NITRIFICATION AND THE IMPACT OF ORGANIC MATTER IN FIXED-FILM BIOFILTERS: APPLICATION TO RECIRCULATING AQUACULTURE SYSTEMS

By

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To the Faculty of Washington State University:

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Abstract

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Inhibition is significant within fixed-film nitrification process in wastewater treatment systems with high carbonaceous material due to the competition between heterotrophic and nitrifying bacteria for limited oxygen and space. The inhibition of organic matter to the nitrification process becomes more critical in recirculating aquaculture systems that contain a low concentration of ammonia and a high concentration of organic matter. Quantitative information on the effect of organic matter upon nitrification is insufficient. Additionally, application of results from pure culture systems to biofilter design can lead to inaccurate estimations. In this research, the effect of organic matter on nitrification biofilters was investigated experimentally with a lab-scale reactor series system and theoretically with a mathematical biofilm model. To extend this research to aquaculture system design and operation, the results from the lab-scale study were compared with pilot and commercial scale systems and biofilter design recommendations were provided for cold water aquaculture systems. The results from these studies showed that the biofilter nitrification rate decreased exponentially with increases in COD/N ratio although

the degree of inhibition on nitrification varied with different types of biofilters. Taking into account these results, a mathematical biofilm model was developed to demonstrate the inhibition due to addition of organic matter on nitrification and a simplified analytical solution was obtained for practical applications. A correction factor of 0.2~1.0 representing the effect of organic matter and a correction factor of 0.2~0.9 associated with the effect of system scale-up were recommended when the nitrification design equations resulted from a pure culture measurement were applied in the design of commercial scale biofilters.

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Dedication

This dissertation is dedicated to my grandma, my mother and father, and my beloved husband.

CHAPTER ONE

INTRODUCTION

1.1. Background

The Nitrogen cycle is one of the most important nutrient cycles that go on within the confines of aquatic and terrestrial ecosystems. It starts with nitrogen in the atmosphere, which can become a part of biological matter and be converted to ammonia through the nitrogen fixation process. Then, ammonia nitrogen can be further converted to nitrite and nitrate by nitrifying bacteria in the process known as nitrification. A reverse process known as denitrification completes the nitrogen cycle by converting nitrate back to nitrogen gas along with some other side products, such as nitrous oxide (N_2O) and nitric oxide (NO).

Within the nitrogen cycle, the nitrification process has been playing a significant role in water quality management (Flora et al., 1999). Ammonia-N concentration is usually regulated by federal or state discharge standards because of its toxicity to aquatic life and problem of eutrophication or plankton bloom in the receiving water (Grady et al., 1999). Ammonia nitrogen, un-ionized ammonia (NH_3) more exactly, is extremely toxic to aquaculture species even at a low concentration. Removal of ammonia nitrogen from aquaculture wastewater therefore is one of the major challenges in the development of water recirculating aquaculture systems (RAS) (Wheaton et al., 1994).

Fixed film biofilters, also called attached growth reactors, play a major role in terms of ammonia removal in both domestic and industrial wastewater treatment (Gujer and Boller, 1986; Lazarova and Manem, 1995; Nogueira et al., 2002; Wheaton et al., 1994). Since the first trickling filter using rock-packed filter media was introduced in the late 1800s (Bovendeur, 1991), different types of fixed film bioreactors have been developed and applied in wastewater treatment (Sharma and Ahlert, 1977; Gujer and Boller, 1986; Furumai and Rittmann, 1994; Cheng and Chen, 1994; Lazarova and Manem, 1995; Liu and Capdeville, 1996; Nogueira et al., 2002). However, information on process mechanism and kinetics relative to nitrification biofilters applied to aquaculture systems is still insufficient, particularly for cold water RAS, which operate at low temperature but with high water quality requirements. Simply employing data from traditional wastewater treatment processes to the design of aquaculture biofilters is not appropriate as nitrification conditions in aquaculture systems differs from domestic and industrial wastewater. For example, compared to domestic and industrial wastewater, aquaculture wastewater usually has a much lower ammonia substrate concentration, with total ammonia nitrogen (TAN) values of 20~50 mg l^{-1} in typical untreated domestic wastewater, 100~800 mg l^{-1} in septage systems (Tchobanoglous and Burton, 1991), and less than 1 and 3 mg l^{-1} of TAN for rainbow trout and catfish aquaculture systems, respectively (Wedemeyer, 2001). Water quality criteria for ammonia are typically written in terms of un-ionized ammonia because of its toxicity. Long term exposure to un-ionized ammonia nitrogen (NH_3-N) of 0.05~0.2 mg l^{-1} are capable of significantly reducing the growth of salmonids. Optimal growth requires an NH_3-N concentration of less than 0.01~0.03 mg l^{-1} for salmonids (Wedemeyer, 2001).

Recirculating aquaculture wastewater normally contains relatively high levels of organic matter. Zhu and Chen (2001) assumed a carbon to nitrogen (C/N) ratio of 2 in aquaculture systems based on a calculation for a typical type of fish feed with 12% of soluble BOD_5 and 3% of TAN. For the water quality measurement of a recirculating system raising yellow perch, the concentration of organic matter was reported as high as 30~60 mg l^{-1} of $CBOD_5$ (Carbonaceous 5-day biochemical oxygen demand) and 10~20 mg l^{-1} of DOC (dissolved organic carbon) (Hall et al., 2002). Summerfelt (2004) also detected high DOC concentrations of 2.7~8.1 mg l^{-1} at the effluent of a full scale fluidized sand filter in a recirculating trout system. Without separate units for biochemical oxygen demand (BOD) treatment, nitrification biofilters used in recirculating aquaculture systems usually have a much lower nitrification rate than those in systems with pure nitrification conditions or with a lower level of organic matter. It was reported that the nitrification rate of submerged filters could decrease 70% with a C/N ratio of 1 or 2 as compared with a pure nitrification system (Zhu and Chen, 2001).

The interaction between a low ammonia and a relatively high organic carbon concentration and associated nitrification inhibition therefore becomes an important consideration for the design and optimization of nitrification biofilters in recirculating aquaculture systems (RAS). Research efforts addressing this issue will be beneficial to both the aquaculture industries and the aquaculture engineering communities.

1.2. Nitrification Kinetics of biofilm

The nitrification process is described as a two-step process, by which toxic ammonia is first oxidized into nitrite (NO_2^-) by *Nitrosomonas sp.* and nitrite is then oxidized to the much less toxic nitrate (NO_3^-) by *Nitrobacter sp*. Equations 1 and 2 show the basic chemical conversions occurring in a nitrification process (USEPA, 1975; WPCF, 1983).

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- + 58\sim84 \text{ Kcal} \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- + 15.4\sim20.9 \text{ Kcal} \quad (2)$$

Given a general chemical expression $C_5H_7O_2N$ for the nitrifying bacteria, a complete nitrification process can be expressed by the following equation (USEPA, 1975):

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (3)$$

This equation can be used for the estimation of oxygen and alkalinity requirements as well as biomass production from the nitrification process. For every gram of TAN oxidized to nitrate nitrogen, approximately 4.57 g of oxygen and 7.07 g of alkalinity (as $CaCO_3$) are consumed and 0.17 g of bacteria biomass is produced.

Compared to suspended growth system, the nitrification kinetics in a fixed-film system (attached growth) is more complex, as the substrate supply into the layer-like aggregation of bacteria film is a diffusion-controlled process driven by concentration gradient across the biofilm (Figure 1.1). Next to the biofilm there is a water film that serves as the

interface between the biofilm and the bulk water. Diffusion resistance occurs at the water biofilm and the kinetics of biofilm reactions is influenced greatly by mass transport (Rasmussen and Lewandowski, 1998). Nitrification rate in a biofilm is then best described as an equilibrium system between substrate demand created by the growth of bacteria biomass and the rate of substrate supply determined by diffusion transport limitation (Rasmussen and Lewandowski, 1998). The substrate demand is determined by the factors that are related to the characteristics of nitrifiers such as the amount of nitrifier biomass, the specific growth rate and yield coefficient. The substrate supply is determined by the transport of essential nutrients. Factors that determine mass transport rate, such as the local chemical environment and flow conditions, influence the rate of substrate supply and subsequently, the extent of biofilm growth. Therefore, the diffusion and transport process should be considered in addition to factors associated with bacterial metabolism in order to better understand the nitrification kinetics of fixed-film biofilters.

1.3. Effect of organic matter on fixed film nitrification process

The most significant impact of organic material upon nitrification is attributed to additional oxygen demand by heterotrophic bacteria. With the addition of organic matter, fast-growing heterotrophic bacteria, which use organic carbon as their energy source, out compete slow-growing nitrifying bacteria, resulting in a decrease in the nitrification rate. It was reported that heterotrophic bacteria have a maximum growth rate of five times and yields of two to three times that of autotrophic nitrifying bacteria (Grady and Lim, 1980). Zhang et al. (1995) used a microelectrode technique and a micro-slicing technique to

study the competition between heterotrophs and autotrophs for substrate and space. It was found that an increase of the organic loading rate would result in a decrease of DO in the biofilm thereafter inhibiting the nitrification rate due to the shortage of oxygen. The presence of organics also affects the composition of the microbial population and the proportion of nitrifiers decreased with an increasing C/N ratio (Ohashi et al., 1995; Satoh et al., 2000). Okabe et al. (1996) also discovered that a higher influent C/N ratio retarded accumulation of nitrifying bacteria and resulted in a considerably longer start-up period for nitrification.

