for my model. Finally, I would thank Food and Agriculture Organization of the United Nations (FAO) provided me the permission to use their report as a support for my thesis.
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ABSTRACT

Recirculating aquaculture systems (RAS) are a type of near zero-discharge fish production system that is used to treat and recirculate aquaculture wastewater and increase the biomass stocking density in the fish tank. The RAS presented in this thesis was a marine system which was operated with two temporally independent cycles, Loop 1, a continuous loop, and Loop 2, an intermittent loop. Flow in the RAS was switched between the two loops by a solenoid valve. During the operation of Loop 1, components involved in the cycle were successively the fish tank for fish production, solids filters for solids removal and moving bed bioreactor (MBBR) for nitrification. During the operation of Loop 2, the solenoid valve directed influent from the fish tank to a cyclic denitrification filter (CDF) for 10min to refresh the water held in the CDF. The time between cycles of the CDF was considered as the hydraulic residence time (HRT) (i.e. 1hr, 2hr, 4hr and 12hr). Two pilot-scale RAS were operated in the laboratory. The system was operated in two phase, a synthetic wastewater phase with varying HRT and a phase that included fish production with an HRT of 12 hrs.

Models for the RAS was developed, calibrated and used to provide a prediction of nitrogen species concentrations and nitrogen removal efficiency in the RAS and CDF. the model incorporated mass balances on particulate organic nitrogen (PON), dissolved organic nitrogen (DON), NH$_4^+$-N and NO$_3^-$-N, and was generally divided into an overall RAS process model and a CDF model. Due to the high salinity in the system, the ionic strength, 0.3M, was calculated based on the experimental data for modification of nitrogen species activity in the RAS.
The overall RAS process model included three primary components, which are the fish tank, solids filters, and the MBBR. Before calibration, the ammonification and nitrification rate constants for MBBR, $k_{MBBR-afc}$ and $k_{MBBR-nfc}$, were determined to be 0.5 and 240 d$^{-1}$ respectively based on the prior literatures. Corresponding to the 240 d$^{-1}$ of $k_{MBBR-nfc}$, the NH$_4^+$-N flux to biofilm was 0.27 g/m$^2$·d, which agrees the literature value ranging from 0.14 to 0.45 g/m$^2$·d. An Excel based matrix was operated to calibrate four parameters, including the ammonification rate constant for the fish tank, $k_{FT-afc}$, the nitrification rate constant for the fish tank, $k_{FT-nfc}$, the porosity of the media in the CDF, $\varepsilon$, and the superficial solids removal efficiency of the solids filters, $f_{SR}$. It was found that $k_{FT-afc} = 0.028$ d$^{-1}$, $k_{FT-nfc} = 4.55$ d$^{-1}$, $\varepsilon = 0.56$, and $f_{SR} = 11.3\%$. The overall RAS process model was primarily used to predict the nitrogen species concentration in the fish tank. It estimated 45.5 mg/L, 0.2 mg/L, 5.8 mg/L and 1.4 mg/L for NO$_3^-$-N, NH$_4^+$-N, DON and PON concentration in the fish tank. The experimental data was observed to fluctuate in narrow neighborhoods of 45.5±4.5, 0.2±0.1, 5.8±4.8, 1.4±0.6, respectively, which proved the validity of the overall RAS process model.

The CDF model was separately developed for operation of Loop 1 and Loop 2. The CDF was treated as a batch reactor during the operation of Loop 1. The denitrification rate based on the sulfur oxidizing microorganisms was assumed to be governed by a half order reaction. The half order reaction constant, $k_{1/2}$, was calibrated to 79 mg$^{1/2}$/L$^{1/2}$·d, and, for the typical influent concentration of 40-45 mg/L in the RAS, the minimum time required to completely remove the NO$_3^-$-N in the influent was approximately 4.45-4.72 hr. During the operation of Loop 2, a hydraulic model was used to determine the effluent flow rate of the CDF over time. The equivalent diameter of the media particles was calibrated to 0.03 mm, which is much smaller than the diameter of the sulfur pellets and expanded clay particles. However, the overall RAS process model indicates a
relatively high porosity, 0.56, of media in the CDF. This might be caused by biofilm that clogged the pore space in the media. Biofilm also possesses an excellent capacity to hold water, which could result in a high porosity of the media. The hydraulic model provided the variable velocity used to model the NO$_3^-$-N concentration in the CDF effluent. The dispersion coefficient was estimated to 0.0051 m$^2$/min, and the estimate for dispersion number range from 0.39 to 1.28. The relatively high dispersion number indicates that dispersion is a significant process occurring in the CDF compared to advection.

The overall RAS process model and CDF model was then used to estimate the nitrogen fate in the RAS and compare it with a previously developed model for calculating the fate of nitrogen (CafaN). Based on the overall RAS model and CDF model, the nitrogen fate was estimated: 25% removed by fish biomass uptake, 25% by solids removal, 42.5% by denitrification in the CDF, 1% by sampling and 6.5% by microbial assimilation or other removal processes. The CafaN model indicated: 7% removed by biomass uptake, 26% by solids removal, 60% by denitrification, 1% by sampling and 6% by passive denitrification. 7% of fish biomass uptake is much lower than the literature information. During the research, the fish bred an amount of offspring, which could be a cause leading to a lower measured fish biomass assimilation rate.

Finally, results of the new developed model in the paper was used to optimize the CDF HRT and active time (i.e. the time the CDF is open during Loop 2). The original CDF design was operated at a HRT = 12hr with an active time = 10min. The CDF only provided 42.5% of total nitrogen removal. The cycle can be optimized to eight hours with a new 7 min of active time for the Loop 2 three times per day. This would enhance the CDF nitrogen removal efficiency to 70% and allow the system to support larger grow out tanks for fish production.
CHAPTER 1: INTRODUCTION

Aquaculture contributes more than 50% of worldwide fish production. It is estimated that the world’s population will reach 9 billion by 2050 (FAO, 2017). This will result in increased demand on the world’s food supply, especially protein. Fortunately, aquaculture fish production has the potential to meet this demand when capture fisheries decline due to overfishing, pollution, and climate change. (Figure 1.1a; FAO, 2010; FAO 2016). However, marine aquaculture is not as well-developed as inland aquaculture because of cultivation technique difficulties (Figure 1.1b). Thus, this field is worthy of further study.

Figure 1.1. Global aquaculture fish production. a) comparison of wild capture fisheries and aquaculture, b) Inland, marine and total aquaculture. (Source: Food and Agriculture Organization of the United Nations, 2016, The State of World Fisheries and Aquaculture. Reproduced with permission).

Conventional methods for aquaculture fish production include cage, raceway and pond cultivation. These methods are all operated as open systems, which means that the nutrients and wastewater generated are discharged into the surrounding environment (Yoge et al., 2017). Consequently, high concentrations of nutrients are discharged to water bodies resulting in eutrophication concerns. Zhang and Liu (2006) indicated that 70% of total nitrogen and 26% of
total phosphorous in the contaminated Changshou Reservoir came from upstream areas where aquaculture is located. It was also reported that eutrophication increased due to mariculture, including fish, shrimp and shellfish cultivation, seriously damaging the sustainable development of the marine environment, particularly near estuaries and coastlines (Liu et al., 2010). Thus, conventional aquaculture is a significant source of excessive nutrients in eutrophic water bodies.

Recirculating aquaculture systems (RAS) are alternative methods that can address this problem. RAS incorporates wastewater-treatment and water reuse into fish cultivation leading to decreased discharges and water inputs (Lin et al., 2003). A number of treatment processes have been studied within RAS to degrade wastes produced by aquatic animals. Yogev and Sower (2017) suggested a RAS with nitrogen removal process in three treatment cycles, including solids removal, nitrification, denitrification and solids stabilization and methane production using an upflow anaerobic sludge blanket (UASB) reactor. Algae have also been used for wastewater purification in RAS due to their ability to consume ammonia and nitrate (Metaxa et al., 2006). However, there are still drawbacks in existing RAS such as the high-power consumption for pumping and aeration, difficulty maintaining water quality and fish disease control. For example, parasitic copepods are common pathogens that affect fish cultivation. The significance of fish disease has become more evident in marine aquaculture due to higher stocking densities in comparison with wild population (Johnson, 2004).

This thesis investigates pilot-scale marine RAS operated at the University of South Florida (Figure 1.2). The RAS system contained four major unit operations, which are fish tank, solids filter, moving bed biofilm reactor (MBBR) and cyclic denitrification filter (CDF).
Figure 1.2. Schematic diagram of the pilot-scale recirculating aquaculture system (RAS).

*Poecilia sphenops* (commonly called Mollys) were cultivated in two 40-gallon fish tanks. Two types of solids filters were operated in the RAS. Solids filter #1 is an upflow bed filter (UBF) for large particle removal followed by solids filter #2, a simplified fiber filter, for small particle removal. The moving bed bioreactor (MBBR) is an attached growth reactor to ensure the efficiency of nitrification. The CDF, a downflow anoxic packed bed bioreactor containing a reactive medium (elemental sulfur), was used for denitrification. This RAS system works in two water-treatment loops. A solenoid valve connected to a timer is used to switch the flow between the two loops. Wastewater from the fish tank was pumped up through solids filters #1 and #2 during the operation of Loop 1, and large and fine particles were removed, respectively. The filtered water was treated...
in the MBBR, where the ammonia is converted to nitrite or nitrate, and then returned to the fish tank. During the operation of Loop 2, the water flowed from solids filter #1 to the CDF (He et al., 2018). Nitrate and nitrite in the wastewater is reduced to nitrogen gas in this reactor. The treated water flowed to the MBBR for further treatment before being returned to the fish tank. The reason for intermittent operation of Loop 2 is to ensure enough hydraulic retention time (HRT) for effective denitrification.

Two studies were conducted based on this experimental configuration to investigate the advantages of different electron donors for denitrification, elemental sulfur granules (study 1) and a mixture of pine wood chips and elemental sulfur (study 2) were respectively utilized in the CDF. Data from study 1 was used for calibration and verification of the validity of the developed model. The model will be modified and applied to simulate processes of the system in study 2 in the future.

Due to the novel RAS design used in this study, it is useful to model the system in order to elucidate nutrient removal mechanisms and as a tool to scale up the system for full-scale operation. Given the near-zero discharge cultivation, modeling this system is feasible because fish food is the sole source of nutrients. Thus, daily food consumption can be determined according to the weight of fish. Sulfur oxidizing denitrification (SOD) results in high denitrification rates with low biomass yield, reducing the amount of saline solids that require further treatment or disposal. Thereby, in addition to nitrogen, phosphorous and organic carbon, sulfur is a significant element that should be taken into consideration because sulfur is the main electron donor for the sulfur oxidizing denitrifying population, as shown in Eq. (1) (Sahinkaya et al., 2011):

\[
55S_0 + 50NO_3^- + 38H_2O + 20CO_2 + 4NH_4^+ \rightarrow 4C_5H_7O_2N + 55SO_4^{2-} + 25N_2 + 64H^+ 
\] (1)

The elemental sulfur is a readily available material. For example, flue gas desulfurization (FGD) process can provide diversity of sulfur products, including sulfate and elemental sulfur (Hao et al.,...
2002). In addition, however, SOD also has a significant drawback. Sulfide produced through sulfate reduction and sulfur disproportionation is a fish health concern due to its toxicity. The thesis will also introduce hydraulic model. The RAS design has the potential to bring economic benefits to aquaculture enterprises and society. The electron donor could be obtained from other industries such as thermal power station. The cooperation will produce more economic efficiency and reduce the environmental impact of industries.

The main goal of this study was to develop models of the RAS to understand the nitrogen removal and transformations in the pilot marine RAS. The objectives can be generalized as:

1. Predict the nitrogen species concentration in the fish tank and CDF nitrogen removal efficiency.
2. Explain the microbial characteristics, reaction kinetics and mechanisms in the RAS.
3. Predict the nitrogen fate in the RAS.
4. Optimize the operating conditions of the CDF to enhance the CDF nitrogen removal efficiency.
5. Scale up the RAS and address the optimal size design of the reactors.
CHAPTER 2: LITERATURE REVIEW

The primary goal of the model developed in this thesis was to track the nitrogen species transformation and nitrogen fate in the RAS. Therefore, knowledge relevant to the nitrogen transformation and cycle are essential for model development. The general nitrogen cycle is first presented followed by specific nitrogen transformations. Subsequently, the RAS process model, sulfur oxidizing denitrification (SOD) kinetic model are introduced.

2.1 Process Microbiology

The general nitrogen cycle is shown in Figure 2.1. Nitrogen gas, which occupies 78% of atmosphere, is only bioavailable to a minority of microorganism species through the microbial metabolism called nitrogen fixation. Ammonia is the most common nitrogen source, which can be

![Figure 2.1. The nitrogen biogeochemical cycle.](image)

Figure 2.1. The nitrogen biogeochemical cycle.
utilized by microorganisms and plants through assimilation. Ammonification, nitrification and denitrification are key processes in the nitrogen cycle employed in RAS for nitrogen removal. ANAMMOX process is an advanced technology, which has the potential to be applied in RAS.

2.1.1 Ammonification

Figure 2.1 shows that ammonia is released when heterotrophic microorganisms decompose organic nitrogen compounds, including nucleotides and amino acids, by the microbial metabolism called ammonification. The majority of these microorganisms are heterotrophs in the water column and other microorganisms, such as invertebrates, in the sediment (Capone et al., 2008). The process is described by Eq. (2):

\[ \text{Organic-N} \rightarrow \text{NH}_4^+ \]  \hspace{1cm} (2)

2.1.2 Nitrification

Nitrification, the biological oxidation of ammonia to nitrite and nitrite to nitrate, plays a key role in the natural nitrogen cycle. Nitrification is also taken advantage in wastewater treatment due to the tight linkage to nitrogen removal. In conventional wastewater treatment, nitrogen removal is completed through denitrification, with the electron acceptor, nitrate, generated by nitrification (Madigan et al., 2014). Nitrification includes two steps, ammonia oxidation and nitrite oxidation. The first step, ammonia oxidation, is driven by aerobic autotrophic microorganisms, including some archaea and bacteria, which are called ammonia oxidizing microorganism (AOM). The overall reaction with oxygen as the electron acceptor is described by Eq. (3) (Ward et al., 2011):

\[ \text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+ \]  \hspace{1cm} (3)

The second step, nitrite oxidation, is driven by nitrite oxidizing bacteria (NOB) (Ward et al., 2011). The overall reaction with oxygen as the electron acceptor is shown in Eq. (4) (Ward et al., 2011):
\[
\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-
\] (4)

A species of complete ammonia oxidizer (Comammox) was reported to directly oxidize ammonia to nitrate (Pinto et al., 2015; Daims et al., 2015). The general reaction is described by Eq. (5):

\[
\text{NH}_4^+ \rightarrow \text{NO}_3^-
\] (5)

In comparison with nitrification in soil and fresh waters, nitrification in marine environments is distinct due to the limited ammonia source in the vast ocean regions (Ward et al., 2011). Therefore, marine microorganisms are able to efficiently utilize the nitrogen to achieve a balance between denitrification and nitrogen fixation.