The C/N ratio therefore is considered one of the main parameters in the design of nitrification processes for wastewater treatment (Carrera et al., 2004). In recirculating aquaculture systems, the fecal material excreted by fish and uneaten feed are organic in nature and thus provide substrates for heterotrophic bacteria which results in significant inhibition on the performance of nitrification biofilters. Quantifiable information on the effect of organic matter upon biofilter nitrification rate subsequently becomes critical to the design of the nitrification process in RAS.

1.4. Mathematical modeling for nitrification biofilm process

Mathematical models have been provided as useful tools for the understanding of the nitrification biofilm process. In contrast to one-dimensional and single species biofilm models developed much earlier, multi-dimensional and multi-species biofilm models have more recently been developed to describe the heterogeneous biofilm structures and

interactions between multiple organism species in biofilms (Noguera et al., 1999; Picioreanu, et al., 1998; Xavier et al., 2004; Laspidou and Rittmann, 2004). The multi-dimensional biofilm models have provided a precise and circumstantial description on biofilm structures and have proved useful as a research tool. However, they have also added to the complexity of model computation, and with too much detailed information included, the complex multi-dimensional models may not be suitable for application to a full-scale biofilm reactor (Morgenroth et al., 2000). For the design of nitrification biofilters in wastewater treatment industries, the practioners paid more attention to the outputs of biofilm models on a "macro" scale, such as the substrate flux through the biofilm, than outputs on a "micro" scale, such as biofilm characterization. Comparison study on multi-species models showed that one-dimensional models could provide very similar results as multi-dimensional biofilm models in terms of bulk substrate concentrations and fluxes into the biofilm (Noguera and Picioreanu, 2004). Simple one-dimensional biofilm models with the advantages for industry application then recaptured the attention of researchers, especially since analytical solutions were difficult to obtain on multi-dimensional biofilm models.

An integrated biofilm model has been developed for nitrification biofilters in water treatment with pseudo-analytical solutions, but competition between bacterial groups was not included and this model was limited to situations where species interaction is not important (Rittmann and Stilwell, 2002). For the nitrification process in aquaculture wastewater treatment, the competition between autotrophs and heterotrophs is intense and

has to be considered. Simplified biofilm models accounting for bacteria competition with computation simplicity are needed for guiding biofilter design in RAS applications.

1.5. Objectives of this research

The overall goal of this dissertation research is to provide technical information on the design and optimization of the fixed-film nitrification process for ammonia removal from aquaculture wastewater, which has low ammonia concentration and relatively high concentration of carbonaceous material. The specific objectives of this research include:

1) Quantifying the impact of organic matter on nitrification rates of different biofilters;

2) Developing a simplified biofilm model for the nitrification process in RAS; and

3) Providing nitrification biofilter design recommendations for cold water RAS.

1.6. Structure of this dissertation

This dissertation is organized in the Washington State University manuscript format. Three manuscripts that reflect the three specific objectives and a general conclusion follow this introductory chapter. The three manuscripts are either previously published, or prepared for publication in peer-reviewed journals.

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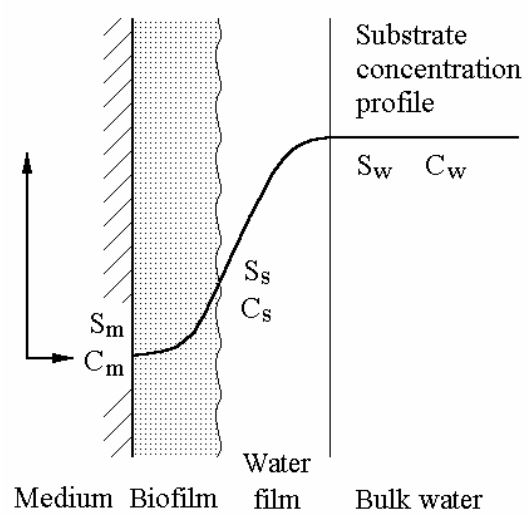

Figure 1.1. Conceptual profile of a biofilm

CHAPTER TWO

IMPACT OF ORGANIC CARBON ON NITRIFICATION PERFORMANCE OF DIFFERENT BIOFILTERS

2.1. Abstract

Nitrification rate as a function of total ammonia nitrogen (TAN) concentration, with and without the interaction of organic matter, was investigated for three types of biofilters of laboratory scale: floating bead filter, fluidized sand filter, and submerged bio-cube filter. The performance of each type of biofilter was evaluated using a 5-reactor series with synthetic solutions containing different carbon/nitrogen ratios (C/N=0, 0.5, and 2.0). The tests were run at representative cold water aquaculture system temperatures of 15 ℃ and 20 ℃. The experimental results showed, within the lower total ammonia concentration range, a first-order nitrification rate with a highly linear regression for all three types of biofilters without the interaction of organic carbon at both test temperatures. However, with the addition of organic carbon, the nitrification rate of all three types of biofilters decreased exponentially. The reduction of nitrification rates of the biofilters was about 60~70% for a substrate concentration of 10 mg TAN l^{-1} when the COD/N ratio increased from 0 to 3. The temperature impact on biofilter nitrification rate was not significant under the two temperatures tested. The results of this study provide useful information to the design of nitrification biofilters for cold water RAS applications.

Keywords: Nitrification; Biofilter; Biofilm; Organic carbon; Series reactor system; RAS.

2.2. Introduction

Biological nitrification, which employs autotrophic nitrifying bacteria for the oxidization of ammonia to nitrate via nitrite, is the key process in the removal of ammonia from wastewater (Satoh et al., 2000). Various parameters influence the nitrification process. Major factors include: dissolved oxygen (DO), temperature, pH, ammonia and nitrite concentrations, organic loading, and hydraulic loading rate (Sharma and Ahlert, 1977). Because DO is a chief factor in limiting the nitrification process, its impact becomes even more significant as organic loading increases in the reactor, allowing fast-growing heterotrophic bacteria to compete with nitrifying bacteria for the limited oxygen. It was reported that heterotrophic bacteria have a maximum growth rate of five times and yields of two to three times that of autotrophic nitrifying bacteria (Grady and Lim, 1980). In addition to competing for DO, heterotrophic bacteria also compete with nitrifying bacteria for space in fixed-film bioreactors, leading to a decrease in nitrification efficiency or even a failure in the system. As a result, inhibition of heterotrophic bacteria on nitrification is of more concern in systems with high organic material, such as aquaculture systems.

Considerable research has been related to the effect of organics on the nitrification process, especially for domestic and industrial wastewater treatment. Cheng and Chen (1994), Fdz-Polanco et al. (2000), Okabe et al. (1996), and Gupta (2001) reported that the bioactivity of nitrifiers were inhibited by an increase in the C/N ratio within fluidized bed reactors, submerged biofilters, and rotating biological contactors (RBC). By applying

fluorescents in situ hybridization (FISH) technique and microelectrodes in the nitrification biofilm analysis, Ohashi et al. (1995) and Satoh et al. (2000) found that the proportion of nitrifiers decreased with an increasing C/N ratio. An exponential decrease of the nitrification rate with an increased influent COD/N ratio was observed in a study on nitrogen removal from high-strength ammonia industrial wastewater (Carrera et al., 2004). The same study also pointed out that the influence of COD/N ratio should be one of the main parameters in the design of biological nitrogen removal process in industrial wastewater treatment. Okabe et al. (1996) discovered that a higher influent C/N ratio retarded accumulation of nitrifying bacteria and resulted in a considerably longer start-up period for nitrification.

Although the organic impact on nitrification biofilters in aquacultural systems is of high concern, very little investigation has been conducted. Zhu and Chen (2001) determined that the nitrification rate of submerged filters could decrease by 70% with a C/N ratio of 1 or 2 as compared to a pure nitrification system (C/N=0). In a study using biofilm material from an operation trickling filter in a catfish recirculation system (25 ℃), a corresponding reduction factor for the simultaneous nitrification process was reported to be -0.015 g m^{-2} d^{-1} NH_4^+-N nitrified per g m^{-2} d^{-1} COD removed as a result of oxygen consumption by oxidation of organic matter (Bovendeur et al., 1990).

As Recirculating Aquaculture Systems (RAS) are increasingly adopted worldwide for commercial fish production, nitrification biofilters are starting to play a more and more important role in aquaculture engineering as a key component of RAS. Typical

nitrification biofilters being utilized in RAS include: submerged filters, trickling filters, rotating biological contactors (RBCs), fluidized bed reactors, and floating bead filters (van Rijn, 1996). These biofilters featured different hydraulic regimes and filtration media and have been investigated for use in intensive aquacultural systems (Manthe et al., 1988; Fdz-Polanco et al., 1995; delos Reyes and Lawson, 1996; Summerfelt and Cleasby, 1996; Golz et al. 1999; Malone and Beecher, 2000; Sandu et al., 2002). Fluidized bed reactors, utilizing media such as sand, can provide a large surface area for nitrifying bacteria to grow with a compact size and therefore have been widely applied in commercial systems (Timmons, 2001). Rittmann (1982) reported that fluidized-bed reactors could achieve superior performance compared to complete-mixed and fixed-bed reactors because the biofilm was evenly distributed throughout the reactor while the liquid regime had plug-flow characteristics. Unfortunately, high energy consumption for fluidization and washout of media has been recognized as a limitation of fluidized bed biofilters (Summerfelt and Cleasby, 1996; Skjølstrup et al., 1998). Since the last decade, floating bead filters have started to show particular potential for aquaculture application because of their ability to remove ammonia and solids simultaneously (Malone and Beecher, 2000). However, the floating bead filters usually have a lower specific surface area compared to fluidized filters and require periodic backwashing. Submerged filters have all of the media immersed in the water and can be operated in a down-flow or an up-flow manner (Wheaton et al., 1994). Submerged filters usually have low head losses through the filter and low energy consumption for pumping. However, the low nitrification efficiency of submerged filters has to be improved for their application in RAS.

Although there have been significant research efforts on bio-filtration systems of RAS, information relative to nitrification kinetics is still lacking, especially for the impact of organic matter on the nitrification process of different types of biofilters. Comparative studies have shown that RBCs, submerged, trickling, or fluidized bed filters all have different performance in terms of TAN removal (mg TAN m^{-2} d^{-1}) (Rogers and Klemeston, 1985; Westerman, et al., 1996; Hall, et al., 2002). Nitrification kinetics varied among filter types due to differences in design and management strategies of the biofilters. Therefore, a series of experiments were conducted to evaluate the effects of organics on nitrification performance of different biofilters: floating bead filters, fluidized sand filters, and submerged bio-cube filters.

2.3. Materials and methods

2.3.1. Experimental set-up

The experimental system (Figure 2. 1) consisted of three types of biofilters: bead filter, sand filter and bio-cube filter. Each type of biofilter series was composed of five biofilters in a sequence, each being connected to an individual aeration sump inside which a submersible pump recycled substrate solution through the biofilter. Each sump was provided with an air diffuser not only to maintain a sufficient oxygen level for the connected biofilter but also to obtain a completely mixed condition in the sump. A multi-channel feeding pump (MASTERFLEX^{R} PUMPS) was used to pump a consistent amount

of substrate solution to the first reactor of each biofilter series. Flexible tubes were set up between sumps to deliver the substrate solution through the entire reactor series by gravity. The whole system was placed in a water bath where temperature was maintained by a heater or chiller. Bead and sand filters were made of clear top and bottom fitted PVC pipes, utilizing plastic beads (Aquaculture Systems Technologies) and 20/35 retaining mesh sieve size sand as filter media. Bio-cube filters were made of flat bottom cylindrical tanks with Bio-cube 650 (Keenton Industries, Inc) as the biofiltration media. The total surface area of the bio-cube filter was maintained at twice the surface area of either the bead or the sand filter (Table 2. 1). The feeding rate in Table 2.1 was the flow rate from the feeding pump as well as the flow rate (solution deliver rate) through the reactor series. However, the flow rates of the biofilters, which were generated from the recirculation submersible pumps inside the aeration sumps, were different from the feeding rates.

2.3.2. Experimental design

Biofilters were inoculated with fish culture water for one week prior to feeding. Then, inoculums were flushed out except for the retention of a small amount of seed inside the filters. A synthetic substrate solution comprised of ammonium chloride, sodium bicarbonate, and other necessary trace elements was continuously fed into the first reactor of each biofilter series (Table 2.2). Sucrose ($C_{12}H_{22}O_{11}$) was used as a carbon source to control the weight ratio of carbon to nitrogen (C/N). The experiment was started with C/N=0, followed by C/N=0.5, and finally C/N=2; each at 15℃ and 20℃. Here, C/N=0 indicates a relatively pure nitrification condition while C/N=2 represents a typical

condition in recirculating aquaculture systems based on an assumption of 12% soluble BOD_5 and 3% of TAN from fish feed (Zhu and Chen, 2001). A C/N of 0.5 instead of 1 in between 0 and 2 was selected because the impact of a C/N of 1 or 2 had no significant effect on nitrification (Zhu and Chen, 2001).

About 5 weeks of continuous feeding was required before the system reached a steady-state culture with a stable TAN concentration established in each biofilter (Zhu and Chen, 1999). During the experimental period, the inner surfaces of aeration sumps and connecting tubes were cleaned daily to prevent biofilm buildup. Bead filters were backwashed every other day to function properly, while the sand and bio-cube filters were not backwashed. After the steady-state condition was established, samples were collected daily over a five-day period from both the solution tank and the effluent of each reactor. Then, all reactors were cleaned to remove biomass before running substrate with a different C/N ratio. The same sampling routine was repeated for each C/N treatment under steady-state culture conditions.

2.3.3. Analysis methods

Collected samples were measured for dissolved chemical oxygen demand (COD), TAN, nitrite, and nitrate at the certified Water Quality Laboratory at Washington State University. Other parameters, such as pH (8.05 ± 0.17) and DO (8.28 ± 0.39 mg l^{-1}), were measured before sampling while the temperature was monitored daily throughout the course of the experiment. To analyze dissolved COD, samples were filtered with a

0.45μm membrane before conducting measurements with the standard method 5220-COD-D (APHA, 1995). TAN, nitrite and nitrate concentrations were determined using a Flow Injection Analyzer (OI Analytical FS3000) according to EPA methods 350.1, 353.2 and 353.2, respectively (US-EPA, 1984).

TAN removal rates were calculated using the following equation (Zhu and Chen, 1999) in a steady-state system:

$$R_i = Q(S_{i-1} - S_i) / A_i \qquad (1 \le i \le 5) \qquad (1)$$

R_i—TAN removal rate of reactor i (mg m^{-2} d^{-1})

Q—feeding rate (m^3 d^{-1})

S_i—concentration of TAN at reactor i or solution tank (S_0) (mg l^{-1})

A_i—biofilm area of reactor i (m^{-2})

A three-factor ANOVA analysis was performed with a SAS program in order to evaluate the impact of organic carbon, biofilter type and temperature on nitrification. However, only results from the first reactor of each reactor series were analyzed, of which all 3 types of biofilters had the same operating conditions and substrate concentration of 10 ± 0.92 mg TAN l^{-1}.

2.4. Results and Discussion

2.4.1. Nitrification kinetics of biofilters without the interaction of organic matter

In aquaculture systems, biofilters are operated at low TAN concentrations and TAN has been considered the rate-limiting factor for the nitrification process (Wheaton et al, 1994; Zhu and Chen, 1999). A modified Michaelis-Menten (M-M) model (Zhu and Chen, 1999) is used to predict the relationship between TAN removal rate and TAN concentration:

$$R = R_{\max} \frac{S - S_{\min}}{K_s + S - S_{\min}} \qquad (2)$$

where R_{max} is the maximum TAN removal rate (mg m^{-2} d^{-1}), K_s is the half saturation constant (mg l^{-1}), and S_{min} is the minimum TAN concentration (mg l^{-1}). When $S \leq S_{min}$, R=0. It needs to be pointed out that in equation (2), the bulk TAN concentration was used instead of the actual TAN concentration at a given point in the biofilm. As a result, this equation differed from the classical Michaelis-Menten equation. In Eq. (2), K_s was a lumped number reflecting both the growth and the diffusion mass transfer within the biofilm.

A minimum TAN concentration of 0.07 mg l^{-1} (Zhu and Chen, 1999) was used in the above model:

$$R = R_{\max} \frac{S - 0.07}{K_s + S - 0.07} \qquad (3)$$

The other two factors, R_{max} and $K_{s,}$ were calculated with experimental data by Lineweaver-Burke plots and the parameters are listed in Table 2.3 for all three types of biofilters under the two temperatures.

The experimental and model results of the effect of TAN concentration on nitrification rates of the three types of biofilters were plotted in Figure 2.2 for 15 ℃ and 20 ℃ without the impact of organic carbon (C/N=0). The plot showed a strong correlation with results predicted by the modified Monod model for a TAN ranging from 0 to12 mg l^{-1} (R^2>0.9 for all combinations).

However, aquaculture systems are usually operated at a TAN much lower than 12 mg l^{-1}. For instance, free ammonia (NH_3) concentration cannot exceed 0.02 mg l^{-1} (Timmons, 2001) in a recirculating rainbow trout system. To achieve a safe NH_3 condition (<0.02 mg l^{-1}) for cold water species, the TAN concentration needs to be kept under 7 mg l^{-1} in a neutral condition (pH=7) at 15 ℃ (Groeneweg et al., 1994) . The design criterion of TAN is 1 mg l^{-1} for cold water species (T = 8~20 ℃) (Timmons, 2001). Therefore, it is very important to investigate the biofilters' nitrification performance at low concentrations for applications in aquacultural systems.