2.1.3 Denitrification

Denitrification is the final step of conventional nitrogen removal process, where nitrite and nitrate are prerequisite electron acceptors for anoxic respiration. Conventionally, heterotrophs are applied in the denitrification process, and the typical electron donor utilized in practical treatment are liquid organic chemicals such as methanol or acetate. The overall reaction with methanol as the electron donor is described by Eq. (6) (Metcalf and Eddy, 2014):

\[
5\text{CH}_3\text{OH} + 6\text{NO}_3^- \rightarrow 3\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} + 6\text{OH}^- 
\] (6)

According to Eq. (3), (4) and (6), denitrification supplies the alkalinity that nitrification destroys, which maintains the system at stable pH.

Autotrophs are also well documented to be utilized for denitrification. For example, sulfur oxidizing microorganisms reduce nitrite and nitrate using elemental sulfur as the electron donor. The process, called sulfur oxidizing denitrification (SOD), is shown in Eq. (7) (Sahinkaya et al., 2011):

\[
55\text{S}^0 + 50\text{NO}_3^- + 38\text{H}_2\text{O} + 20\text{CO}_2 + 4\text{NH}_4^+ \rightarrow 4\text{C}_5\text{H}_7\text{O}_2\text{N} + 55\text{SO}_4^{2-} + 25\text{N}_2 + 64\text{H}^+ 
\] (7)
In comparison with conventional denitrification, SOD continues to destroy alkalinity following nitrification. In addition, due to the insolubility of elemental sulfur, suspended growth biological treatment is unsuitable for SOD processes. Sulfur oxidizing bacteria must attach to the surface of sulfur particles to directly obtain sulfur from the electron donor (Reyes et al., 2007). Thus, attached growth or biofilm treatment is applied to SOD processes.

The majority of denitrifying microorganisms are facultative. According to the redox tower, the NO$_3^-$/$N_2$ couple has an oxidization/reduction potential (ORP) of 0.75 V while the O$_2$/H$_2$O has an ORP of 0.82 V. The higher ORP leads to the preferential aerobic respiration in the presence of oxygen. Thus, it is necessary to ensure anaerobic environments for denitrification.

**2.1.4 Anaerobic Ammonium Oxidation (ANAMMOX)**

The combination of AOMs and ANAMMOX bacteria can be used to reduce oxygen and electron donor requirements in wastewater treatment plants (WWTPs), as shown in Figure 2.2. Nitrification can be controlled during oxidation from ammonia to nitrite by low DO and relatively high ammonia concentration or nitrite concentration, which is called nitritation. The microbial reaction was shown previously in Eq. (3)

Theoretically, ANAMMOX bacteria should convert the electron acceptor, nitrite, and the electron donor, ammonia, at the ratio of 1:1, to nitrogen gas. However, the true ratio is approximately 1.3, as shown in Eq. (8) (Kuenen, 2008):

$$\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- +$$
2.03H_2O + 0.066CH_2O_0.5N_0.15 \quad (8)

Figure 2.2. The overall ANAMMOX process.

2.1.5 Effects of Salinity on Microbiological Metabolism

High salinity has been shown to inhibit the performance and colony growth in nitrification process. Moreover, high salinity could change the microbiological structure of the biofilm due to inhibiting Candidatus Brocardia and Nitrosomonas but favoring some halophiles such as Marinobacter and Limnobacter (Garcia-Ruiz et al., 2018). Nitrifying microorganisms are sensitive to salinity changes. The specific nitrification rate was shown to decrease by 86% when the concentration of NaCl increased to 10.0 mg/L. However, high-salinity-acclimated nitrifying microorganisms possess similar nitrification efficiency as non-halophilic microorganisms (Cui et al., 2008; Li et al., 2008). Some cations present in saline water change the kinetics of ammonia transfer between the liquid phase and solid phase. For example, Zn^{2+} and Cu^{2+} can react with free aqueous NH_3 to form ammine complexes. The complexing reaction of Zn^{2+} and aqueous NH_3 is described by Eq. (9) (Benjamin, 2015):

\[
Zn^{2+} + 4NH_3\cdot H_2O \rightarrow [Zn(NH_3)_4]^{2+} + 4H_2O \quad (9)
\]
High salinity also enhances the ionic strength which can decrease the activity of \( \text{NH}_4^+ \). Both complexation and ionic strength lower \( \text{NH}_4^+ \) activity, the effective concentration, in the water and weaken the kinetics of \( \text{NH}_4^+ \) mass transfer from liquid to biofilm. Thus, nitrification is inhibited in saline water, as indicated in Equation (10) (Crittenden et al., 2012):

\[
M_{\text{ammonia}} = k_f (C_b - C_s)V
\]

where, \( M_A \) is the mass flow of \( \text{NH}_4^+ \) (mg/s), \( k_f \) is the mass transfer coefficient (m/s), \( C_b \) is the \( \text{NH}_4^+ \) concentration in bulk solution mg/L; \( C_s \) is the \( \text{NH}_4^+ \) concentration at interface, mg/L.

2.2 RAS Designs and Reactors

RAS mainly include fish tanks, filters and reactors. Solids, organic matter and nutrient removal are significant goals of RAS wastewater treatment processes. Yogev (2016) designed a RAS, including a fish tank, a nitrification reactor, a solids filter, a denitrification mixed tank, and a upflow anaerobic sludge blanket (UASB) reactor. The UASB was implemented for bioenergy production from waste solids. Solids treatment is not a part of the RAS process in our research. Thus, this section focuses on the designs and reactors for solids removal, nitrification and denitrification.

2.2.1 Solids Filters

Upflow media filters and drum filters are the typical reactors used in RAS. Drum filters are designed in the shape of a drum. The wastewater is introduced to the inside of the drum, and, as the filter rotates, the water flows through the drum surface, which is typically made of cloth or stainless steel (Metcalf and Eddy, 2014). When the depth of the water reaches a specific point, a backwash system is activated. A high-pressure water spray dislodges and removes the accumulated solids to a backwash collection trough. Drum filters can be installed in concrete, stainless steel, or fiberglass tankage structures. The pore size of the cloth drum ranges from 10 μm to 1 mm. Ali
(2013) reported that the drum filters, consisted of a 100 μm woven metal mesh, were used to treat the wastewater with a solids loading rate of 10 kg/m²·min.

In comparison with the drum filter, the upflow media filters possess a simple configuration and are easily operated. The floating carriers are applied in the column of filters. During filtration, the wastewater is pumped from the bottom to the top of the filter. The solids are removed by the carriers. When the solids accumulate to specific level in the pore space of media, the filter is backwashed is. However, due to the low density of the carriers, it is not effective to backwash the upflow media filters using backwashing water at the conventional rate that is used for activated carbon or sand filters. Therefore, a turbulent-flow backwashing method was developed to tackle this problem (Xie et al., 2004). The upflow media filters was selected as the solids filter #1 in the RAS and is shown in Figure 1.2.

2.2.2 Nitrification Systems in RAS

A specific challenge in RAS is the low NH₄⁺-N concentration, which limits mass transfer and biodegradation rates. The suspended growth treatment is unsuitable in RAS because the low density of sludge cannot be retained in the reactor. Attached growth treatment is a solution for this problem although only a thin biofilm can form on the surface of attachment. (Pfeiffer, 2011)

Moving bed bioreactor (MBBR) technology is typically applied as a treatment processes for BOD removal and nitrification. MBBRs are particularly useful for nitrification when small reactor size is required due to lack of available land area. Polyurethane foam or plastic biofilm carriers are typical media alternatives in the MBBR process. Generally, plastic carriers share a specific density ranging from 0.96 to 0.98 g/cm³ and a bulk specific surface area of 500 to 700
m²/m³ (Metcalf and Eddy, 2014). The carriers used in this study (Wholesale Kio Farm, USA) and the conventional MBBR configuration for nitrification are shown in Figure 2.3.

![MBBR for nitrification. a) K1 size plastic carriers, b) the conventional configuration of MBBR for nitrification.](image)

By acclimation in aerobic reactor, the microbes attach to surfaces of the carriers and form biofilms. Over time the biofilm thickens on the media surface, while aeration increases the sloughing and keeps the biofilm thin enough to avoid forming an anaerobic zone inside. Therefore, MBBR has the ability to efficiently self-regulate the biofilm thickness for nitrification. During the operation, the flow drives the carriers rotating and completely mixes the effluent with the wastewater in the reactor. Generally, MBBR process possesses several essential characteristics (Metcalf and Eddy, 2014):

1. No return activated sludge.
2. Floating carriers for microbial attachment.
3. High media fill volume (up to 70%).
4. Small space requirements.

In comparison with MBBR, fluidized bed reactors (FBR) possess distinct characteristics: FBR allows denser carriers or particles, and a fixed media configuration is implemented for the
reactor. Wills (2015) reported that a FBR charged with aragonite was applied to control the alkalinity and treat wastewater in a RAS. FBR also has the advantages similar as MBBR, which are no returned sludge, floating carriers, high media filled volume, and small footprint. Physically, the bed expansion of FBR ranges from 50 to 100 percent (Metcalf and Eddy, 2014). Aerobic FBR is applied in nitrification. The bed expansion in the FBR is mainly driven by the fluid velocity. A lower flow rate leads to less energy consumption of pumping but a lower carrier expansion and a slower sloughing rate of biofilm, which also increases the energy consumption due to aeration for denser biomass on the surface of the carriers.

The bed depth of FBR for nitrification ranges from 5 to 6 m. It was reported that a typical FBR had an approximately removal rates of 0.24g TAN/m², which is not a good performance in comparison with other types of treatment applications in RAS (i.e. rotating biological contactor (0.19-0.79g TAN/m²)) (Crab, 2007; Miller and Libey, 1985). The typical configuration of FBR is shown in Figure 2.4:

![Figure 2.4. The conventional configuration of FBR for nitrification.](image-url)
2.2.3 Denitrification Systems in RAS

It is a significant cost concern that large amounts of organic chemicals are used during conventional nitrogen removal processes. Thus, studies have been conducted to explore methods for decreasing the chemical addition or less expensive substitutes for organic chemicals. Autotrophic denitrification is an alternative that utilizes an inexpensive inorganic compound as the electron donor and leads to a low sludge production because inorganic substrates are not as thermodynamically favorable as organic compound metabolism (Sahinkaya and Dursun, 2012). Elemental sulfur (S\(^0\)) is the potential candidate as an inorganic electron donor due to not only its reducing power but also its availability as a waste product from flue gas desulfurization (FGD) industries (Hao and Ma, 2002). SOD process also results in efficient denitrification and low sludge production, which leads to lower costs of sludge treatment and disposal. Because saline sludge causes considerable environmental problems (Klas et al., 2006), SOD is particularly attractive for marine RAS application. Several drawbacks of SOD must be taken into consideration:

1. Alkalinity consumption, which is the opposite of conventional denitrification.
2. Production of a toxic anion (S\(^2-\)) and hazardous gas (H\(_2\)S).
3. Lack of knowledge of SOD for marine systems.

According to Eq. (1), 1.28 mole of protons (H\(^+\)) are formed when reducing one mole of NO\(_3^-\). More alkalinity is required for replenishing alkalinity consumption during nitrification and denitrification. Oyster shells have been shown to be an excellent alkalinity source for autotrophic denitrification. In comparison with limestone, oyster shells contain more compact CaCO\(_3\) in the crystalline phase, which leads to lower dissolution of the components and consequently lower turbidity (He et al., 2018). He et al. (2018) also showed that oyster shell addition could maintain a RAS at approximately pH 8 without adding extra alkalinity. In addition, oyster shells can function
as a biofilm carrier. SOD bacteria can form biofilms on the surface of the shell and conduct efficient nitrogen removal (He et al., 2018).

In the presence of oxygen, $S^0$ is rapidly converted to oxidized states such as $SO_4^{2-}$. Therefore, pecked bed bioreactors with intermittent operation can be used to prevent frequent oxygen injection from the influent while controlling the residence time, packed bed reactor is a feasible configuration for SOD process (Sahinkaya and Dursum, 2015). During the treatment phase, the media is submerged by wastewater. The biological contact process provides a near-complete denitrification using the nitrite and nitrate in influent.

The redox tower indicates that $SO_4^{2-}$ possesses lower reduction potential than oxygen, $NO_3^-$, fumarate and $Fe^{3+}$ (Madigan et al., 2014). When $NO_3^-$ is exhausted in the RAS, $SO_4^{2-}$ reduction dominates and converts $SO_4^{2-}$ to $S^{2-}$. In this process fish waste or dislodged biofilm can serve as an electron donor to sulfate reducing bacteria. $S^0$ can also produce the $S^{2-}$ through disproportionation under alkaline conditions, and the overall reaction is shown in the Eq. (11) (Bottcher and Thamdrup, 2001):

$$4H_2O + 4S^0 \rightarrow 3H_2S + SO_4^2- + 2H^+ \quad (11)$$

High concentrations of $S^{2-}$ could lead to the release of $H_2S$, causing odor problems. The residual $S^{2-}$ will be toxic to fish in the fish tanks. Therefore, reasonable HRT control is important for decreasing or avoiding $S^{2-}$ and $H_2S$ production because excess retention might lead to $NO_3^-$ depletion and $SO_4^{2-}$ reduction.

Currently, the majority of SOD studies have been conducted in the freshwater RAS (He et al., 2018) and, drinking water and wastewater treatment (Sahinkaya et al., 2011; Graaf et al., 1996). There is a research gap in SOD application for the marine RAS, and the marine aquaculture
contributes large part to global food supply. Thus, it is worthy researching the feasibility of SOD in marine RAS.

2.3 RAS Models

2.3.1 Process Models for RAS

North Carolina State University (NCSU) has put efforts into the evaluation, analysis and development of RAS since 1989. During this research, engineering spreadsheets were created for design of new RAS and estimation of treatment efficiency. Using spreadsheets based on the data from Carolina Power and Light company (CP&L), Losordo and Hobbs (1999) set up an estimating tool for a RAS in their Fish Barn project. The mass balances developed by Losordo and Hobbs were used to assist describing the RAS process. This RAS model is aimed at estimating the required reactor size and necessary flow rates through the system. Recycling flows can be also calculated by the model to maintain water quality desired by the users, including total ammonia nitrogen (TAN), dissolved oxygen (DO), and suspended solids (SS). The spreadsheet contains five parts—tank size and fish biomass, TAN mass balances, biofilter size, solids mass balance and oxygen mass balance. With respect to TAN, the required data are feed protein content, desired TAN concentration in the recirculating water, passive nitrification, maximum NO₃⁻ concentration desired and biofilter efficiency for TAN removal. Passive nitrification was carried out by the biofilm growing on the surface of the system rather than media in the biofilter. Thus, the tool makes it possible to predict the treatment efficiency and cost before building the full-scale facility.

Yogeve et al. (2017) developed a process model based on mass balances in a novel near-zero discharge saline RAS. The RAS process incorporated three loops, including solids removal followed by nitrification, denitrification using fish waste as an electron donor, and an upflow
anaerobic sludge blanket (UASB) for solids digestion and methane production. The model was developed to achieve four main goals:

1. Simulate the fate of nitrogen in the RAS
2. Simulate the fate of organic carbon in the RAS
3. Estimate the energy consumption of the RAS
4. Favor the development of the full-scale system

The nitrogen fate was focused on TN but was divided into several categories such as protein, nitrate, TAN and mineral nitrogen precipitation. The model can be used to calculate the nitrogen assimilated by the fish biomass, trapped in solids removal, and wasted or excreted by fish. Based on the mass balance, the nitrogen removal and solid disposal efficiency of the RAS can be estimated. Similarly, the carbon fate was focused on TOC. However, the model includes the carbon dioxide and biogas in the UASB related to the carbon removal of the RAS. The model can be used to calculate carbon accumulated by fish, captured in the solids filter, degraded during denitrification, and respiration of fish. Additionally, the results from the model can be used to estimate required water exchanges.