At low ammonia substrate concentrations ($S<<K_s$), the Monod nitrification kinetics can be simplified into a first order reaction model, so Eq. 3 turns into Eq. 4.

$$R = \frac{R_{\max}}{K_s}(S - 0.07) \qquad (4)$$

Therefore, the biofilters nitrification rates were simply considered to increase linearly with the increase of ammonia concentration for applications in cold water aquaculture systems. For TAN under 1 mg l^{-1} ($<K_s$) that represents the culture conditions for cold water species, the 1^{st} order kinetics model can be applied to` all the three types of biofilters (Table 2. 3).

These results are consistent with other reports. Easter et al (1994) studied the performance of three RBC systems used for aquaculture where water temperature ranged from 24 to 30 ℃ and observed first order nitrification kinetics (linear relationship) at low ammonia concentrations (Figure 2.3). The areal nitrification rate (mg m^{-2} d^{-1}) of the small scale RBC is comparable to the results from the reactor series system in this study (Table 2.3), fitting in between the bio-cube filter and the sand and the bead filter. Other researchers (Watanabe et al., 1980; Surampalli and Baumann, 1989) have also found that a first-order reaction can be developed for RBC reactors at extremely low ammonia concentrations and low organic loading rates.

It can be seen from Figure 2.2 and Table 2.3 that the areal nitrification rate (mg m^{-2} d^{-1}) of the bio-cube filter was much lower than the bead and the sand filter. It was the authors' speculations that this was due to the difference in hydraulic conditions among the different biofilters. With a relatively low flow rate, the bio-cube filter may have undergone insufficient turbulence within the reactor and was unable to transfer nutrients into the biofilm. Conversely, the high flow rate of the sand filter and the frequent

backwashing of the bead filter contributed to the increase of the diffusion rate through the sand biofilm, keeping an active biofilm on the bead surface, and resulting in a productive nitrifying bacterial population.

The K_s and R_{max} values were also compared to the results from the previous studies (Table 2.3). The maximum nitrification rate (mg m^{-2} d^{-1}) of the bio-cube filter was consistent with the submerged filter in an earlier study, while the half saturation constants were higher in this study. In the previous study (Zhu and Chen, 2002), the diffusers were placed inside the biofilters leading to better mixing and a higher mass transfer flux when compared to the biofilters in this study, which used a separate sump for aeration. Therefore, the bio-cube filters in the former system might have experienced less substrate diffusion resistance across the water film, resulting in a lower half saturation constant in the bulk liquid. Other factors, such as the system setup, flow rate, and system management, could also have contributed to the variations of the half saturation constants and maximum removal rate (mg m^{-2} d^{-1}).

2.4.2. Effect of organic matter on biofilter nitrification rates

The impact of organics on the biofilters' nitrification performance was evaluated by running the three biofilter series under two different C/N ratios (0.5 and 2). However, it is not easy to draw the nitrification kinetics corresponding to TAN substrate concentrations with this set of experimental results since the C/N ratios were not consistent through the five reactors in a biofilter series. To evaluate the effect of organic matter, the nitrification

rates of the first reactor from each reactor series with the same operating conditions and substrate concentration (10 ± 0.92 mg TAN l^{-1}) under the interaction of organic matter were analyzed and compared to the results without organic interaction.

With a TAN concentration of 10 mg l^{-1}, an exponential decrease of the nitrification rate with an increase of the COD/N ratio was observed for all three types of biofilters under both temperatures. Here, the C/N ratios were converted to COD/N ratios for the convenience of comparison with other previous studies. The C/N ratios of 0, 0.5 and 2, are equal to COD/N=0, 1.4 and 5.4, respectively, according to a ratio of COD/C=2.68 with sucrose as the organic carbon source (Zhu and Chen, 2001). The relationship between COD/N and nitrification rate in this study is very similar to the results obtained from an activated sludge system (Carrera et al., 2004). Figure 2.4 shows the effect of COD/N ratios on nitrification rates with the comparison of the bead filter (20 ℃) and an activated sludge system (25 ℃). The error bar was defined as the standard deviation of the mean value. The inhibition of organic carbon on the nitrification rate is apparent and similar for both systems. As COD/N increased from 0 to 1.5 (C/N≈0.5), the nitrification rate declined rapidly with the increase of the COD/N ratio, but the degree of organic inhibition became less and less with a higher COD/N ratio. If the COD/N ratio was higher than 3 (C/N≈1) the nitrification rates of both systems tended to remain unchanged at a minimum value. This indicates that the inhibition of organic matter on nitrification could be maximized as the growth of heterotrophic bacteria reached a saturation level with a certain COD/N ratio (COD/N=3 in this study).

The relationship between the bead filter nitrification rate and influent COD/N ratios at 20 ℃ can be defined by an exponential function ($r^2 = 1.0$) according to equation 5, which is similar to equation 6 of the relationship developed in suspended growth systems (Carrera et al., 2004).

$R = 0.67 + 2.27\ e^{(-1.38(COD/N))}$ (This study) (5)

$R_{nitrification} = 0.0323 + 0.334e^{(-1.660(COD/N))}$ (Carrera et al., 2004) (6)

where the nitrification rate in the fixed film process was defined by g TAN removal per unit biofilm surface area per day, while the nitrification rate in the suspended growth system was defined by g TAN removal per g volatile suspended solids (VSS) per day (Eq. 7).

$$R_{nitrification} = \frac{Q_{in}([NH_4^+ - N]_{in} - [NH_4^+ - N]_{out})}{V_{reactors}[VSS]_{reactors}} \quad (7)$$

Q_{in} — Influent flow rate, $L^3\ T^{-1}$.

$V_{reactors}$ — Reactor working volume, L^3.

$[VSS]_{reactors}$ — VSS concentration in reactor, $M\ L^{-3}$.

In the same manner, the relationship between nitrification rates and COD/N ratios can be developed for the bead filter at 15 ℃ and the other two types of filters at both 15 and 20 ℃ (Table 2.4).

The results in Table 2.4 can be used as references in terms of the design of nitrification biofilters which are operated in mixed culture conditions like aquaculture systems. The

reduction of nitrification rates of the three types of biofilters were about 60~70% under the tested conditions when the COD/N ratio increased from 0 to 3. In a previous study, Zhu and Chen (2001) also reported a 70% reduction of the nitrification rate when the C/N ratio increased from 0 to 1 (COD/N=2.7), while the nitrification rate difference between C/N=1 and 2 was determined to not be significant. The consistency of the two studies again confirmed the inhibition of organic matter on biofilter nitrification performance. Consequently, the effects of the COD/N ratio on biofilter nitrification capacity need to be taken into account when installing an aquaculture system. Nitrification capability of aquacultural biofilters may be overestimated if test data from a pure nitrification condition were used in the design of biofilters. It also needs to be addressed that this COD/N vs. nitrification rate relationship was obtained at a TAN concentration of $10mgl^{-1}$, which is higher than the allowable TAN concentration in most aquaculture systems. Correction factors should be considered that compensate for the substrate effects on nitrification rates when the equations in Table 2.4 are applied for lower TAN concentrations. Based on this point, more work needs to be done to investigate the organic effect on nitrification rates at a lower TAN concentration that is more applicable to aquaculture operations.

2.4.3. Statistical analysis of organic matter, biofilter type and temperature impacts on nitrification

Statistical analysis showed that the filter type, C/N ratio, and the interaction between both had highly significant ($p<0.0001$) effects on nitrification, while temperature had no

significant (p=0.283) impact on nitrification at 15℃ and 20℃. Zhu and Chen (2002) also reported that the impact of temperature was insignificant on nitrification rates of fixed-film filters with temperatures ranging from 14℃ to 27℃ under a limitation of dissolved oxygen. As saturation DO concentration decreases with an increase of temperature, it reduces the normally positive temperature effect on the nitrification rate that results because of the improvement in biofilm bacteria growth.

The interaction between biofilter type and organics was also analyzed statistically. In Table 2.5, capital letters indicate the impact of organic carbon on the nitrification rates of different biofilters. The results show that when C/N increased from 0 to 0.5, the nitrification rate of all three types of biofilters decreased significantly and when C/N increased from 0.5 to 2, the nitrification rate of the bead filter continued to decrease while the nitrification decrease was insignificant for the sand and the bio-cube filters. The lowercase superscripts show the effect of biofilter type on nitrification. The nitrification difference among biofilters varied with an increase of organics in the system. At C/N=0, the bead filter had the best nitrification performance, followed by the sand filter and the bio-cube filter. At C/N=0.5, there was no significant difference between the nitrification rate of the sand filter and the bead filter even though both of them had a higher nitrification rate than the bio-cube filter. And at C/N=2 the nitrification difference among the three types of biofilters became insignificant. This implies that the nitrification difference decreased as organic inhibition increased and became dominant in the nitrification process. It also has to be mentioned that the nitrification rate being compared here is based on unit media surface area. Therefore, in biofilter selection for cool water

RAS, biofilters utilizing media with high specific surface area are particularly important as they are more efficient and can be built with a compact size. However, the nitrification performance of biofilters may vary greatly due to the differences resulting from their specific designs and operating conditions. Specific conditions that limit the nitrification performance of biofilters need to be considered when applying these comparison results to RAS biofilter design.

2.5. Acknowledgements

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Table 2.1. Specifications of three biofilter series

Biofilter type	Floating bead filter	Fluidized sand filter	Submerged bio-cube filter
Water volume (l)	0.51	0.29	3.24
Expansion	--	50%	--
Media specific surface area (SSA, $m^2 m^{-3}$)	1310	6070	361
Total biofilm area (m^2)	0.40	0.40	0.80
Flow rate (l min^{-1})	1.78	2.26	1.11
Cross-sectional area (cm^2)	20	11	182
Feeding rate ($m^3 d^{-1}$)	0.216	0.216	0.216

Table 2.2. Composition of substrate nutrients (Zhu and Chen, 1999)

Ingredient	Composition
NH_4Cl	50.6 g
$NaHCO_3$	129 g
$MgSO_4 \cdot 7H_2O$	1.32 g
Na_2HPO_4	5.85 g
KH_2PO_4	5.63g
$FeCl_3 \cdot 6H_2O$	0.18 g
Water	1325 l

Table 2.3. Biofilters nitrification kinetic constants and first order reaction rates at low TAN concentration (TAN <1 mg l^{-1})

Temperature (℃)	Biofilter type	R_{max} (mg m^{-2} d^{-1})	K_s (mg l^{-1})	R (mg m^{-2} d^{-1})
20	Floating bead	5000	8.5	R=588*S-41
	Fluidized sand	3330	5.3	R=625*S-44
	Submerged bio-cube	1670	5.5	R=345*S-24
15	Floating bead	5000	9.5	R=526*S-37
	Fluidized sand	3330	6.0	R=556*S-39
	Submerged bio-cube	1670	6.0	R=278*S-19
				Reference
8	Submerged	1550	5.5	Zhu and Chen (2002)
14	Submerged	1690	2	Zhu and Chen (2002)
20	Submerged	1720	2	Zhu and Chen (2002)
27	Submerged	1860	2	Zhu and Chen (2002)

Table 2.4 Expressions of the COD/N effect on nitrification rate R ($g\ m^{-2}\ d^{-1}$)

Biofilter type	Temperature (℃)	
	20	15
Bead filter	$R=0.67+2.27e^{(-1.38(COD/N))}$	$R=0.85+1.88e^{(-1.36(COD/N))}$
Sand filter	$R=1.0+1.16e^{(-0.92(COD/N))}$	$R=0.90+0.93e^{(-1.21(COD/N))}$
Bio-cube filter	$R=0.33+0.65e^{(-1.38(COD/N))}$	$R=0.51+0.62e^{(-2.22(COD/N))}$

Table 2.5. Statistic analysis of organic impacts on nitrification rate (mg m^{-2} d^{-1}) and comparison of biofilters (α=0.05)

C/N	Biofilter types			Pr
	Bead	Sand	Bio-cube	
0	2800±390 (A^a)	2000±300 (A^b)	1100±140 (A^c)	<0.0001
0.5	1100±110 (B^a)	1000±220 (B^a)	450±160 (B^b)	<0.0001
2	730±350 (C^a)	810±420 (B^a)	370±270 (B^a)	0.073
Pr	<0.0001	<0.0001	<0.0001	

Capital letters: C/N as an impact; Lowercase superscript: biofilter type as an impact. The same letter indicates no significant difference.

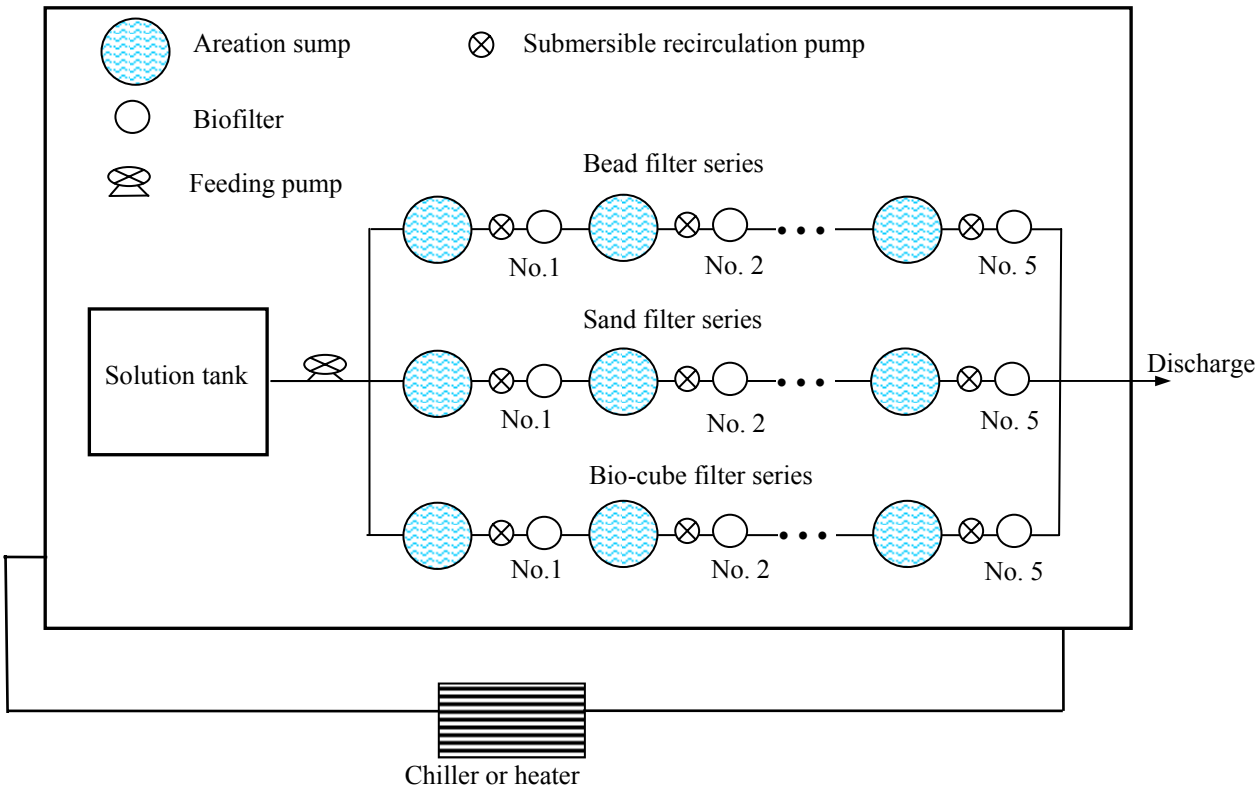

Figure 2.1. Schematic of the series reactor system

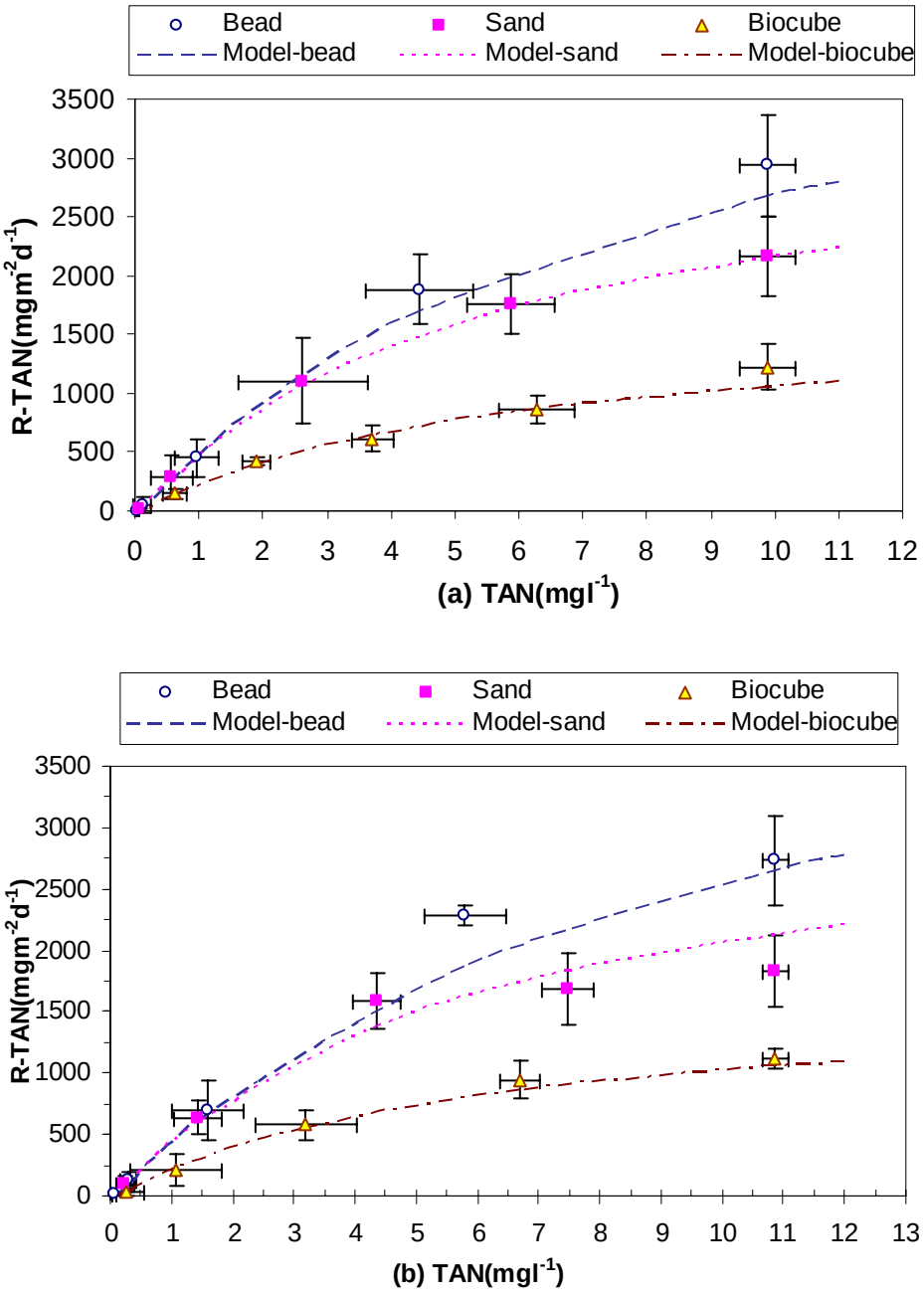

Figure 2.2. Impacts of TAN concentration on biofilters TAN removal rates (R-TAN) for C/N=0 (no organic interaction); (a) T=20 ℃; (b) T=15 ℃.