2.3.2 Kinetic Models

Several nitrification kinetic models are presented in this section. Leyva-Diaz et al. developed models for MBBR and membrane bioreactor (MBR) systems. In their research, three independent systems were parallelly operated. The first system was a MBR. The second system was a MBBR-MBR combined plant that was operated under both aerobic and anaerobic conditions. The third system was a MBBR-MBR combined plant that was only operated under aerobic conditions. To model the kinetics of the microbial reaction, the Monod model was applied. The
biomass decay was also taken into the consideration. Therefore, the net microbial growth rate was described by Eq. (12):

\[ r'_X = \frac{\mu_m S_X}{K_s + S} - k_d X \]  

(12)

The letter H and A represent heterotrophic and autotrophic, B represents active biomass, NH represents ammonia nitrogen, and M and S represent organic matter. Where \( \mu_{m,H} \) and \( \mu_{m,A} \) are maximum specific growth rates (d\(^{-1}\)), the \( X_{B,A} \) and \( X_{B,H} \) are active biomass density (mg/L), \( K_{NH} \) is the half saturation coefficients for ammonia nitrogen (mg/L), the \( K_M \) is the half saturation coefficients for organic matter (mg/L), \( S_{NH} \) is the ammonium concentration (mg/L), \( S_S \) is the organic matter concentration, \( k_d \) is the microbial decay constant (d\(^{-1}\)).

The experimental data were processed using BM-Advance software. The mixed liquid was taken from the three plants and then transferred to the BM-Advance analyzer called respirometer. The \( X \) and \( S \) values were previously measured. Before each test, the mixed liquid was completely aerated for 18hr to ensure that the biofilm was under endogenous conditions and could consume any substrates. After that, a pulse of substrate was spiked into the liquid. In the software, the DO consumption, OC (mg/L), was monitored. According to the Monod equation, the parameters including \( K_s \), \( \mu_m \) and \( k_d \) were estimated.
CHAPTER 3: MATERIALS AND METHODS

The research included two different phases. The first phase was carried out with synthetic saline water. The second phase was carried out stocking with *Poecilia Sphenops* (mollies) in the RAS. This chapter presents how the RAS was operated and monitored during the two phases.

3.1 RAS System Description

Two pilot-scale the RASs were set up at University of South Florida (USF) and operated in duplicate. As shown in Figure 3.1, each RAS contained four primary components, which are the fish tank, solids filters (filter#1 and filter#2), moving bed biofilm reactor (MBBR), and cyclic denitrification filter (CDF).

Figure 3.1. Schematic diagram of the pilot-scale recirculating aquaculture system (RAS).
The parameters of these components are provided in Table 3.1. Duplicates of RASs, System A and System B, were set up for experimental comparison. Each fish tank was aerated by two air stone bars to ensure that dissolved oxygen (DO) concentration > 5 mg/L. A submersible heater (HL-338, MWGears) was used to keep the aquarium temperature between 26°C and 27.5°C, and a thermometer served as the temperature monitor. The overall recirculating flow rate throughout the system was maintained at approximately 220 mL/min by a Masterflex C/L Dual Channel Pump (Cole Palmer; Vernon Hills, IL). To offset water loss by evaporation and stabilize the salinity of water in the system, approximately 2 liter tap water was replenished into each fish tank daily.

Table 3.1. Description of reactors in the RAS

<table>
<thead>
<tr>
<th>Item</th>
<th>Dimensions</th>
<th>Reactor Volume (L)</th>
<th>Media and Carrier Volume (L)</th>
<th>Configuration Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Tank</td>
<td>75 cm Length × 30 cm width × 50 cm depth</td>
<td>112.5 L</td>
<td></td>
<td>Two air stone bars; One heater.</td>
</tr>
<tr>
<td>Solids filter #1</td>
<td>35 cm filter height with 25 cm bed depth, 6 cm diameter</td>
<td>0.99L</td>
<td>0.71L</td>
<td>Upflow configuration; Mini-size plastic carrier with 60% fill fraction</td>
</tr>
<tr>
<td>Solids filter #2</td>
<td>12 cm bed depth with 9 cm diameter</td>
<td>0.76L</td>
<td>0.76L</td>
<td>Filter with fiber between two layers of sponge</td>
</tr>
<tr>
<td>MBBR</td>
<td>24 cm reactor height with 8 cm diameter, 12 cm bed depth for system A and 15 cm system B</td>
<td>1.21L</td>
<td>0.6L for system A and 0.75L for system B</td>
<td>Moving bed configuration; Kaldnes media (K1-size) with 60% fill fraction</td>
</tr>
<tr>
<td>CDF</td>
<td>20 cm of media height with 12.5 cm of diameter</td>
<td>&gt;3L</td>
<td>2.45L</td>
<td>Intermittent submerged packed bed configuration with a mixture of elemental sulfur pellets, shells, and clay for study 1; wood chips, elemental sulfur pellets, and clay for study 2</td>
</tr>
</tbody>
</table>
The systems were designed to switch between two loops, the nitrification cycle and denitrification cycle. The first cycle, Loop 1, the nitrification loop, was considered as a continuous system because the first cycle ran continuously for 11hr 50min out of each 12hr. It consisted of an upflow bed filter (UBF, filter #1) and a fiber filter (filter #2) followed by the MBBR. The UBF was filled with mini-size plastic media (60% fill fraction; Wholesale Kio Farm, USA). Weekly backwashing was conducted on the UBF for sludge removal, and the supernatant liquid of the settled sludge was returned to the MBBR. The fiber filter was comprised of three layers, a fiber layer (Acurel 100% Polyester Filter Fiber, Acurel LLC) between two sponge layers (Aquarium Biochemical Cotton Filter Foam Fish Tank Sponge, Liannmarketing). The sponges were washed by tap water, and the middle layer was periodically replaced with intact fiber for avoiding clogging. The MBBR was packed with K1-size plastic carriers, Kaldnes media (60% fill fraction; AMBTM media, EEC, Blue Bell, PA, USA), and aerated using a compressor and air stone to promote nitrification.

In comparison with Loop 1, the second loop, Loop 2, employed an intermittently operated submerged denitrification reactor, the CDF. In study 1, The media of the CDF was composed of 1200 g elemental sulfur pastille pellets (1-2 mm), 700 g crushed oyster shell (2-4 mm) and 1300 g expanded clay (3-5 mm). The intermittent flow was controlled by combination of a timer (Fisherbrand™ Traceable™ Digital Outlet Controller) and three-ways solenoid valve (CSA Certified, NEMA 4X, UL Listed). The second loop was activated for 10 minutes every 12hr given that the results of a preliminary study showed that a HRT of 12hr ensured the complete removal of nitrate in the influent to the CDF (He, et al., in review). It also made the manual operation feasible because the system required inspection and sampling especially during the active time of 10 min. The treated water in the CDF was discharged into the MBBR and then the fish tank. When
the valve was closed, the fresh influent was treated in the reactor under warm and anoxic conditions. When clogging was observed, the CDF was backwashed with water from the fish tank at a rate of 400 mL/min. Then, air flow was pumped through the CDF media for 5 min. The supernatant liquid of the settled backwash water was returned to the system.

3.2 Study Description

Two studies were conducted based on this experimental configuration to investigate the advantages of different electron donors for denitrification, elemental sulfur granules (study 1) and a mixture of pine wood chips and elemental sulfur (study 2) were respectively utilized in the CDF. Thus, study 2 also employed heterotrophic microorganisms. Data from study 1 were used for calibration and verification of the validity of the established model. The model will be modified and applied to simulate processes of the system in study 2 in the future.

3.2.1 Study 1: The RAS Based on Autotrophic Sulfur Oxidation Denitrification

Study 1 was carried out in two phases, Phase 1 and Phase 2. The general experimental phases and operational conditions are shown in Table 3.2 (He et al., in review):

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Fish biomass density (kg/m³)</th>
<th>Nitrogen supplementation</th>
<th>Denitrification reactors (YES/NO)</th>
<th>HRT for denitrification reactors (h)</th>
<th>Days of operation (d)</th>
<th>Solids Filter II (YES/NO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td></td>
<td></td>
<td>NO</td>
<td>N/A</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td></td>
<td></td>
<td></td>
<td>12, 8, 6, 5, 4, 2, 1</td>
<td>42, 5 (one day/HRT)</td>
<td></td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>0.44</td>
<td></td>
<td>NO</td>
<td>N/A</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td></td>
<td>NO</td>
<td>N/A</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>0.88</td>
<td></td>
<td>60 mg NO₃⁻-N/d</td>
<td>YES</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>
The first phase was subdivided 2 stages. In the first stage, synthetic water was recirculated in the system without denitrification for 25 days. A synthetic salt solution, which was composed of 1.91 mg ammonium chloride (NH₄Cl), 84 mg sodium bicarbonate (NaHCO₃) and 15 g Instant Ocean Sea Salt (Instant Ocean®) per liter tap water, was initially added into the system. Subsequently, the researchers supplemented the fish tank by daily addition to simulate the growth of 0.8 kg/m³ biomass density, which included NH₄Cl at a rate of 191 mg/day (TAN loading = 0.42 mg N/L·d), fish food pellets (1.5 mm; 45% protein, Skretting Classic Fry, Skretting USA, Utah) at a rate of 1.0 g/day using an automatic feeder (Fish Mate F14, with 14 individual meals), and NaHCO₃ at a rate of 588 mg per day. In the second stage, the denitrification was activated and explored at different hydraulic retention times (HRTs), 24hr, 12hr, and 8hr. Additionally, due to the decrease of NO₃⁻ at 8hr HRT, an incremental loading of TAN (2 mg N/L·d) was applied to maintain the concentration of NO₃⁻ above 65 mg NO₃⁻-N/L in the fish tank.

The second phase incorporated Poecilia sphenops (mollies) into the system. Four stages were carried out during this phase, which the former two were operated without denitrification while latter two were connected to the intermittent CDF. In the first stage, 20 saline water-acclimated mollies (i.e. 0.44 kg/m³ biomass) were cultivated in each fish tank. The population was increased to 40 mollies per tank, and the biomass was also doubled to 0.88 kg/m³ in the second stage. The fish fed on food pellets (0.6 mm Purina AquaMax Fingerling 300) per day at a rate of 2.5 g/(tank·d). The nutrient list of of the fish food was described as following: 50.00% crude protein (min.), 16.00% crude fat (min.), 3.00 % crude fiber (max.), 2.85% calcium (max.), 1.30% phosphorus, 0.60% sodium (max.) and 12.00% ash (max.). After the 55 days, Loop 2 was included throughout stage 3 and 4 at a 12hr HRT, which was selected based on the HRT research of the first phase. During these two stages, an additional 6 g NO₃⁻-N/L of KNO₃ solution was supplied to fish.
tank due to the high efficiency of the nitrogen removal of denitrification. To abate the impact of suspended solids on fish health, filter #2 was added following filter #1 during stage 4.

3.2.2 Study 2: The RAS Based on Wood Chip-Elemental Sulfur Mixed Denitrification

The study contained two stages. In the first stage, the system was isolated from denitrification. The new media included wood chips and sulfur. The acclimation was carried out, and the water with denitrifying bacteria was seeded into the media. Subsequently, the media was packed into the CDF to mature for 2 weeks. When acclimation was completed, the CDF was connected to the system. The HRT, feeding rate, and flow rate were maintained uniform as in the previous study.

3.2 Analytical Methods

Total nitrogen (TN), NH$_4^+$, NO$_3^-$, NO$_2^-$, SO$_4^{2-}$, S$_2^-$, total suspended solids (TSS), volatile suspended solids (VSS), total phosphorous (TP), chemical oxygen demand (COD), and alkalinity were measured throughout the research. Dissolved oxygen (DO), conductivity, pH and temperature in the fish tanks were also measured on site to ensure the stability of the RAS. On site measurements were made using a DO meter (Mettler Toledo, USA), pH meter (Oakton™ Handheld Ion Meter), conductivity meter (Oakton™ CON 6+ Portable Conductivity Meter), and an electronic thermometer. All the experiments were conducted either following Standard Methods (APHA et al., 2012) or using HACH test kits except ammonia and NO$_x$ tests. An ammonia analyzer (TL-2800, Timberline Instrument, USA) was used to measure ammonia and NO$_x$ concentration. Table 3.3 shows the method description of each measurement for different parameters.
Table 3.3. Method description of measurements for parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Filtered through the 0.45 µm membrane filter</th>
<th>Method Detection Limit (MDL) (mg/L)</th>
<th>Method description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>YES</td>
<td></td>
<td>HACH method 10071 adapted from <em>Standard Methods</em> 4500C</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>YES</td>
<td>0.05</td>
<td>Ammonia Analyzer (TL-2800, Timberline Instrument, USA)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>YES</td>
<td>0.05</td>
<td>Calculated from the difference between NO₃⁻-N and NO₂⁻ concentration</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>YES</td>
<td>0.01</td>
<td>A combination of <em>Standard Methods</em> 4500 and Strickland and Parsons (1972)</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>YES</td>
<td>2</td>
<td>SulfaVer 4 Method (<em>Standard Method</em> 4500E) according to HACH method 10248</td>
</tr>
<tr>
<td>S²⁻</td>
<td>NO</td>
<td>0.005</td>
<td>Methylene Blue Method (<em>Standard Method</em> 4500D) according to HACH method 8131</td>
</tr>
<tr>
<td>TSS</td>
<td>NO</td>
<td></td>
<td>Standard Methods 2540 D &amp; E</td>
</tr>
<tr>
<td>VSS</td>
<td>NO</td>
<td></td>
<td>Standard Methods 2540 D &amp; E</td>
</tr>
<tr>
<td>TP</td>
<td>YES</td>
<td>1</td>
<td>HACH method 10127 adapted from <em>Standard Methods</em> 4500 B-C</td>
</tr>
<tr>
<td>COD</td>
<td>NO</td>
<td>3.1</td>
<td>HACH method 8000 adapted from <em>Standard Methods</em> 5220D with addition of 0.5 g of HgSO₄ per vial to inhibit chlorine ions</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>NO</td>
<td>20</td>
<td><em>Standard Methods</em> 2320 B titrated with 0.02 N HCl to a pH 4.5 end point (865 Dosimat plus and 827 pH Lab, Metrohm AG, Switzerland)</td>
</tr>
</tbody>
</table>
CHAPTER 4: MODEL DEVELOPMENT

The flow diagram for the pilot recirculating aquaculture system (RAS) operated in the USF labs is shown in Figure 4.1. Section 4.2 presents a simple nitrogen mass balance model that tracks the fate of nitrogen in the pilot RAS based on the experimental data. Individual models were developed for the fish tank, solids filters, moving bed bioreactor (MBBR) and cyclic denitrification filter (CDF). The RAS process model was based on mass balances, with integrated kinetic models. In Loop 1, the flow rate was treated as constant, Q, because flow was redirected to Loop 2 for only 10 min every 12hr. However, in Loop 2 the effluent flow rate, Q_E, from the CDF was directly affected by the head loss, which leads to non-steady state conditions.

Four major assumptions were made when developing the model, which are discussed further below:

1. No hydrolysis of particulate organic nitrogen (PON) from the uneaten fish food occurred in the system. Previous research indicates that PON hydrolysis is hindered at increasing pH, and all components of the RAS were operated under a weak alkaline condition. Hence and Odegaard (1993) showed that PON hydrolysis was almost completely inhibited under aerobic conditions at a pH of 7 and 25°C.

2. Nitrite concentrations were neglected throughout the RAS, due to the low measured nitrite concentrations in the pilot RAS (He et al., in review).

3. Intense aeration causes rapid mixing in the fish tank and MBBR. Therefore, they can be treated as CMFRs.
4. NH$_3$ volatilization is negligible at the pH of the RAS. Therefore, nitrogen losses from the system were due to solids wasting and denitrification.

![Flow diagram for the RAS](image)

Figure 4.1. Flow diagram for the RAS.

Based on assumption 1 above, PON was assumed to only be removed by the solids filters. Thus, fish excreta was the sole source of dissolved organic nitrogen (DON) without consideration of PON hydrolysis. The ammonia and nitrate concentrations are the important parameters for water quality and are transformed by biochemical reactions, especially nitrification and denitrification. Therefore, the nitrogen indicators modeled were concentrations of PON, DON, NH$_4^+$-N and NO$_3^-$-N. Note that all the nitrogen species concentration are presented in mg N/L throughout the thesis.
4.1 Effect of Ionic Strength on Biochemical Reactions

Given that the RAS water has a high salt concentration, the ionic strength was a significant concern because it can dramatically affect microbiological reactions and, thus, was taken into consideration in the model development. The ionic strength and activity coefficients were estimated using Eq. (13) and Eq (14) (Benjamin, 2002):

\[ I_{\text{total}} = \frac{1}{2} \sum c_i z_i^2 \]  \hspace{1cm} (13)

\[ \log y = -A z^2 \left( \frac{\sqrt{I_{\text{total}}}}{\sqrt{I_{\text{total}}+1}} - 0.3 I_{\text{total}} \right) \]  \hspace{1cm} (14)

where \( I_{\text{total}} \) is the total ionic strength (mole/L), \( c_i \) and \( z_i \) are the concentration (mole/L) and charge of each ionic species, respectively, \( y \) represents the activity coefficient, and \( A \) is a constant that is equal to 0.505 at 27°C. Eq. (2) is applicable \( 0.1 \text{M} < I_{\text{total}} < 0.5 \text{M} \). The actual ionic strength of the RAS water was approximately 0.3 (15ppt):

Nonelectrolytes are also affected by ionic strength, due to a salting out phenomenon. The salting out coefficients were estimated using Eq. (15):

\[ \log y = K_s I \]  \hspace{1cm} (15)

where \( y \) is the salting out coefficient, and \( K_s \) is the salting out constant, which typically ranges from 0.01-0.1 (Zhu, 2006).

The activity and salting-out coefficients were used to estimate the activity of nitrogen ionic species and nonelectrolytes using Eq. (16) and Eq. (17), respectively:

\[ \{A\} = y[A] \]  \hspace{1cm} (16)

\[ \{B\} = y[B] \]  \hspace{1cm} (17)

where \( \{A\} \) and \( \{B\} \) are the activity of ion A and nonelectrolyte B (mole/L), and \( [A] \) and \( [B] \) are the molar concentration of ion A and nonelectrolyte B (mole/L). Note that the activities were only used in kinetic expressions.
4.2 Fish Tank Mass Balance

Figure 4.2 shows the overall fate of nitrogen in the fish tank. The fish food was considered as the only source of nitrogen and was fed by an automatic feeder at a rate of 2.5 g/d for each tank. Part of the fish food protein is utilized by fish to synthesize biomass. The other part of the protein is wasted in the form of uneaten fish food or fish excreta. Fish excreta is divided into solid and liquid fractions. The solid waste corresponds to PON while the liquid waste contains DON and NH$_4^+$-N. According to a previous study, PON in fish excreta is rapidly hydrolyzed to DON; therefore, the uneaten food was treated as the only source of PON, which accounted for 20%-30% of nitrogen in food waste and fish excreta (McCarthy and Gardner, 2003). Chen and Fornshell (2000) reported that 20%-30% of the nitrogen from fish food can be assimilated by biomass, which indicates that approximately 75% of the added nitrogen will be discharged into the fish tank environment. They also reported that 70%-90% of excreta is metabolized to NH$_4^+$-N.

![Figure 4.2. The fate of nitrogen from fish food in the fish tank.](image-url)
A mass balance on PON in the fish tank is described by Eq. (18):

$$V_{FT} \frac{dC_{FT-PON}}{dt} = QC_{MBBR-PON} - QC_{FT-PON} + f_{PON}m_{feed-N} - m_{sample-PON-1}$$  \hspace{1cm} (18)

where $C_{FT-PON}$ is the PON concentration in the fish tank (mg/L), $C_{MBBR-PON}$ is the PON concentration in the MBBR (mg/L), $f_{PON}$ is the fraction of total nitrogen in the fish food that is converted to PON, $Q$ is the flow rate throughout Loop 1 (L/d), $V_{FT}$ is the volume of fish tank (L), $m_{feed-N}$ is the feeding rate (mg/d), and $m_{sample-PON-1}$ is the PON loss rate from the fish tank from sampling, (mg/d).

The only source of DON is from the fish excreta, and DON can be converted to NH$_4$+-N through ammonification. Ammonification was treated as a first order reaction because the DON concentration is relatively low. The mass balance is given by Eq. (19), and the ammonification rate is shown in Eq. (20):

$$V_{FT} \frac{dC_{FT-DON}}{dt} = QC_{MBBR-DON} - QC_{FT-DON} + f_{DON}m_{feed-N} - r_{FT-afc}V_{FT} - m_{sample-DON-1}$$  \hspace{1cm} (19)

$$r_{FT-afc} = k_{FT-afc}C_{FT-DON}^*$$  \hspace{1cm} (20)

where $C_{FT-DON}$ is the DON concentration in the fish tank (mg/L), $C_{MBBR-DON}$ is the DON concentration in MBBR (mg/L), $f_{DON}$ is the fraction of the total nitrogen in the fish food that converted to DON, $r_{FT-afc}$ is the ammonification rate in the fish tank (mg/L·d), $C_{FT-DON}^*$ is the modified DON concentration in the fish tank based on the ionic strength (mg/L), $k_{FT-afc}$ is the first order ammonification rate constant in the fish tank (d$^{-1}$), and $m_{sample-DON-1}$ is the DON loss rate to the fish tank from sampling, (mg/d).

Ammonification of DON is a source of NH$_4$+-, which can be converted to NO$_3$-N through nitrification. Although the NH$_4$+-N concentration was comparable to the half saturation constant, Nitrification in this RAS can be considered as a first order reaction because the NH$_4$+-N
concentration changed within a small range. Thus, the average \(\text{NH}_4^+-\text{N}\) activity was used to calculate the first order nitrification reaction constant. The mass balance for \(\text{NH}_4^+-\text{N}\) is given by Eq. (21), and the nitrification rate is shown in Eq. (22):

\[
V_{FT} \frac{dC_{FT-A}}{dt} = QC_{MBBR-A} - QC_{FT-A} + f_A m_{feed-N} + r_{FT-a_{fc}} V_{FT} - r_{FT-nfc} V_{FT} - m_{sample-A-1}
\]

\[
r_{FT-nfc} = \frac{\mu_{ni,max} X}{Y_{ni}(K_{ni} + C_{FT-A}^{*})} C_{FT-A}^{*} = k_{FT-nfc} C_{FT-A}^{*}
\]

where \(C_{FT-A}\) is the \(\text{NH}_4^+-\text{N}\) concentration in the fish tank (mg/L), \(C_{MBBR-A}\) is the \(\text{NH}_4^+-\text{N}\) concentration in the MBBR (mg/L), \(f_A\) is the fraction of the total nitrogen in the fish food that converted to \(\text{NH}_4^+-\text{N}\), \(r_{FT-nfc}\) is the nitrification rate in the fish tank (mg/L·d), \(m_{sample-A-1}\) is the \(\text{NH}_4^+-\text{N}\) loss rate to the fish tank from sampling, (mg/d), \(\mu_{ni,max}\) is the specific maximum growth rate for nitrifiers (d\(^{-1}\)), \(X\) is the nitrifier biomass concentration in the fish tank (mg/L), \(Y_{ni}\) is yield coefficient for nitrifiers, \(K_{ni}\) is the half saturation constant for \(\text{NH}_4^+-\text{N}\) (mg/L), \(C_{FT-A}^{*}\) is the average modified \(\text{NH}_4^+-\text{N}\) concentration based on ionic strength (mg/L), \(C_{FT-A}^{*}\) is the modified \(\text{NH}_4^+-\text{N}\) concentration based on ionic strength (mg/L), and \(k_{FT-nfc}\) is the first order nitrification rate constant in the fish tank (d\(^{-1}\)).

During the experimental program, the rate of denitrification in the CDF was higher than the rate of nitrate production. As the goal of this project was to maintain a steady-state nitrate concentration, KNO\(_3\) was supplemented into the fish tanks. Due to the intense aeration in the fish tank, fish tank passive denitrification was ignored in the RAS model. Therefore, nitrification of \(\text{NH}_4^+-\text{N}\) and NO\(_3^-\)-N supplementation were assumed as the only NO\(_3^-\)-N sources as the feed does not contain nitrate and fish do not excrete nitrate. The mass balance is given by Eq. (23):

\[
V_{FT} \frac{dC_{FT-NT}}{dt} = QC_{MBBR-NT} - QC_{FT-NT} + r_{FT-nfc} V_{FT} + m_{sp} - m_{sample-NT-1}
\]
where $C_{FT-TN}$ is the NO$_3^-$ concentration in the fish tank (mg/L), $C_{MBBR-NT}$ is the NO$_3^-$ concentration in the MBBR (mg/L), and $m_{sample-NT-1}$ is the NO$_3^-$ loss rate related to the fish tank from sampling, (mg/d).

### 4.3 Solids Filter Mass Balance

Nitrification occurred in the two solids filters as observed in the experimental data. However, it was assumed that no material transformations occurred in the solids filters. Thus, for modeling the fate of nitrogen other than PON, the solids filter was not taken into consideration. DON, NH$_4^+$-N and NO$_3^-$-N concentrations were treated as constant throughout the solids filters. Figure 4.3 shows a conceptual diagram illustrating how the solids filters worked.

![Solids Filter Mass Balance Diagram](image)

Figure 4.3. Conceptual diagram of solids filtration process.

A mass balance for PON in the filters is shown in Eq. (24):

\[
Q C_{SFs-POn} = Q C_{FT-POn} - Q f_{SR} C_{FT-POn}
\]  

(24)

where $C_{SFs-POn}$ is the PON concentration in the filter effluent (mg/L), and $f_{SR}$ is the superficial solids removal efficiency of the two filters. Note that, the superficial solids removal efficiency is not the true removal of newly produced PON. Under steady state condition, no accumulation of PON occurred in the fish tank, which indicates that all newly generated PON was removed in the solids filters.
4.4 MBBR Mass Balance

A conceptual diagram of the MBBR is shown in the Figure 4.4. The MBBR installed in the RAS is categorized as an attached growth bioreactor. The microorganisms are cultivated in form of a biofilm on the surface of carriers rather than as suspended biomass as in activated sludge systems. The biofilm configuration maximizes the sludge retention time (SRT) and avoids the need for sedimentation and recycle of activated sludge. In this research, the MBBR was packed with K1-size plastic carriers, Kaldnes media (60% fill fraction; AMBTM media, EEC, Blue Bell, PA, USA), and operated with compressed aeration using an air stone to promote nitrification. The K1 carriers have a surface area to volume ratio (S/V) of 350 m²/m³. It was assumed that the DO concentration was not a limiting factor for nitrification because the DO was > 5 mg/L (Metcalf and Eddy, 2014). Intensive aeration was assumed to cause rapid and complete mixing of the liquid in the reactor, and the MBBR was treated as a CMFR.

Figure 4.4. Conceptual diagram of MBBR process.

Given that it is inconvenient to model the intermittent cycle in Loop 1, the intermittent flow from the CDF to the MBBR was converted to a equivalent continuous flow rate based on the daily active time of the two cycles (Note that, in this research, the ratio of flow rate from the solids filters, \( Q_{SF} \), to that from the CDF, \( Q_{CDF} \), was \( 12 \text{hr} \times \frac{60 \text{min}}{\text{hr}} - 10 \text{min} \): \( 10 \text{min} = 71:1 \)) and treatment efficiency of the CDF. Because excess wastewater was pumped into the CDF, it directly flowed out of the reactor without treatment while the nitrate in the wastewater remaining in the CDF was perfectly removed. Thus, the treatment efficiency here was used to represent the proportion of the CDF influent that can be treated, and the CDF effluent nitrate concentration after treatment can be
considered as zero. Due to the minority compared to the flow in Loop 1 and the minute concentration change in PON, DON, and NH$_4^+$-N in the CDF, only the NO$_3^-$-N concentration change in CDF was taken into consideration.

Mass balances for PON, DON, NH$_4^+$-N and NO$_3^-$-N in the MBBR are given by Eq. (25), (26), (27), and (28), respectively. The ammonification rate was determined by Eq. (29) (Di Toro et al., 1997), and the nitrification rate was determined by Eq. (30) (Metcalf and Eddy., 2014).

\[
V_{MBBR} \frac{dC_{MBBR-PON}}{dt} = QC_{SFS-PON} - QC_{MBBR-PON} - m_{sample-PON-2}
\]  

(25)

\[
V_{MBBR} \frac{dC_{MBBR-DON}}{dt} = QC_{FT-DON} - QC_{MBBR-DON} - r_{MBBR-afc}V_{MBBR} - m_{sample-DON-2}
\]  

(26)

\[
V_{MBBR} \frac{dC_{MBBR-A}}{dt} = QC_{FT-A} - QC_{MBBR-A} + r_{MBBR-AFC}V_{MBBR} - r_{MBBR-nfc}V_{MBBR} - m_{sample-A-2}
\]  

(27)

\[
V_{MBBR} \frac{dC_{MBBR-NT}}{dt} = Q(1 - f_{treat})C_{FT-NT} - QC_{MBBR-NT} + r_{MBBR-nfc}V_{MBBR} - m_{sample-NT-2}
\]  

(28)

\[
r_{MBBR-afc} = 0.002TC_{MBBR-PON}^*
\]  

(29)

\[
r_{MBBR-nfc} = \left[ a \left( \frac{3.3g}{m^d} \right) \frac{C_{MBBR-A}^*}{K_A + C_{MBBR-A}^*} \right] \cdot C_{MBBR-A}^* = k_{MBBR-nfc}C_{MBBR-A}^*
\]  

(30)

where $V_{MBBR}$ is the volume of MBBR (L), $r_{MBBR-afc}$ is the ammonification rate in MBBR (mg/L·d), $r_{MBBR-nfc}$ is the nitrification rate in the MBBR (mg/L·d), $Q_{SFS}$ is the flow rate from solids filter #2 (L/d), $Q_{CDF}$ is the flow rate from the CDF (L/d), and $C_{CDF}$ is the NO$_3^-$-N concentration in the effluent from the CDF (mg/L). $m_{sample-PON-2}$, $m_{sample-DON-2}$, $m_{sample-A-2}$ and $m_{sample-NT-2}$ are the PON, DON, NH$_4^+$-N and NO$_3^-$-N loss rate related to the MBBR from sampling, (mg/d), $C_{MBBR-A}^*$ is the average modified NH$_4^+$-N concentration in the MBBR based on ionic strength (mg/L), $C_{MBBR-PON}^*$ is the modified PON concentration in the MBBR based on ionic strength (mg/L), $C_{MBBR-NT}^*$ is the modified NH$_4^+$-N concentration in the MBBR based on ionic strength (mg/L), $f_{treat}$ is the
proportion of the CDF influent that can be treated, T is the temperature in the MBBR (°C), a is the specific surface area of the media for mass transfer of \( \text{NH}_4^+ \)-N in the MBBR (m\(^{-1}\)), \( K_A \) is the half saturation rate constant for nitrification in MBBRs (mg/L), and \( k_{\text{MBBR-nfc}} \) is the first order nitrification rate constant in the MBBR (d\(^{-1}\)). Note that Eq. (29) and (30) are both semi-empirical equations, 0.002 and 3.3 are parameters determined by experiments, and \( K_A \) equals to 2.2 g/m\(^3\) in Eq. (18).