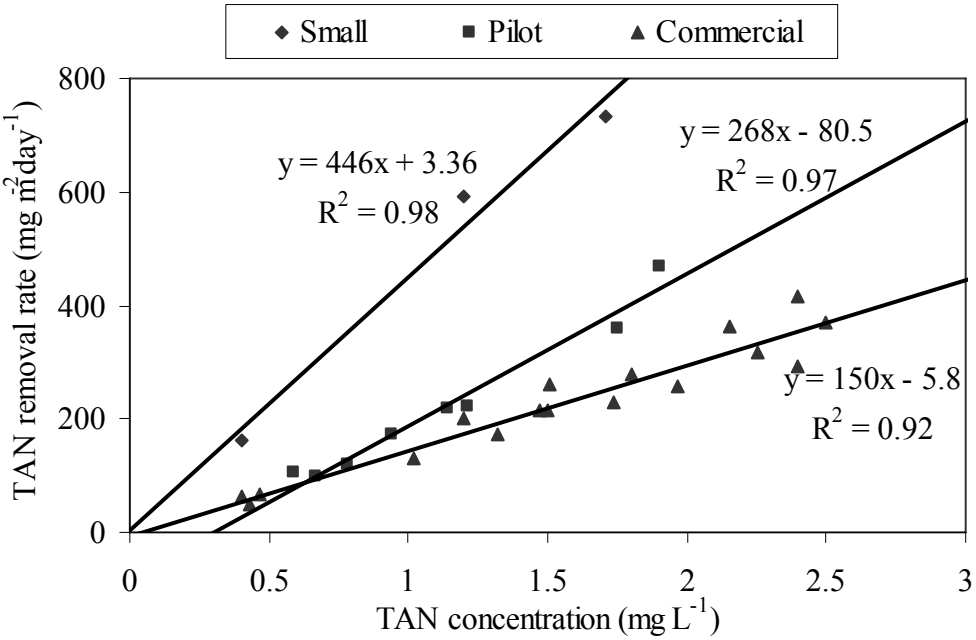

Figure 2.3. Performance data for RBCs applied to recirculating aquaculture systems. Small= laboratory scale RBC (0.9 m diameter); pilot = large laboratory scale RBC (1.8m diameter); commercial = industrial application (3.7m diameter) (Easter et al., 1994).

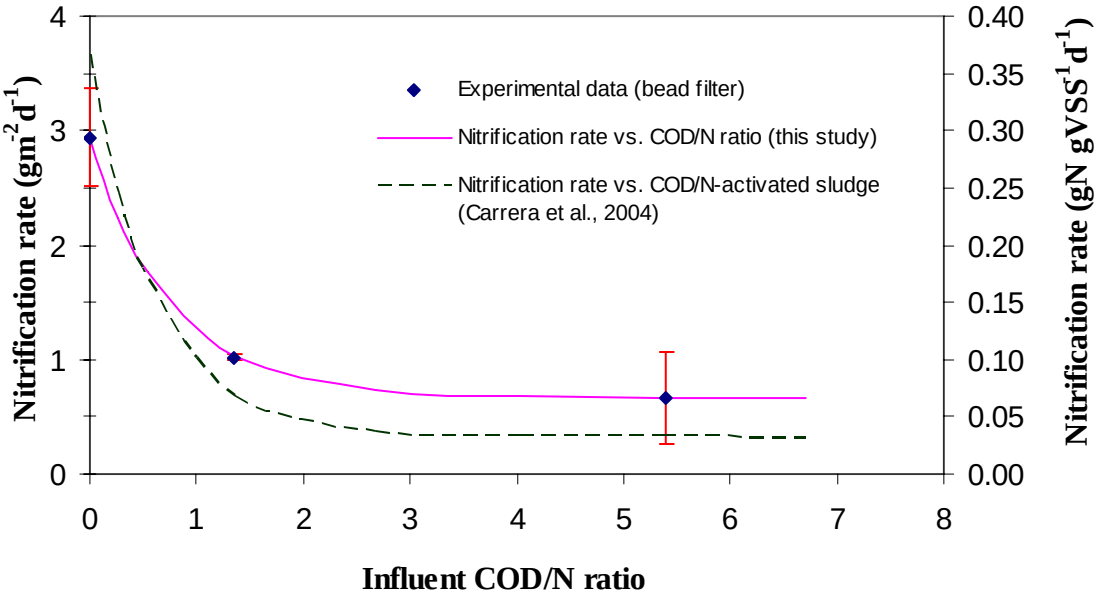

Figure 2.4. Relationship between the nitrification rate and the influent COD/N ratio

CHAPTER THREE

SIMPLIFIED BIOFILM MODEL APPLICABLE FOR NITRIFICATION PROCESS IN WASTEWATER TREATMENT AND RECIRCULATING AQUACULTURE SYSTEMS

3.1 Abstract

Although a variety of biofilm models have been developed for the nitrification process in wastewater treatment, very few are suitable for practical application due to computation complexity. Therefore it is necessary to establish proper but simple biofilm models for practical use in wastewater treatment and aquaculture systems. This research aimed at developing a simplified but well constructed model by incorporating the major physical and chemical input parameters and biofilm kinetics to provide useful information for the optimization of biofilter design. Simplifications on the biofilm physical characteristics accomplished through mathematical derivations on the intermediate parameters helped to achieve simplified analytical solutions on the substrate mass fluxes in a multi-substrate and multi-species biofilm. The accuracy of this simplified model on predicting biofilter nitrification rates was verified against predictions derived from more complex numerical models resulting in predictive deviations of less than 10%. This model was also deployed within a spreadsheet program so that the model implementation could more practically be accomplished.

Key words: biofilm; model; multi-species; nitrification; aquaculture.

3.2. Introduction

Biofilm processes have been found to be effective in terms of nutrient removal from domestic and industrial wastewater. Compared to suspended growth systems such as the activated sludge process, the fixed-film process is considered less energy-intensive and more resistant to changes in the process parameters such as shock loads (Chaudhry and Beg, 1998) and temperature drop. Although fixed-film bioreactors have been well adopted in wastewater treatment and recirculating aquaculture systems (RAS) for the removal of ammonia nitrogen, design is often based on empirical data or trial-and-error experimentation under particular conditions (Rittmann and Stilwell, 2002). When empirical data is lacking or operating conditions change, biofilters are usually oversized or undersized for the system leading to either a waste of energy and material resources or system failure.

Employing a mathematical model as a design tool can overcome these problems and improve the efficiency of the biofilters. Numerous mathematical models have been developed and applied to the nitrification biofilm processes. The early biofilm models were based on the conventional conceptual model with a homogenous biofilm structure, which consists of a base film and a surface film (Characklis and Marshall, 1990). In this concept, the transport of substrates, nutrients, and products, etc. in and out of the biofilm is by molecular diffusion only. Transport between the bulk fluid and the biofilm, on the other hand, is dominated by advection and turbulent diffusion (Grady et al., 1999).

Mathematical models based on this conceptual model were mostly one-dimensional (1D) with the assumption of an evenly distributed biofilm structure and biomass and usually only the base film was considered (Noguera et al., 1999b). Grady et al. (1999) presented a comprehensive review about biofilm modeling in wastewater treatment till the middle of the 1990s. In this summary, a detailed description is provided on the modeling methods for one-dimensional biofilm models, including effectiveness factor approach, pseudo-analytical approach, and limiting-case solution approach. Most of the early biofilm models only considered single species biofilm to simplify the computation with analytical solutions. Although some of the biofilm models might have considered competitions with mixed culture they either required complex numerical solutions (AQUASIM, Wanner and Morgenroth, 2004) or were associated with limiting-case conditions to obtain model solutions.