4.5 CDF Models

In comparison with the fish tank, solids filter, and MBBR, the CDF is an intermittently operated reactor that receives influent only when the valve controlling Loop 2 is open. Loop 2 was operated for 10 minutes two times per day. Another distinct characteristic of the CDF is the variable flow rate of the effluent. Thus, it is important to establish a model to estimate the effluent conditions, including the flow rate and concentration of nitrate. When Loop 2 was closed, the denitrification process occurred over a 12hr HRT. Due to the configuration of the CDF, the reactor was modeled using a 1-dimensional form of the advection-dispersion equation when Loop 2 was activated and as a batch reactor when Loop 2 was closed. It was assumed that the intermittent operation provided ideal anoxic conditions for denitrification. Although DO was present in the influent, it was assumed that it was used quickly by aerobic heterotrophic and sulfur oxidizing bacteria. Thus, the model development was divided into two parts, an effluent model and a process model. A hydraulic model was developed to calculate the variable flow rates, and a NO\(_3^\)-N mass balance model was used to evaluate the NO\(_3^\)-N removal efficiency.

4.5.1 CDF Hydraulic Model

The variable effluent flow rate of the CDF resulted from the varying headloss through the media. The influent cannot rapidly flow out the reactor due to the resistance of media, which is
mainly composed of sulfur pellets, crushed oyster shells and expanded clay and contains large amounts of microbial biofilm. Given that the recirculating flow throughout the system was very low, a laminar flow pattern was assumed in the CDF. The differential equations related to headloss and Poiseuille’s law (Posieuille, 1841) are given by Eq. (31) and (32):

\[
\frac{d\Delta p}{dt} = \frac{Q - Q_E}{A} \quad (31)
\]

\[
\frac{\Delta p}{L} = \frac{32\mu v}{\rho_wgd^2} \quad (32)
\]

where, \(\Delta p\) is the headloss (m), \(Q_E\) is the effluent flow rate from the CDF (m\(^3\)/s), \(L\) is the depth of the media in the CDF (m), \(A\) is the cross-sectional area of the CDF (m\(^2\)), \(\mu\) is the dynamic viscosity, which is equal to 0.789\(\times\)10\(^{-3}\) kg/m\(\cdot\)s at 30\(^\circ\)C, \(v\) is the vertical flow velocity (m/s), \(\rho_w\) is the density of water, which equal to 995.7 kg/m\(^3\) at 30\(^\circ\)C, \(g\) is the gravitational acceleration on earth, which is equal to 9.81 m/s\(^2\), and \(d\) is the media equivalent diameter (m).

Combining Eq. (31) and (32), and solving and integrating the resulting differential equation, (Note that check the equation solution in Appendix B.), the headloss as a function of time is shown by Eq. (33) and (34).

For \(t \leq 10\) min (during the active time of Loop 2):

\[
\Delta p = \left(1 - e^{-\frac{60\rho_wgd^2}{32L\mu}t}\right) \frac{32QL\mu}{A\rho_wgd^2} \quad (33)
\]

For \(t > 10\) min (after closing Loop 2):

\[
\Delta p = \Delta p_{10\text{min}} e^{\frac{60\rho_wgd^2}{32L\mu}(t-10)} \quad (34)
\]

where \(\Delta p_{10\text{min}}\) is the headloss at \(t=10\) min.

According to Eq. (32), \(Q_E\) can be calculated by Eq. (35):

\[
Q_E = \frac{\Delta p\rho_wgd^2A}{32\mu L} \quad (35)
\]
4.5.2 CDF Kinetic Model

For developing the kinetic model, the following assumptions were made:

1. Diffusion of material to the biofilm follows Fick’s law.

2. The biofilm is evenly attached to the sulfur pellets for direct uptake of elemental sulfur because it is insoluble in water.

3. Sufficient sulfur can be utilized by the microbes, and sulfur is not a limiting factor for denitrification.

4. The water film adjacent to biofilm was ignored, and the nitrate concentration on the outer surface of biofilm equals to the nitrate concentration in the bulk liquid.

5. The denitrification rate is independent of the nitrate concentration when nitrate penetrates the biofilm to attachment surface because the denitrification rate in the biofilm can be assumed to be uniform only in presence of nitrate (Liu et al., 1994).

6. Diffusion in the vertical direction of flow is ignored.

7. DO is rapidly consumed to achieve anoxic conditions.

8. The biofilms reach steady state.

Given that diffusion only occurs when substrates in the bulk liquid penetrate biofilm, the overall mass balance located at a specific depth of biofilm under steady-state is given by Eq. (36):

\[ 0 = D \frac{\partial^2 C_{NT}}{\partial z^2} + r_{NT} \]  

(36)

where D is diffusion coefficient (m²/d), C_{NT} is the NO₃⁻-N concentration inside the biofilm (mg/L), z is spatial coordinate of biofilm (m), and r_{NT} is the NO₃⁻-N depletion rate inside the biofilm, (mg/L·d).
Based on the assumptions above, two conditions, nitrate concentrations fully penetrating the biofilm and not, were considered. The two different conditions are shown in Figure 4.5. NO₃⁻-N conversion rate along the depth of biofilm can be treated as a constant, \( k_0 \) (Liu et al., 1994).

![Diagram of nitrate distribution along the direction of concentration gradient](image.png)

Figure 4.5. Nitrate distribution along the direction of the concentration gradient. The diagram on the left shows the nitrate distribution along the direction of the concentration gradient under saturated conditions. The diagram on the right shows the nitrate distribution along the direction of concentration gradient under unsaturated conditions. Note that \( Z' \) is the maximum depth of biofilm that the NO₃⁻-N in bulk liquid can penetrate, and \( \delta \) is the thickness of the biofilm.

Based on the assumption 8 above, the reaction rate reach equilibrium with nitrate transfer. When NO₃⁻-N fully penetrates the biofilm \( \left( \frac{2DCCDF_{NT}}{k_0\delta^2} \geq 1 \right) \), the boundary conditions are:

1. NO₃⁻-N concentration at the surface of biofilm is equal to the concentration in the bulk liquid. That is:
   \[ z = 0, C_{NT} = C_{CDF-NT}^* \]

2. NO₃⁻-N concentration gradient decreases along the direction of diffusion. The diffusion will conduct nitrate through the biofilm until the gradient is equal to zero. Because no
reaction occurs after the substrate penetrates the biofilm (i.e. at the attachment surface),
the NO$_3^-$-N concentration gradient at the biofilm thickness is zero. That is:

$$z = \delta, \frac{dC_{NT}}{dt} = 0$$

Integrating Eq. (36), the nitrification kinetic model is given by Eq. (37) and (38) under this condition:

$$r_{DN} = k$$  \hspace{1cm} (37)

$$k = wk_0\delta$$  \hspace{1cm} (38)

When NO$_3^-$-N cannot fully penetrate the biofilm ($\frac{2DC^*_{CDF-NT}}{k_0\delta^2} < 1$), the boundary conditions are:

1. As indicated above, the first boundary condition is:

$$z = 0, C_{NT} = C^*_{CDF-NT}$$

2. Diffusion will conduct nitrate through the biofilm until the gradient is equal to zero.

Meanwhile, the concentration is also equal to zero, and the reaction ceases. That is:

$$z = z', \frac{dC_{NT}}{dt} = 0, z' = \left(\frac{2DC^*_{CDF-NT}}{k_0}\right)^{\frac{1}{2}}$$

Integrating Eq. (36), the nitrification kinetic model was given by Eq. (39) and (40) under this condition. Liu et al (1994) also indicated the results:

$$r_{DN} = k_{1/2}C^*_{CDF-NT}^{\frac{1}{2}} = k_{1/2}\gamma_{charge}C^*_{CDF-NT}^{\frac{1}{2}} = k_{1/2}'C^*_{CDF-NT}^{\frac{1}{2}}$$  \hspace{1cm} (39)

$$k_{1/2} = w\sqrt{2Dk_0}$$  \hspace{1cm} (40)

where, $r_{DN}$ is the denitrification rate (g/m$^3$.d), $C^*_{CDF-NT}$ is the modified NO$_3^-$-N concentration in the CDF based on ionic strength (mg/L or g/m$^3$), $k_0$ is the denitrification rate constant per unit volume of biofilm (g/m$^3$.d), $\gamma_{charge}$ is the activity coefficient for NO$_3^-$ in the RAS, $\delta$ is the thickness
of the biofilm (m), k is the modified denitrification rate constant under fully penetrating condition (g/m³·d), w is the surface area per unit volume of the media (m⁻¹), k₁/₂ is the modified denitrification rate constant under unsaturated conditions (g¹/₂/m³⁻²·d), and k₁/₂' is the observed denitrification rate constant under unsaturated conditions based on the ionic strength (g¹/₂/m³⁻²·d).

4.5.3 CDF Process Model

When developing the mass balance for the CDF during the active time of Loop 2, the denitrification was ignored due to short active time of 10 min. Thus, the general mass balance for the CDF during operation of Loop 2 is given by Eq. (41):

\[
\frac{\partial c_{CDF-NT}}{\partial t} = -v_x \frac{\partial c_{CDF-NT}}{\partial l} + D_L \frac{\partial^2 c_{CDF-NT}}{\partial l^2}
\]  

(41)

where l is the depth at a specific location in the CDF (m), D_L is the dispersion coefficient (m²/s) and v_x is the superficial velocity at specific location in the CDF (m/s).

As indicated from prior literature, dispersion coefficient is a parameter related to fluid characteristic and hardly affected by reactor geometry (Crittenden et al., 2012). Crittenden et al. (2012) provided Eq. (42) to explain the relationship between dispersion and liquid fluid in their paper.

\[
D_L = \frac{k_B T_l}{3 \pi \mu_l d_m}
\]  

(42)

where k_B is the Boltzmann’s constant, 1.381×10⁻²³ J/K, T_l is the fluid absolute temperature (K), \( \mu_l \) is the viscosity of liquid (kg/m·s), and d_m is the solute molecule diameter (m). Given that the condition of the system was steady, the dispersion coefficient can be treated as a constant.

For determining a unique solution, initial and boundary conditions must be provided. The initial condition is the condition for the CDF at the end of last cycle. Thus, it is more critical to discuss the boundary conditions. Engineering systems can be treated as closed systems when no
significant dispersion occurs before liquid enters and after discharging (Crittenden et al., 2012). Thus, at the entrance of the CDF, the boundary condition is described by:

\[ Q|_{l=0^-} = Q|_{l=0^+} - A \cdot D_L \frac{\partial C_{CDF-NT}}{\partial l}|_{l=0^+} = \frac{\partial C_{CDF-NT}}{\partial l}|_{l=0^+} = \frac{v_x}{D_L} (C|_{l=0^+} - C_{FT-NT}) \]

Similarly, at the outlet of the CDF the boundary condition is described by:

\[ Q|_{l=L^-} - A \cdot D_L \frac{\partial C_{CDF-NT}}{\partial l}|_{l=L^+} = Q|_{l=L^+} \]

However, the concentration across the outlet should show continuity, which is described by:

\[ C|_{l=L^-} = C|_{l=L^+} = \frac{\partial C_{CDF-NT}}{\partial l}|_{l=L} = 0 \]

Based on these conditions, the equation was solved using MATLAB 2016a.

The process model part for Loop 1 was utilized to estimate the nitrogen removal of the CDF. When Loop 2 was closed, the CDF was isolated from the RAS. Thus, the same depth in the isolated reactor was treated as a batch reactor. Eq. (37) and (39) were also utilized to establish mass balances in the RAS process model. Thus, using \( \frac{dC_{CDF-NT}}{dt} = -r_D N \), the residual NO\textsubscript{3}-N concentration was calculated by Eq (43) and (44).

For NO\textsubscript{3}-N fully penetrates the biofilm \((\frac{2Dc_{CDF-NT}}{k_0 \delta^2} \geq 1)\):

\[ C_{CDF-NT} = C_{FT-NT} - kt \]  
(43)

For NO\textsubscript{3}-N cannot penetrate the biofilm \((\frac{2Dc_{CDF-NT}}{k_0 \delta^2} \leq 1)\):

\[ C_{CDF-NT}^1 = \frac{C_{FT-NT}^1 \cdot \frac{1}{2} k_{1/2}}{\gamma_{charge}} t \]

\[ C_{CDF-NT}^2 = \frac{C_{FT-NT}^2 \cdot \frac{1}{2} k_{1/2}}{\gamma_{charge}} t \]

(44)

where \( C_{CDF-NT}^1 \) is the NO\textsubscript{3}-N concentration at time \( t \) in the CDF (mg/L), and \( t \) is the HRT of the CDF (d).

The NO\textsubscript{3}-N mass residual in the CDF is \((C_{CDF-NT,t} \times V_{CDF}) \) mg. Given that NO\textsubscript{3}-N was the predominant nitrogen species compared to NO\textsubscript{2}-N in the CDF, the difference between NO\textsubscript{3}-N
residual and original mass was a significant indicator, which can evaluate the nitrogen removal efficiency of the CDF.

4.6 A Model for Calculating Fate of Nitrogen (CafaN)

Before the model in the paper was developed, the CafaN had been established for the RAS (He et al., in review), and is shown in Eq. (45):

$$\frac{dm_N}{dt} = m_{feed-N} + m_{sp} - f_{Bio} m_{feed-N} - r_{DN} V_{CDF} - m_{sr} - m_{sample-N} - r_{passive} V$$ (45)

where $m_N$ is the mass of nitrogen in the RAS (mg), $m_{feed-N}$ is the nitrogen feeding rate (mg/d), $m_{sr}$ is the PON removal rate (mg/d), $m_{sample-N}$ is the nitrogen loss rate from sampling (mg/d), $r_{passive}$ is the passive denitrification rate in the fish tank (mg/L·d), $m_{sp}$ is the NO$_3^-$-N supplement rate (mg/d), $f_{Bio}$ is the fraction of the total nitrogen in fish food that is converted to biomass, $r_{DN}$ is the denitrification rate in the CDF (mg/L·d), $V$ is the volume of Loop 1 and $V_{CDF}$ is the volume of the CDF.

Live *Poecilia* bidies commonly contain approximately 75% water and 12% protein by mass (Miliou et al., 1998), and nitrogen accounts for 16% of the total crude protein in the fish feed. Thus, the efficiency of fish food utilization can be estimated by Eq. (46):

$$f_{Bio} = \frac{12\% \times n_{fish} \times \Delta m_{fish}}{50\% \times 80\% \times r_{feed} \times t_{grow}}$$ (46)

where $f_{Bio}$ is fraction of the total nitrogen in the fish food that is converted to fish biomass, $t_{grow}$ is the time for fish growth (d), $\Delta m_{fish}$ is the average change in mass for each fish during the growth period (g), $r_{feed}$ represents the feeding rate (g/d), $n_{fish}$ is the number of fish in each tank, 50% is the fraction of crude protein in the fish food, and 80% is the fraction of fish food eaten.

The PON removal rate and nitrogen loss from sampling were also estimated from the experimental data. The nitrogen loss resulting from passive denitrification was assumed to be due
to denitrification that occurred in anoxic zone in the fish tank, solids filters and MBBR. However, other nitrogen losses may have occurred such as NH$_3$ volatilization.

The parameter applied in the general mass balance model were all attained from the experimental data or literature review. Although the mass balance model was useful for understanding the fate of nitrogen in the pilot RAS, it has the limits in explaining the mechanisms and scaling up the RAS to full scale fish production. Thus, it is meaningful to develop the overall RAS process model and CDF model. The results are also compared with the newly developed models in the next chapter.

4.7 Sulfur Consumption Estimate

The project comprised of study 1 and study 2 as shown in the section 3.2. Sulfur consumption model was prepared to estimate the nitrate reduced by sulfur oxidizing bacteria because, in study 2, both sulfur and wood chip leachate worked as the electron donor. The CDF mainly involved two types of reactions related to sulfur species, sulfur oxidization (S$^0$ → SO$_4^{2-}$) and sulfate reduction (SO$_4^{2-}$→S$^2$) (That is, the sulfur disproportionation is ignored). Thus, the sulfur consumption during operation of Loop 1 can be calculated by Eq. (47):

$$S_u = (C_{sft-e} + C_{sfd-e} - C_{sft-in})V_{CDF-pore}$$ (47)

where $S_u$ is sulfur consumption during the operation of Loop 1, $C_{sft-e}$ is the CDF effluent sulfate concentration after a specific Loop 1, $C_{sfd-e}$ is the CDF effluent sulfide concentration after a specific Loop 1, $C_{sft-in}$ is the CDF effluent sulfate concentration before a specific Loop 1, and $V_{CDF-pore}$ is the pore volume of the CDF.
CHAPTER 5: RESULTS AND DISCUSSION

A comparison between the experimental data and the CDF and overall RAS process model curves are presented and discussed in this chapter. The objective of the CDF model was to predict the kinetics of denitrification and nitrogen removal efficiency. In this part, MATLAB 2016a was utilized to solve the equations for the NO$_3^-$-N concentration in the CDF effluent. The objective of overall RAS process model was to predict the steady-state nitrogen species concentrations. In this part, a matrix based on Excel was used to calibrate unknown parameters and solve for the nitrogen species concentrations. The results were compared with the model for calculating fate of nitrogen (CafaN) to verify the rationality of the models.

5.1 Calibration

Calibration data used was from RAS-A, which was one of the replicate RAS operated during the research. Model parameters used in the overall RAS process and CDF models are shown in Table 5.1 and Table 5.2, respectively. The models included three categories of parameters: calibrated, calculated and literature based. Several parameters from the literature are also shown in the two tables were not applied in the models to determine the rationality of the calibrated parameters.

In the overall RAS process model, the calibrated parameters included the ammonification rate constant in the fish tank, the nitrification rate constant in the fish tank, the porosity of the packed media in the CDF and the superficial solids removal efficiency. The calibration was conducted in Excel. Solver was used to minimize the squared residual between the modeled and experimented data. The ammonification and nitrification rate constants in the MBBR were
calculated using Eq. (29) and Eq. (30), respectively. The ammonia species distribution and activity coefficients were calculated using reaction equilibrium and ionic strength, and then used to correct the concentrations. Parameters affecting the fate of the nitrogen in the fish tank were determined from the literature. The calibrated and calculated nitrification coefficients were compared with literature values. The activity coefficients for the non-electrolytes were also obtained from the literature.

In the CDF model, as shown in Table 5.2, there were no parameters obtained by calculation. The calibrated parameters included the media equivalent diameter, dispersion coefficient in the CDF during the operation of Loop 2 and the denitrification coefficient. $R^2$ value were used to indicate the goodness of fitting between model and observed data instead of the variances used in the overall RAS process model because, during Loop 2, the CDF was operated under unsteady-state conditions. The water viscosity and density were hydraulic-related parameters obtained from the literature.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Source</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{FT-afc}, \text{d}^{-1}$</td>
<td>First order ammonification rate constant in the fish tank</td>
<td>Calibrated</td>
<td>0.0284</td>
</tr>
<tr>
<td>$k_{FT-nfc}, \text{d}^{-1}$</td>
<td>First order nitrification rate constant in the fish tank</td>
<td>Calibrated</td>
<td>4.55</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Porosity of the media in the CDF</td>
<td>Calibrated</td>
<td>0.558</td>
</tr>
<tr>
<td>$f_{SR}$</td>
<td>Superficial solids removal efficiency of the solids filters</td>
<td>Calibrated</td>
<td>11.3%</td>
</tr>
<tr>
<td>$k_{MBBR-afc}, \text{d}^{-1}$</td>
<td>First order ammonification coefficient in the MBBR</td>
<td>Calculated</td>
<td>0.5</td>
</tr>
<tr>
<td>$k_{MBBR-nfc}, \text{d}^{-1}$</td>
<td>First order nitrification coefficient in the MBBR</td>
<td>Calculated</td>
<td>240</td>
</tr>
<tr>
<td>$\gamma_{\text{charge1}}$</td>
<td>Activity coefficient for the ions with single charge</td>
<td>Calculated</td>
<td>0.736</td>
</tr>
<tr>
<td>$f_{\text{a}+}$</td>
<td>$\text{NH}_4^+$ concentration fraction to total ammonia</td>
<td>Calculated</td>
<td>0.978</td>
</tr>
<tr>
<td>$y$</td>
<td>Activity coefficient for the non-electrolyte</td>
<td>Benjamin (2015)</td>
<td>1.05</td>
</tr>
<tr>
<td>$f_{\text{PON}}$</td>
<td>Fraction of total nitrogen in the fish food that is converted to PON</td>
<td>Chen and Fornshell (2000)</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Table 5.1. (Continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Source</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{\text{DON}} )</td>
<td>Fraction of total nitrogen in the fish food that is converted to DON</td>
<td>Chen and Fornshell (2000)</td>
<td>0.1</td>
</tr>
<tr>
<td>( f_A )</td>
<td>Fraction of total nitrogen in the fish food that is converted to ( \text{NH}_4^+ )-N</td>
<td>Chen and Fornshell (2000)</td>
<td>0.4</td>
</tr>
<tr>
<td>( \mu_{\text{ni, max}}, \text{d}^{-1} )</td>
<td>Maximum specific growth rate for nitrifiers</td>
<td>Wynn and Liehr (2001)</td>
<td>0.1</td>
</tr>
<tr>
<td>( Y_{\text{ni}} )</td>
<td>Yield coefficient for nitrifiers</td>
<td>Wynn and Liehr (2001)</td>
<td>0.1</td>
</tr>
<tr>
<td>( K_{\text{ni}}, \text{mg/L} )</td>
<td>Half saturation constant for ammonium</td>
<td>Wynn and Liehr (2001)</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5.2. Parameters used in the CDF model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Source</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d, \text{m} )</td>
<td>Equivalent diameter of the media particles in the CDF</td>
<td>Calibrated</td>
<td>( 2.9 \times 10^{-5} )</td>
</tr>
<tr>
<td>( D_L, \text{m}^3/\text{s} )</td>
<td>Dispersion coefficient of in the CDF during Loop 2 operation</td>
<td>Calibrated</td>
<td>0.0051</td>
</tr>
<tr>
<td>( k_{1/2}, \text{mg}^{1/2}/\text{L}^{1/2} \cdot \text{d} )</td>
<td>Half order denitrification coefficient in the CDF</td>
<td>Calibrated</td>
<td>79.44</td>
</tr>
<tr>
<td>( \mu, \text{kg/m} \cdot \text{s} )</td>
<td>Water viscosity at 25°C</td>
<td>Crittenden et al (2012)</td>
<td>( 7.98 \times 10^{-4} )</td>
</tr>
<tr>
<td>( \rho_w, \text{kg/m}^3 )</td>
<td>Water density at 25°C</td>
<td>Crittenden et al (2012)</td>
<td>995.7</td>
</tr>
</tbody>
</table>

5.2 CDF Model Analysis

The CDF model was developed for both Loop 2 and Loop 1, and focused on the nitrate concentration. This is the key part because the nitrogen removal efficiency indicated by nitrate reduction was used to evaluate the performance of our CDF. During the operation of Loop 2, both the flow rate and nitrate concentration are functions of time, and the overall flow conditions were fit to the 1-dimesional advection and dispersion model. During the operation of Loop 1, the influent to the CDF was interrupted, and the reactor was treated as a batch reactor.
5.2.1 CDF Model Analysis during Operation of Loop 2

During the operation of Loop 2, the flow rate did not stay at a constant value and had an impact on the pattern of the flow in the CDF. In other words, the change in flow rate will alter the ratio of advection to dispersion and, consequently, affect the nitrate concentrations at the reactor outlet. The experimental data and model results for flow rate over time are shown in Figure 5.1.

![Figure 5.1](image)

Figure 5.1. The plot comparing the hydraulic model and the experimental data.

The activated time for Loop 2 was 10 min. During this period, the hydraulic head above the reactor bed increased, and water was forced to flow out more rapidly over time. After 10 min, the influent was interrupted, and the hydraulic head decreased as the CDF drained. As shown in Figure 5.1, as the water discharged, the flow rate decreased very slowly. It is estimated that the flow rate would not reach 1 ml/min after an hour. The calibrated equivalent diameter of the media particle was only 0.03 mm; however, the reactor bed porosity reaches to 0.558. The equivalent particle diameter differs from the true average diameter of sulfur pellets and clay particles because of the presence of the biofilm which clogged the pore space in the media. However, biofilms
possess an excellent capacity to hold water, which could result in a high porosity. Effluent was collected from the CDF outlet every 30 seconds and the volume of liquid (ml) collected was divided by 0.5 minute to calculate the average flow rate. The last experimental data point does not follow the model result, most likely due to data collection error. In addition, the value of the model at $t=0$ is not exactly zero because even after a cycle of 12 hours, there is still a small quantity of effluent from the CDF.

Figure 5.2 shows the relationship between the CDF effluent NO$_3$-N concentration versus time. Both modeled and observed data showed the same pattern. A low NO$_3$-N concentration was observed at the beginning of Loop 2 operation as water that had been retained in the pores of the CDF was discharged. After that, an accelerated increasing NO$_3$-N concentration was observed between 2 and 10 min as fresh RAS water mixed with treated water. Eventually, the NO$_3$-N concentration reached a constant value. The pattern fits the effluent flow rate in the hydraulic model well. The accelerated increasing NO$_3$-N concentration corresponds to the high hydraulic head when at 10 min because advection brings more concentrated wastewater from the influent to the effluent. Although the model and observed data agreed with the pattern, there was a small gap, approximately 2.7 mg/L, between the final effluent concentration. This is likely due to denitrification carried out by the sulfur oxidizing bacteria and other microbes during the active phase of Loop 2.

The goodness of fit, $R^2$, is 0.98. The dispersion coefficient was estimated as 0.0051 m$^2$/min, and the estimate for dispersion number mainly ranges from 0.39 to 1.28. The dispersion number can be determined by Eq. (48):

$$d_x = \frac{D_L}{Lv} \quad (48)$$
Commonly, the engineering processes control dispersion number is lower than 0.025 (Crittenden et al., 2012). The relatively high dispersion number indicates that dispersion is a significant process occurring in the CDF compared to advection. The initial effluent NO$_3^-$-N concentration was very low because the CDF removed almost all the nitrate during the operation of Loop 1 when the CDF was modeled as a batch reactor. After 15 min, the effluent concentration remained constant because the influent completely replaced the water held in the pore volume from the previous cycle.

![Figure 5.2. Comparison of modeled CDF effluent NO$_3^-$-N concentration model versus time with the experimental data.](image)

The NO$_3^-$-N concentration curve for the CDF effluent does not have significant meaning for the overall process of the RAS; yet, the model can be used for other similar application such
as, bioretention systems. Bioretention systems are used to treat stormwater, which contains excess nitrogen (Lynn, 2014). In these systems stormwater flow is not constant. Thus, bioretention systems are frequently used under unsteady non-ideal state conditions. Thus, it is worth studying the application of the CDF model to bioretention systems.

5.2.2 CDF Model Analysis during Operation of Loop 1

Because the nitrate concentration penetrating the single layer cell membrane of sulfur oxidizing microorganism ranges from 35 to 45 mg/L NO$_3^-$-N (Liu et. al., 1994), the NO$_3^-$-N concentration in the CDF cannot penetrate the biofilm. Thus, it was assumed that the process could be modeled using a half order reaction. The CDF effluent NO$_3^-$-N concentrations at different influent concentrations is shown in Figure 5.3.

![Figure 5.3. CDF effluent NO$_3^-$-N concentrations versus influent NO$_3^-$-N concentration for the CDF operated at different HRTs.](image-url)
The CDF effluent samples were collected at approximately 2 min after opening the Loop 2 to avoid collecting the water in the CDF outlet pipe and influent from the fish tank. After that, the CDF effluent NO$_3$-N concentration was tested and recorded as the eventual NO$_3$-N concentration of the treated water in the CDF. Due to the lack of the experimental data, $k_{1/2}$ could only be estimated at HRT=1 hr and HRT=2 hr (Note that, the data were collected from the synthetic water treatment research, stage II in Phase 1). The $k_{1/2}$ was calibrated to 79 mg$^{1/2}$/L$^{1/2}$·d, and, for the typical influent concentration of 40-45 mg/L in the RAS, the minimum time required to completely remove the nitrate is approximately 4.5-4.7 hr which is close to 4 hr, the experimental minimum time for completely depleting 45 mg NO$_3$-N/L.