As electron and confocal microscopes were more and more involved in the study of biofilm structures, it was revealed that biofilm systems were much more complex and diverse than that was believed previously (Noguera et al., 1999a; Wanner, 2002). Some observations described biofilms as non-uniform structures consisting of discrete cell clusters attached to each other and to the solid support with extracellular polymeric material (Costerton, 1995). Others defined biofilms as being made of microcolonies separated by interstitial voids where microconlonies were compact aggregates of extracellular polymers with densely packed microorganisms (Lewandowski, 2000). Recent mathematical models attempt to consider these experimental observations into model developments. Therefore, two-dimensional (2D) and three-dimensional (3D) models have been worked on using approaches such as “biomass-based modeling” and

"individual-based modeling" (Noguera et al., 1999a; Xavier et al., 2004). However, it is currently difficult to apply the microscale information provided by the complex 3D model to a macroscale level due to the lack of experimental data on the behavior of individual cells as well as on the availability of their kinetic parameters (Wanner, 2002). Many biofilm models have concentrated mainly on describing organism populations that also makes today's models less relevant to the real world applications, especially for engineering practices. From a practitioner perspective, the physical operation of the plant (e.g., backwashing procedures, flow distribution) may have a greater impact on the systems performance than population dynamics and micro-scale transport processes in the biofilm. Moreover, with too many details on microscale biofilms included, models may not be able to reproduce the behavior of a full-scale biofilm reactor (Morgenroth et al., 2000). Some of the input parameters may not be measurable, which makes calibrations impossible for many mathematical biofilm models. Finally, the expensive computation cost also limits the applications of multi-dimensional models. It is impractical to apply multi-dimensional biofilm models in the design of biofilters for wastewater treatment and RAS at the present time.

To extend the application of biofilm models in wastewater treatment practices, efforts had been made on simplified solutions for one-dimensional multi-species biofilm models. Based on the pseudo-analytical approach, Rittmann and Stiwell (2002) presented an integrated biofiltration model (IBM) for the drinking water treatment process by dividing a plug flow reactor into three completely mixed biofilm segments. Although multiple species were included in this model, each species was considered separately and the total biofilm was the combination of the separate species. This limited the application of the

IBM model for biofilms with low substrate loadings like water treatment, where species competition was not important. While with high substrate loading or with substrate ratios that strongly favor the growth of one type of organism over another, this model may lead to significant errors (Rittmann and Stiwell, 2002). Perez et al. (2005) made a good attempt to simplify the calculation of multi-species biofilm models by introducing a weighted ratio analysis for zero and first order reactions. However, external mass transfer was neglected in this model, which also limited the application of this model to certain types of biofilms, such as thin biofilms, in which the substrate utilization rate is not diffusion limited.

The purpose of this research is to develop a simplified but well constructed model by incorporating the major physical and chemical input parameters and biofilm kinetics of the reactor to provide useful information for the optimization of biofiter design. To achieve analytical solutions for the model, mathematical simplification was applied to determine the intermediate parameters in addition to the simplifications on the physical characteristics of the biofilm model. This model was developed as a spreadsheet so that it could be more practically utilized. The accuracy of the simplified model was evaluated in comparison to numerical solutions and parameter estimation guidelines were also provided for the application of this model in wastewater treatment and aquaculture systems practice.

3.3. Model development

The biofilm model developed in this research is based on a one-dimensional steady-state model with consideration of multiple substrates and multiple species. From an engineering aspect, the 1D model is sufficient because the thickness of the biofilm is much smaller compared to the surface area. Because the substrate utilization and diffusion rate occurring within the biofilm are very fast compared to bacteria growth rate in the biofilm, steady-state mass balance on substrate at any point in the biofilm is appropriate (Rittmann and Manem, 1992). As a result, the assumptions for this model are as follows,

1) The reactor is operated under steady-state conditions; the substrate concentrations, biofilm thickness, and biomass density remain constant at a stable operating state.

2) The biofilm has a uniform thickness and the biomass density is uniform within the biofilm.

3) Transport of dissolved substrates in the biofilm is by molecular diffusion only. Mass diffusion is considered perpendicular to the substratum.

4) All mass transfer resistance occurs at the boundary layer.

5) The physicochemical conditions in the reactor do not change with time and space.

As illustrated in Figure 3.1, the mass transfer of substrates into the biofilm is composed of two processes: the external mass transfer from bulk liquid to the biofilm surface at the

diffusion boundary layer, and the internal mass transfer within the biofilm. To obtain simplified analytical solutions, this model emphasized only solving the mass fluxes into the biofilm while the substrate profiles within the biofilm were not targeted. To achieve this goal, the simplified model was developed in two steps. Firstly, mass balance equations of substrates (TAN, COD and DO) were developed for both the external and internal diffusion layers of the biofilm. Then, the mass balance equations from both layers were combined and solved for the mass transfer flux into the biofilm based on the fact that an equilibrium mass flux exists at the interface of the two layers (also called liquid-biofilm interface). The obtained substrate mass flux, the flux of TAN more specifically, was then used for nitrification biofilter design. The effect of temperature and hydrodynamic conditions on mass transfer was also included in the biofilm model so as to reflect the variation of flow regime in different types of biofilters as well as the variation in operating temperature. With its simplicity and convenience for application, this model could work as a useful tool in guiding the design and operation of biofilters in wastewater treatment and recirculating aquaculture systems.

In the development of the model, COD was used for the substrate associated with organic matter for heterotrophs and TAN (total ammonia nitrogen) was used for the substrate of autotrophs. Also for model simplicity, the intermediate nitrite was not considered and the nitrifiers were treated as one "species" that ammonia was oxidized directly to nitrate (Rittmann et al., 2004).

3.3.1 External mass transfer

The transport of substrate across the diffusion boundary layer from bulk liquid into the biofilm can be described with Fick's first law for all three interested substrates.

For dissolved oxygen, $J_C = \frac{D_{bC}}{L_w}(C_b - C_S)$ (1)

For ammonia substrate, $J_1 = \frac{D_{b1}}{L_w}(S_{1b} - S_{1s})$ (2)

For organic substrate, $J_2 = \frac{D_{b2}}{L_w}(S_{2b} - S_{2s})$ (3)

J_c, J_1, J_2 = Mass flux of dissolved oxygen (DO), TAN, and COD at the diffusion layer, g m^{-2} d^{-1};

D_{bc}, D_{b1}, D_{b2}, = diffusion coefficients of DO, TAN, and COD in bulk liquid, m^2 d^{-1};

C_b, S_{1b}, S_{2b} = DO, TAN, and COD concentrations in bulk solution, g m^{-3};

Cs, S_{1s}, S_{2s} = DO, TAN, and COD concentrations at biofilm surface, g m^{-3};

L_w = thickness of the diffusion boundary layer, m.

The diffusion boundary layer thickness can be calculated with the following equation (Hamdi, 1995),

$$L_w = 1.23(\mu/\rho)^{1/3}(d/V)^{0.5}\,\varepsilon^{1.5}\,(D_{bC}/86400)^{1/3}$$

μ = water viscosity, g m^{-1} d^{-1};

ρ = water density, g m^{-3};

d = characteristic length of biofloc, biofilm thickness in fixed-fixed film processes, m;

V = water flow velocity or water superficial velocity, m s^{-1};

ε = void fraction, dimensionless.

3.3.2 Mass transfer within biofilm

Within the biofilm, the accumulation of substrate can be expressed with Fick's Second law, and the mass balance equations for the interested substrates are,

for dissolved oxygen, $$\frac{\partial C_f}{\partial t} = D_c \frac{\partial^2 C_f}{\partial Z^2} - r_c \qquad (4)$$

for ammonia nitrogen, $$\frac{\partial S_{1f}}{\partial t} = D_1 \frac{\partial^2 S_{1f}}{\partial Z^2} - r_1 \qquad (5)$$

for organic matter (COD), $\frac{\partial S_{2f}}{\partial t} = D_2 \frac{\partial^2 S_{2f}}{\partial Z^2} - r_2$ (6)

C_f, S_{1f}, S_{2f} = concentrations of DO, TAN and COD within biofilm, g m^{-3};

D_c, D_1, D_2 = diffusion coefficients of DO, TAN and COD within biofilm, m^2 d^{-1};

r_c, r_1, r_2 = utilization rates of DO, TAN and COD within biofilm, g m^{-3} d^{-1};

Z = distance perpendicular to the biofilm support media surface, m.

Double Monod kinetics can often be used to model dual nutrient limitations of substrate consumption (Wanner and Gujer, 1984; Chen et al., 1989).