5.3 Overall RAS Process Model Analysis

Figure 5.4, 5.5, 5.6 and 5.7 shows model of four nitrogen species versus their observed concentration in the fish tank. In the overall RAS process model, the system was modeled as steady state. In the RAS, the system environment in the fish tank is the most significant concern due to the high fish biomass density and toxicity of NH$_3$. To solve for the concentrations of PON, DON, NH$_4^+$-N and NO$_3$-N, the coefficients for the terms in the eight mass balance equations, Eq. (18), (19), (21), (23), (25), (26), (27) and (28), were input into a matrix. The concentrations can be easily calculated using matrix operations.
Figure 5.4. Comparison of modeled fish tank NO$_3$-N concentration versus time with experimental data.

Figure 5.5. Comparison of modeled fish tank NH$_4^+$-N concentration versus time with experimental data.
Figure 5.6. Comparison of modeled fish tank DON concentration versus time with experimental data.

Figure 5.7. Comparison of modeled fish tank PON concentration versus time with experimental data.
The overall RAS process model estimated 45.5 mg/L, 0.2 mg/L, 5.8 mg/L and 1.4 mg/L for NO$_3^-$-N, NH$_4^+$-N, DON and PON concentration in the fish tank. The experimental data was observed to fluctuate in narrow neighborhoods of 45.5±4.5, 0.2±0.1, 5.8±4.8, 1.4±0.6, respectively, which proved the validity of the overall RAS process model. The goodness of fit of the data is indicated in Figure 5.8.

![Box and whisker plot for NO$_3^-$-N, NH$_4^+$-N, DON and PON.](image)

During the research, the TAN, TN, nitrite and nitrate concentrations in the filtered samples from the fish tank were monitored. The experimental DON data were attained from the difference between the TN and total inorganic nitrogen (TIN) which mainly comprises of TAN, nitrite and nitrate. The TSS concentration in the fish tank was also monitored to calculate the PON concentration based on the measured nitrogen fraction in the sludge discharged from the solids filters. Figure 5.4 shows a regular fluctuation of NO$_3^-$-N concentration evenly distributed in the neighborhood of the fixed value predicted by the model. Similarly, the NH$_4^+$-N, DON and PON concentration also followed the same pattern as NO$_3^-$-N concentration. The fluctuations might be caused by slight changes in operating parameters such as temperature and feeding rate.
The calibrated value of $k_{\text{FT-afc}}$ and $k_{\text{FT-nfc}}$ were 0.028 and 5.6 d$^{-1}$. The calculated value of $k_{\text{MBBR-afc}}$, and $k_{\text{MBBR-nfc}}$ were 0.05 and 240 d$^{-1}$. 240 d$^{-1}$ is a rational parameter and can be validated by calculation. From literature data, the biofilm concentration in the MBBR was estimated based on Eq. (49):

$$a \cdot \frac{0.2 \text{mg/L}}{2.2 \text{mg/L} + 0.2 \text{mg/L}} \cdot \frac{3.3 \text{g}}{2 \text{g}} \cdot \frac{\text{X}}{\mu_{\text{max}} \times 0.2 \text{mg/L} / \text{Y}(K_{\text{nl}} + 0.2 \text{mg/L})} = d$$

(49)

The 0.2 mg/L was the overage NH$_4$$^+$$$-N concentration. The biofilm concentration in the MBBR was determined to 290 mg/L, and the NH$_4$$^+$$$-N flux to biofilm in the MBBR was 0.27 g/m$^2$.d.

Camstra et al. (2017) reported serial NH$_4$$^+$$$-N flux value ranging from 0.14 to 0.45 depending on the size of the MBBR and fish species. The relatively low biofilm concentration in the MBBR might be caused from the low NH$_4$$^+$$$-N concentration.

By changing different parameters, the degree of variability of NH$_4$$^+$$$-N concentration can be visually reflected. The change in ammonification rate constant had almost no effect on the predicted ammonia concentration. However, due to the sensitivity of NH$_4$$^+$$$-N concentrations to the nitrification rate constant, nitrification was suggested to be the dominant process which has a larger impact on the fish compared to ammonification.

5.4 Sensitivity Analysis

Because ammonia is toxic to fish, it is significant to know how the ammonia concentration in the fish tank response to change of the $k_{\text{FT-afc}}$, $k_{\text{FT-nfc}}$, $k_{\text{MBBR-afc}}$, and $k_{\text{MBBR-nfc}}$. By changing the value of the parameters, the concentration response is shown in Table 5.3.
Table 5.3. Fish tank response to change of the $k_{FT-nfc}$, $k_{FT-afc}$, $k_{MBBR-afc}$, and $k_{MBBR-nfc}$.

<table>
<thead>
<tr>
<th>$k_{FT-nfc}$</th>
<th>Response</th>
<th>$k_{MBBR-nfc}$</th>
<th>Response</th>
<th>$k_{FT-afc}$</th>
<th>Response</th>
<th>$k_{MBBR-afc}$</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.55</td>
<td>0.135</td>
<td>340</td>
<td>0.191</td>
<td>0.043</td>
<td>0.201</td>
<td>0.069</td>
<td>0.201</td>
</tr>
<tr>
<td>6.55</td>
<td>0.152</td>
<td>250</td>
<td>0.2</td>
<td>0.038</td>
<td>0.201</td>
<td>0.064</td>
<td>0.201</td>
</tr>
<tr>
<td>5.55</td>
<td>0.173</td>
<td>241</td>
<td>0.201</td>
<td>0.033</td>
<td>0.201</td>
<td>0.059</td>
<td>0.201</td>
</tr>
<tr>
<td>4.55</td>
<td>0.201</td>
<td>240</td>
<td>0.201</td>
<td>0.028</td>
<td>0.201</td>
<td>0.054</td>
<td>0.201</td>
</tr>
<tr>
<td>3.55</td>
<td>0.241</td>
<td>239</td>
<td>0.201</td>
<td>0.023</td>
<td>0.201</td>
<td>0.049</td>
<td>0.201</td>
</tr>
<tr>
<td>2.55</td>
<td>0.299</td>
<td>230</td>
<td>0.203</td>
<td>0.018</td>
<td>0.201</td>
<td>0.044</td>
<td>0.201</td>
</tr>
<tr>
<td>1.55</td>
<td>0.395</td>
<td>140</td>
<td>0.218</td>
<td>0.013</td>
<td>0.201</td>
<td>0.039</td>
<td>0.201</td>
</tr>
</tbody>
</table>

Based on the table above, ammonification has little effect on the ammonia concentration because of the low DON concentration and slow ammonification rate. The nitrification in the fish tank has a larger impact on the fish than in the MBBR. This may result from higher volume of the fish tank in comparison with the MBBR. The suspended solids in the fish tank can play a role as the media for microbial attached growth, and the aeration facilitated the nitrification occurred on the biofilm. Thus, microbial growth in the fish tank can help improve the environment for the aquatic animals. However, high TSS concentration also increase the oxygen consumption and compromise the water quality. A treatment limitation in the MBBR showed up at approximately 0.31 mg/L TAN with an assumption that no nitrification happened in the fish tank (i.e. $k_{FT-nfc}=0$). Thus, the volume or specific surface of the carriers should be increased under this condition. Generally, appropriate TSS concentration is favorable in the RAS.

5.5 Comparison of Nitrogen Fate with Experimental Data

As shown in the Figure 5.9, based on the overall RAS process and CDF model, the overall fate of nitrogen can be conveniently estimated: 25% removed by fish biomass uptake, 25% by solids removal, 42.5% by denitrification in the CDF, 1% by sampling and 6.5% by microorganisms assimilation or other removal processes. Note that no passive denitrification occurred throughout the RAS in the overall RAS process model. The CaFaN model indicated: 7% removed by biomass uptake, 26% by solids removal, 60% by denitrification, 1% by sampling and 6% by passive
denitrification. The two models reported 42.5% and 60% nitrogen removal by denitrification in the CDF. 7% of fish biomass uptake is much lower than the literature information. During the research, the fish bred an amount of offspring, which could be a cause leading to a lower fish measured biomass assimilation rate.

Figure 5.9. Comparison of nitrogen fate estimated by the new RAS model on the right with CafaN model on the left.
5.6 Optimal CDF HRT and Active Time

During the active time of Loop 2, more water was pumped into the CDF than that could be held in the pore volume. Therefore, a significant portion of the wastewater directly flowed out of the CDF without treatment. If the influent during the 20 min active time of Loop 2 can be completely treated by the CDF, there should be 76% of nitrogen removed by denitrification, calculated by:

\[
\frac{Q \times t_{\text{ACT}} \times C_{\text{FT-NT}}}{M_{\text{add}}} = \frac{0.22L}{\text{min}} \times \frac{20\text{min}}{d} \times \frac{45\text{mg/L}}{260\text{mg/d}}
\]

where \( t_{\text{ACT}} \) is the active time for the CDF per day (min/d), \( M_{\text{add}} \) is the nitrogen loading per day (mg/d).

However, only 42.5% nitrogen was removed by the CDF. Porosity of the media was calibrated to 0.56, which means 1.4L (2.45Lx0.56) could be held in the CDF in a single cycle. Thus, 6.4 min (\( \frac{1.4L}{0.22L/\text{min}} \)) of influent can fill up the pore space of the media. The CDF model also indicated that the influent \( \text{NO}_3^-\text{-N} \) concentration can be almost completely removed at a HRT>4hr. Therefore, the cycle can be optimized to eight hours with a new 7 min of active time for the Loop 2 three times per day. This strategy will enhance the nitrogen removal to more than 70% and increase the allowable RAS stocking density.

5.7 Full-scale System Design

Some full-scale RAS facilities has established worldwide. Revivim catfish farm is a pioneer producer in the fishery industry and realized a super high-intensive catfish cultivation in a RAS with a stocking density of 300 kg/m\(^3\) (Appelbaum, 2011). In comparison, Mote system has a much lower stocking density which is approximately 22 kg/m\(^3\). The final goal of our model is to design a full-scale system and predict its performance. Based on a spreadsheet developed by staff
at Mote Aquaculture Research Park, a spreadsheet (Table 5.4) was established to design a full-scale RAS with a same configuration of our pilot one. The blue terms in the table represent the fixed values of the parameter from experimental, literature or calibrated data in this thesis.

Table 5.4. Excel spreadsheet for full-scale design of RAS.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microbial parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$k_{FT-afc}, d^{-1}$</td>
<td>0.028359109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$k_{MBBR-afc}, d^{-1}$</td>
<td>0.054</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$k_{FT-nfc}, d^{-1}$</td>
<td>4.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$k_{MBBR-nfc}, d^{-1}$</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Value</td>
<td>Unit</td>
<td>Calculation Formula</td>
<td>Note</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Fish Tank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Tank water depth</td>
<td>1 m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Tank radius</td>
<td>3 m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Tank volume</td>
<td>28.27431388 m^3</td>
<td></td>
<td>B8<em>2</em>PI()</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Desired biomass density</td>
<td>22 kg/m^3</td>
<td></td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Fish biomass</td>
<td>622.0353454 kg</td>
<td></td>
<td>B9*B10</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Feeding rate of body weight</td>
<td>3% /d</td>
<td></td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Feeding rate</td>
<td>18.66106036 kg/d</td>
<td></td>
<td>B11*B12</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Turnover time</td>
<td>37.87878788 min</td>
<td></td>
<td>B9*1000/B32</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Nitrogen species calculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Fish food protein content</td>
<td>50%</td>
<td></td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>% of TAN from fish food</td>
<td>3.20%</td>
<td></td>
<td>50%*16%*40%</td>
<td>16% is the nitrogen content in crude protein</td>
</tr>
<tr>
<td>19</td>
<td>Desired TAN concentration</td>
<td>0.6 mg/L</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>TAN from fish food</td>
<td>0.597153932 kg/d</td>
<td></td>
<td>B17*B13</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Passive nitrification</td>
<td>10%</td>
<td></td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Real TAN loading to MBBR</td>
<td>0.537438538 kg/d</td>
<td></td>
<td>B19*(1-B20)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Desired nitrate concentration</td>
<td>50 mg/L</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Minimum TAN concentration in the MBBR</td>
<td>0.1 mg/L</td>
<td></td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>MBBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Estimate of nitrification rate</td>
<td>84 mg/L-d</td>
<td></td>
<td>D3*B18</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Media volume required</td>
<td>6.398077839 m^3</td>
<td></td>
<td>B21/B26*1000</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Maximum HRT</td>
<td>0.005952381 d</td>
<td></td>
<td>(B18-B23)/B26</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Flowrate</td>
<td>1074.877077 m^3/d</td>
<td></td>
<td>(B18-B23)/B26<em>24</em>60</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>HRT</td>
<td>746.4424145 L/min</td>
<td></td>
<td>B27/B28</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Media density</td>
<td>1 m</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>MBBR radius</td>
<td>1.427084941 m</td>
<td></td>
<td>5*SQRT(B28/B33)/PI()</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>CDF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Porosity</td>
<td>0.6</td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Volume required</td>
<td>6.635043684 m^3</td>
<td></td>
<td>B20/B23*1000/B37/3</td>
<td>3 means that Loop 2 open for 3 times each day</td>
</tr>
<tr>
<td>36</td>
<td>HRT</td>
<td>8 hr</td>
<td></td>
<td>24/3</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Minimum activated time</td>
<td>5.333333333 min</td>
<td></td>
<td>B38<em>B37/B31</em>24*60</td>
<td>i.e. 8 min may be more reasonable because it can ensure that treated water in the CDF is completely squeezed out</td>
</tr>
<tr>
<td>38</td>
<td>Height/radius</td>
<td>4</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>CDF radius</td>
<td>0.808248004 m</td>
<td></td>
<td>POWER(B38/PI(),1/3)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>CDF Height</td>
<td>3.232992016 m</td>
<td></td>
<td>4*B42</td>
<td></td>
</tr>
</tbody>
</table>

When increasing the stocking density to 300 kg/m^3, the MBBR and CDF will require an approximate volume of 90 m^3 that is over three times the size of the fish tank, and the flowrate also increase to over 10000 L/min, which means that the capital and power cost will become a big concern. Thus, the tradeoff between production and investment should be analyzed in the future.
CHAPTER 6: CONCLUSIONS AND RECOMMENDATION FOR FUTURE RESEARCH

The cyclic denitrification filter (CDF) is a highly efficient reactor for nitrogen removal when being incorporated into a recirculating aquaculture system (RAS). Sulfur-based denitrification is a type of autotrophic biological reduction process which possesses several advantages, compared to heterotrophic denitrification, such as less sludge production, no extra carbon source and low cost.

Models for the RAS was developed, calibrated and used to provide a prediction of nitrogen species concentrations and nitrogen removal efficiency in the RAS and CDF. The models are divided into the overall RAS process model and CDF model. The primary goal of the overall RAS process model is to estimate the particulate organic nitrogen (PON), dissolved organic nitrogen (DON), NH$_4^+$-N and NO$_3^-$-N concentrations in the fish tank. The CDF model was developed for Loop 1 and Loop 2. During the operation of Loop 1, the CDF was disconnected from the RAS, and treated the wastewater remained in the reactor. During the operation of Loop 2, the fresh influent was pumped from the fish tank into the CDF to replace the treated water. The primary goal of the CDF model was to monitor the CDF effluent NO$_3^-$-N concentration and nitrogen removal efficiency. The main findings are:

- In comparison with the fish tank, a more intensive nitrification occurred in the MBBR. The first order denitrification rate constant was 240 d$^{-1}$ in MBBR and 5.38 d$^{-1}$ in the fish tank.
The PON, DON, NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}--N concentrations were expected to fluctuate within a narrow neighborhood of a value predicted by the model under near steady-state conditions. In the RAS, the NH\textsubscript{4}\textsuperscript{+}-N concentration was estimated at approximately 0.2 mg/L.

The models predicted a smaller media diameter in the CDF compared to sulfur pallets and expanded clay packed in the CDF, and relatively high porosity because the biofilms clogged the pore space in the media; however, biofilms possess an excellent capacity to hold water, which could result in a high porosity.

The NO\textsubscript{3}--N concentration cannot fully penetrate the denitrifying biofilm formed in the CDF, and denitrification carried out by the biofilm that follows a half order reaction.

The CDF model can be used to estimate minimum time, approximately 4.5hr, for the RAS, for complete consumption of specific influent NO\textsubscript{3}--N concentration.

For the CDF and other similar systems, dispersion is a significant process, compared to advection, when liquid flows through the reactor.

The overall RAS process model and CDF model was also used to estimate the nitrogen fate in the RAS and compared with the previously developed model for calculating the fate of nitrogen (CafaN). The main findings are:

- The CDF has a high nitrogen removal efficiency accounting for over 40% of the total nitrogen.

- The estimates of the models are affected by the parameters used in the models such as fish biomass nitrogen and feed conversion ratios. The difference of biomass nitrogen-nitrogen feeding conversion ratio between experimental and literature data cause a large gap in denitrification rate estimated by the CafaN and the new models.
• The reproduction of the fish compromises the accuracy of measurement of fish biomass growth rate.

Results of the new developed model in the paper was also used to optimize the CDF HRT and active time:

• The cycle can be optimized to eight hours with a new 7 min of active time for the Loop 2 three times per day. This would enhance the CDF nitrogen removal efficiency to 70% and allow the system to support a larger fish production rate.

Finally, our RAS and research still have some places to be improved. Six recommendations are provided below:

• Change the CDF configuration to a upflow submerged bed reactor to mitigate the congestion in the media.

• Improve the solids removal reactor to reduce the TSS concentration in the fish tank.

• Research on how to address the sulfide production and sulfate accumulation in the RAS.

• Research on the oxygen transfer and consumption in the RAS.

• Analyze the tradeoff between fish production and investment in a full-scale RAS.

• Carry out fundamental study on unit process to help validate our model. (i.e. tracer study on the CDF during Loop 2)
REFERENCES


APPENDIX A: LIST OF SYMBOLS

Table A.1. List of symbols.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physical Meaning</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C^*_{\text{FT-DON}}$</td>
<td>the modified DON concentration in the fish tank under ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C^*_{\text{FT-A}}$</td>
<td>the modified $\text{NH}_4^+\text{-N}$ concentration under ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C^*_{\text{FT-NT}}$</td>
<td>the modified $\text{NO}_3^-\text{-N}$ concentration in the fish tank under ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C^*_{\text{MBBR-PON}}$</td>
<td>the modified PON concentration in the MBBR based on ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C^*_{\text{MBBR-A}}$</td>
<td>the modified $\text{NH}_4^+\text{-N}$ concentration in the fish tank under ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$\overline{C^*_{\text{FT-A}}}$</td>
<td>the average modified $\text{NH}_4^+\text{-N}$ concentration based on ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$\overline{C^*_{\text{MBBR-A}}}$</td>
<td>the average modified $\text{NH}_4^+\text{-N}$ concentration in the MBBR based on ionic strength</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{CDFe}}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration in the effluent from the CDF</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{CDF-NT}}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration in the CDF</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{CDF-NT,}t}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration at HRT of $t$ in the CDF</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{FT-PON}}$</td>
<td>the PON concentration in the fish tank</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{FT-DON}}$</td>
<td>the DON concentration in the fish tank</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{FT-A}}$</td>
<td>the $\text{NH}_4^+\text{-N}$ concentration in the fish tank</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{FT-TN}}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration in the fish tank</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{MBBR-PON}}$</td>
<td>PON concentration in the MBBR</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{MBBR-DON}}$</td>
<td>the DON concentration in MBBR</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{MBBR-A}}$</td>
<td>the $\text{NH}_4^+\text{-N}$ concentration in the MBBR</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{MBBR-NT}}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration in the MBBR</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{NT}}$</td>
<td>the $\text{NO}_3^-\text{-N}$ concentration inside the biofilm</td>
<td>mg/L</td>
</tr>
<tr>
<td>$C_{\text{SFs-PONe}}$</td>
<td>the PON concentration in the effluent from filter #2</td>
<td>mg/L</td>
</tr>
<tr>
<td>$K_{\text{ni}}$</td>
<td>the half saturation constant for $\text{NH}_4^+\text{-N}$</td>
<td>mg/L</td>
</tr>
<tr>
<td>$K_A$</td>
<td>the half saturation rate constant for nitrification</td>
<td>mg/L</td>
</tr>
<tr>
<td>$X$</td>
<td>the nitrifier biomass concentration in the fish tank</td>
<td>mg/L</td>
</tr>
<tr>
<td>$f_{\text{PON}}$</td>
<td>the fraction of the total nitrogen in the fish food that converted to PON</td>
<td></td>
</tr>
<tr>
<td>$f_{\text{DON}}$</td>
<td>the fraction of the total nitrogen in the fish food that converted to DON</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Physical Meaning</td>
<td>Unit</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>------</td>
</tr>
<tr>
<td>$f_A$</td>
<td>the fraction of the total nitrogen in the fish food that converted to $\text{NH}_4^+-\text{N}$</td>
<td></td>
</tr>
<tr>
<td>$f_{\text{SR}}$</td>
<td>the solids removal efficiency of the two filters</td>
<td></td>
</tr>
<tr>
<td>$f_{\text{treat}}$</td>
<td>the proportion of the CDF influent that can be treated</td>
<td></td>
</tr>
<tr>
<td>$Y_{ni}$</td>
<td>yield coefficient for nitrifiers</td>
<td></td>
</tr>
<tr>
<td>$\mu_{ni,max}$</td>
<td>the specific maximum growth rate for nitrifiers</td>
<td>$d^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{FT-afc}}$</td>
<td>the first order ammonification rate constant in the fish tank</td>
<td>$d^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{FT-nfc}}$</td>
<td>the first order nitrification rate constant in the fish tank</td>
<td>$d^{-1}$</td>
</tr>
<tr>
<td>$k$</td>
<td>the modified denitrification rate constant under fully penetrating condition</td>
<td></td>
</tr>
<tr>
<td>$k_0$</td>
<td>the denitrification rate constant per unit volume of biofilm</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$k_{1/2}$</td>
<td>the modified denitrification rate constant under unsaturated condition</td>
<td>mg$^{1/2}$/L$^{1/2}$·d</td>
</tr>
<tr>
<td>$m_{\text{feed-N}}$</td>
<td>the nitrogen feeding rate</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-PON-1}}$</td>
<td>the PON loss rate from the fish tank from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-DON-1}}$</td>
<td>the DON loss rate to the fish tank from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-A-1}}$</td>
<td>the $\text{NH}_4^+-\text{N}$ loss rate to the fish tank from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-NT-1}}$</td>
<td>the NO$_3^-$-N loss rate related to the fish tank from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-PON-2}}$</td>
<td>the PON loss rate from the MBBR from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-DON-2}}$</td>
<td>the DON loss rate to the MBBR from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-A-2}}$</td>
<td>the $\text{NH}_4^+-\text{N}$ loss rate to the MBBR from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sample-NT-2}}$</td>
<td>the NO$_3^-$-N loss rate related to the MBBR from sampling</td>
<td>mg/d</td>
</tr>
<tr>
<td>$m_{\text{sp}}$</td>
<td>the NO$_3^-$-N supplement rate</td>
<td>mg/d</td>
</tr>
<tr>
<td>$Q$</td>
<td>the flow rate throughout Loop 1</td>
<td>L/d</td>
</tr>
<tr>
<td>$Q_{\text{CDFe}}$</td>
<td>the flow rate from the CDF</td>
<td>L/d</td>
</tr>
<tr>
<td>$Q_{\text{SFc}}$</td>
<td>the flow rate from the solids filter #2</td>
<td>L/d</td>
</tr>
<tr>
<td>$Q_E$</td>
<td>the effluent rate from the CDF</td>
<td>m$^3$/s</td>
</tr>
<tr>
<td>$r_{\text{FT-afc}}$</td>
<td>the ammonification rate in the fish tank</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$r_{\text{FT-nfc}}$</td>
<td>the nitrification rate in the fish tank</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$r_{\text{MBBR-afc}}$</td>
<td>the ammonification rate in MBBR</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$r_{\text{MBBR-nfc}}$</td>
<td>the nitrification rate in MBBR</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$r_{\text{DN}}$</td>
<td>the dentrification rate</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$r_{\text{NT}}$</td>
<td>the NO$_3^-$-N depletion rate inside the biofilm</td>
<td>mg/L·d</td>
</tr>
<tr>
<td>$v$</td>
<td>the vertical flow velocity</td>
<td>m/s</td>
</tr>
<tr>
<td>$V_{\text{FT}}$</td>
<td>the volume of fish tank</td>
<td>m$^3$</td>
</tr>
<tr>
<td>$V_{\text{MBBR}}$</td>
<td>the volume of MBBR</td>
<td>m$^3$</td>
</tr>
<tr>
<td>$A$</td>
<td>the cross-sectional area of the CDF</td>
<td>m$^2$</td>
</tr>
<tr>
<td>$L$</td>
<td>the depth of the media in the CDF</td>
<td>M</td>
</tr>
<tr>
<td>$l$</td>
<td>the depth of specific location of the CDF</td>
<td>M</td>
</tr>
<tr>
<td>$z$</td>
<td>the depth of biofilm</td>
<td>M</td>
</tr>
<tr>
<td>Parameter</td>
<td>Physical Meaning</td>
<td>Unit</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>------</td>
</tr>
<tr>
<td>δ</td>
<td>the thickness of the biofilm</td>
<td>m</td>
</tr>
<tr>
<td>Δp</td>
<td>the headloss</td>
<td>m</td>
</tr>
<tr>
<td>d</td>
<td>the media grain equivalent diameter</td>
<td>m</td>
</tr>
<tr>
<td>a</td>
<td>is the specific surface area of the media for mass transfer of NH₄⁺-N in the MBBR</td>
<td>m⁻¹</td>
</tr>
<tr>
<td>T</td>
<td>the temperature in the MBBR</td>
<td>°C</td>
</tr>
</tbody>
</table>
APPENDIX B: SOLUTION FOR HYDRAULIC DIFFERENTIAL EQUATION

The differential equations related to headloss and Poiseuille’s law (Posieuille, 1841) are given by Eq. (31) and (32):

\[ \frac{d\Delta p}{dt} = \frac{Q - Q_E}{A} \]  \hspace{1cm} (31)

\[ \frac{\Delta p}{L} = \frac{32\mu v}{\rho g d^2} \]  \hspace{1cm} (32)

Using the relationship, \( v = \frac{Q_E}{A} \), combine Eq. (31) and (32), and determine the differential equation (*):

\[ \frac{d\Delta p}{A \frac{\rho g d^2}{32L\mu - \Delta p}} = dt \]  \hspace{1cm} (*)

For \( t \leq 10 \) min (during the active time of Loop 2), the initial headloss, \( \Delta p_{in} \), was treated as zero:

\[ \int_{0}^{\Delta p_t} \frac{d\Delta p}{A \frac{\rho g d^2}{32L\mu - \Delta p}} = \int_{0}^{t} dt, \ t \in [0, 10], \text{ where } \Delta p_t \text{ is the headloss at time } t, \rightarrow \]

\[ \rightarrow \Delta p = \left(1 - e^{-\frac{60\rho g d^2}{32L\mu}}\right) \frac{32QL\mu}{A \rho g d^2} \]  \hspace{1cm} (33)

For \( t > 10 \) min (after closing Loop 2), the influent, \( Q \), was cut off (That is, \( Q = 0 \)), and the initial headloss, \( \Delta p_{in} \), equaled to that at \( t=10 \) min:

\[ \int_{10}^{\Delta p_t} \frac{d\Delta p}{\frac{\rho g d^2}{32L\mu}} = \int_{0}^{t} dt, \ t \in (10, \infty], \rightarrow \]

\[ \rightarrow \Delta p = \Delta p_{10 \min} e^{\frac{60\rho g d^2}{32L\mu}(t-10)} \]  \hspace{1cm} (34)

where \( \Delta p_{10 \min} \) is the headloss at \( t=10 \) min.
APPENDIX C: MATLAB CODE

clear
clc
%establish array-------------------------------------------
nt=zeros(2501,21);
ntds=zeros(2501);
%----------------------------------------------------------
%assignment zone-------------------------------------------
var=0; vards=0;
%parameter assignment
nt0=0; E=0.001; Cin=45; Q=3.67*10^-6;
vis=7.98*10^-4; pw=995.7; g=9.81; L=0.2;
A=0.003068; d=3*10^-5;
%mesh ratio
dt=0.01; dl=0.01;
r1=dt/dl; r2=dt/dl^2;
%initial concentration assignment
nt(1,:)=nt0;
%observed data
i= [0 1 2 3 5 7 9 11 13 15 17 19];
j= [1.01 0.35 3.32 5.95 21.28 31.15 37.38 39.66 40.09 42.26 42.28 41.88];
%----------------------------------------------------------
%main procedure--------------------------------------------
while (var<vards)||(E<=0.0011)
   E=E+0.0001;
   %data storage
   vards=var;
   ntds=nt(:,21);
   %forward difference
   for n=(2:2501)
      if n<=1001
         v=(1-exp(-(60*pw*g*d^2*(n-1)*dt)/(32*L*vis)))*100*Q/A;
      else
         v=(1-exp(-(600*pw*g*d)/(32*L*vis)))*100*Q/A*exp
            (-60*pw*g*d^2*((n-1)-10)*dt)/(32*L*vis));
      end
      nt(n,1)=(1-2*E*r2*(1+v/E*dl)+v*r1)*nt(n-1,1)+(2*E*r1)*nt(n-1,2)+2*r2*dl*v*Cin;
   end
end
for m=(2:20)
    nt(n,m)=(E*r2-v/2*r1)*nt(n-1,m+1)+(1-2*E*r2)*nt(n-1,m)+(E*r2+v/2*r1)*nt(n-1,m-1);
end
nt(n,21)=(1-2*E*r2-v*r1)*nt(n-1,21)+(2*E*r2+v*r1)*nt(n-1,20);
end
% variance test
var=0;
for n=(1:12)
    var=var+(nt(i(n)/dt+1,21)-j(n))^2;
end
var=var/11;
end
% calculate the goodness of fitting
ave=mean(j);jsq=0;
for n=(1:12)
    jsq=jsq+(ave-j(n))^2;
end
Rsq=1-vards*11/jsq;
% plot and output
x=0:0.01:20;
figure;
cdscatter(i,j);
hold on;
plot(x,ntds(1:2001));
E=E
Rsq=Rsq
xlabel('Time, min');
ylabel('Nitrate-N concentration, mg/L');
legend('Observed data','CDF model');
%
APPENDIX D: COPYRIGHT PERMISSION

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Rushika