The utilization rates of TAN and COD can be represented as:

$$r_1 = \frac{\mu_{1,\max}\phi_1 X}{Y_{s1}} \frac{S_{1f}}{K_{s1} + S_{1f}} \frac{C_f}{K_{c1} + C_f} \quad (7)$$

$$r_2 = \frac{\mu_{2,\max}\phi_2 X}{Y_{s2}} \frac{S_{2f}}{K_{s2} + S_{2f}} \frac{C_f}{K_{c2} + C_f} \quad (8)$$

$\mu_{1,max}$, $\mu_{2,max}$ = the maximum specific growth rate of autotrophs and heterotrophs, d^{-1}; For temperature other than 20 ℃, $\mu_{1,\max} = \mu_{1,\max}^{20}\theta^{(T-20)}$, $\mu_{2,\max} = \mu_{2,\max}^{20}\theta^{(T-20)}$, θ=1.10 (Gujer and Boller, 1986);

Y_{s1}, Y_{s2} = the true yield coefficients of autotrophs and heterotrophs, g COD_x g^{-1} (TAN or COD_s;

K_{s1}, K_{s2} = the half saturation constants for TAN and COD, g m^{-3};

K_{c1}, K_{c2} = the half saturation constants of oxygen associated with TAN and COD oxidation, g m^{-3};

ϕ_1 = biomass volume fraction of autotrophs, dimensionless;

ϕ_2 = biomass volume fraction of heterotrophs, dimensionless;

X = total viable biomass density, g m^{-3}.

$$\phi_1 + \phi_2 = 1$$

The utilization rate of DO can be expressed with stoichiometric coefficients and the utilization rates of TAN and COD. For biofilters operated under low substrate concentrations, the consumption of DO due to biomass decay was small compared to DO consumption due to the growth of bacteria. The consumption of DO because of the decay of biomass was neglected here as a result. Therefore,

$$r_c = Y_{c1} r_1 + Y_{c2} r_2$$

$$r_c = Y_{c1}\frac{\mu_{1,\max}\phi_1 X}{Y_{s1}}\frac{S_{1f}}{K_{s1}+S_{1f}}\frac{C_f}{K_{c1}+C_f} + Y_{c2}\frac{\mu_{2,\max}\phi_2 X}{Y_{s2}}\frac{S_{2f}}{K_{s2}+S_{2f}}\frac{C_f}{K_{c2}+C_f} \quad (9)$$

Where, Y_{c1}, Y_{c2} = stoichiometric yield coefficients O_2/TAN = 4.57, O_2/COD=1.

At steady state, $\frac{\partial}{\partial t} = 0$

So, Eqs (4), (5), and (6) turn into,

$$D_c\frac{d^2C_f}{dZ^2} = Y_{c1}\frac{\mu_{1,\max}\phi_1 X}{Y_{s1}}\frac{S_{1f}}{K_{s1}+S_{1f}}\frac{C_f}{K_{c1}+C_f} + Y_{c2}\frac{\mu_{2,\max}\phi_2 X}{Y_{s2}}\frac{S_{2f}}{K_{s2}+S_{2f}}\frac{C_f}{K_{c2}+C_f} = r_c$$

(10)

$$D_1\frac{d^2S_{1f}}{dZ^2} = Y_{c1}\frac{\mu_{1,\max}\phi_1 X}{Y_{s1}}\frac{S_{1f}}{K_{s1}+S_{1f}}\frac{C_f}{K_{c1}+C_f} = r_1 \quad (11)$$

$$D_2\frac{d^2S_{2f}}{dZ^2} = Y_{c2}\frac{\mu_{2,\max}\phi_2 X}{Y_{s2}}\frac{S_{2f}}{K_{s2}+S_{2f}}\frac{C_f}{K_{c2}+C_f} = r_2 \quad (12)$$

3.3.3 Mass fluxes at the biofilm surface

The procedure to obtain substrate mass fluxes at the biofilm surface can be demonstrated with the derivation of the mass flux of dissolved oxygen. The derivation of TAN and COD fluxes can be done in the same manner.

Because of the flux continuity at the liquid-biofilm interface, the mass flux of DO at the biofilm surface must equal the mass flux in the diffusion layer.

$$J_{cfs} = D_c \frac{dC_f}{dZ}\Big|_{z=L_f} = J_c$$

Eq. (10) can be used to derive the expression for J_{cfs},

$D_c \frac{d^2C_f}{dZ^2} = r_c$ cannot be solved analytically but can be integrated once to solve for $D_c \frac{dC_f}{dZ}$ (Frank-Kamenetskii, 1969).

Set $\frac{dC_f}{dZ} = P$

$$\frac{d^2C_f}{dZ^2} = \frac{dP}{dZ} = P\frac{dP}{dC_f} = \frac{1}{2}\frac{d}{dC_f}P^2$$

$$P = \sqrt{\frac{2}{D_c}\int r_c dc + const.} \qquad (13)$$

C_0 = DO concentration at the bottom of biofilm (media surface).

From the boundary condition of, Z=0 (at the biofilm bottom), P=0, we have

$\frac{dP}{dZ} = 0$, r_c=0 (Z=0). Substitute this into eq. (13), then const.= 0.

$$P = \frac{dC_f}{dZ} = \sqrt{\frac{2}{D_c}\int r_c dc}$$

$$J_c = D_c \frac{dC_f}{dZ}\Big|_{z=L_f} = \sqrt{2D_c \int_{C_0}^{Cs} r_c dc}$$

$$\int_{C_0}^{Cs} r_c dc = \int_{C_0}^{Cs} Y_{c1} r_1 dc + \int_{C_0}^{Cs} Y_{c2} r_2 dc$$

$$\int_{C0}^{Cs} r_c dc = Y_{c1} q_{1,\max} \phi_1 \frac{XS_{1f}}{K_{s1} + S_{1f}} [C_s - C_0 - K_{c1} \cdot Ln(\frac{K_{c1} + C_s}{K_{c1} + C_0})]$$
$$+ Y_{c2} q_{2,\max} \phi_2 \frac{XS_{2f}}{K_{s2} + S_{2f}} [C_s - C_0 - K_{c2} \cdot Ln(\frac{K_{c2} + C_s}{K_{c2} + C_0})]$$

Where, $q_{1,\max} = \frac{\mu_{1,\max}}{Y_{s1}}$ and $q_{2,\max} = \frac{\mu_{2,\max}}{Y_{s2}}$ are the maximum specific substrate utilization rate for TAN and COD, g_{CODS}/g_{CODX}-d.

Therefore, the mass flux of DO at the liquid-biofilm interface was obtained.

$$J_c = D_c \frac{dC_f}{dZ}\Big|_{Z=L_f}$$

$$= \sqrt{\begin{aligned} &2D_c\{Y_{c1}q_{1,\max}X_1\frac{S_{1s}}{K_{s1}+S_{1s}}[C_s - C_0 - K_{c1}\cdot Ln(\frac{K_{c1}+C_s}{K_{c1}+C_0})] \\ &+Y_{c2}q_{2,\max}X_2\frac{S_{2s}}{K_{s2}+S_{2s}}[C_s - C_0 - K_{c2}\cdot Ln(\frac{K_{c2}+C_s}{K_{c2}+C_0})]\} \end{aligned}} \quad (14)$$

In the same manner, the mass fluxes of TAN and COD,

$$J_1 = \sqrt{2D_{s1}q_{1,\max}X_1\frac{C_s}{K_{c1}+C_s}[S_{1s} - S_{10} - K_{s1}\cdot Ln(\frac{K_{s1}+S_{1s}}{K_{s1}+S_{10}})]} \quad (15)$$

$$J_2 = \sqrt{2D_{s2}q_{2,\max}X_2\frac{C_s}{K_{c2}+C_s}[S_{2s} - S_{20} - K_{s2}\cdot Ln(\frac{K_{s2}+S_{2s}}{K_{s2}+S_{20}})]} \quad (16)$$

$X_1 = \phi_1 X$ = density of autotrophic bacteria, g m^{-3};

$X_2 = \phi_2 X$ = density of heterotrophic bacteria, g m^{-3}.

3.3.4 Derivation of substrate concentrations at media and biofilm surface

To solve eqs. (14) through (16) for the mass fluxes of DO, TAN, and COD, the substrate concentrations at the media surface (C_0, S_{10}, S_{20}) and biofilm surface (Cs, S_{1s}, S_{2s}) as well as other necessary kinetic constants need to be known. Therefore, the only unknowns of this set of equations are the three substrate mass fluxes.

3.3.4.1 Substrate concentrations at biofilm surface

The substrate concentrations at the biofilm surface (C_s, S_{1s}, S_{2s}) can be expressed with mass fluxes. From eq. (1), (2), and (3), the concentrations of DO, TAN, and COD at the biofilm surface are,

$$C_s = C_b - \frac{J_c L_w}{D_{bc}} \tag{17}$$

$$S_{1s} = S_{1b} - \frac{J_1 L_w}{D_{b1}} \tag{18}$$

$$S_{2s} = S_{2b} - \frac{J_2 L_w}{D_{b2}} \tag{19}$$

3.3.4.2 Calculation of C_0, S_{10}, S_{20}

To simplify the derivation procedure for the substrate concentrations at the media surface, the mathematical derivation was first presented in a general manner and the derivation was then specified to each substrate species.

According to the mass balance of biomass at a steady-state biofilm, there is no net accumulation of biomass on the biofilm, and the following equation can be obtained (Rittmann and McCarty, 1980; Rittmann and Stilwell, 2002).

$$0 = \frac{d(X_f dz)}{dt} = (\frac{\mu_m S_f}{K + S_f})(X_f dz) - b' X_f dz$$

where, μ_m is the maximum specific utilization rate (d^{-1}); b' is the decay rate coefficient (d^{-1}); X_f is the biomass density (g m^{-3}).

Because the flux of substrate into the steady-state biofilm is equal to the sum of the reaction within the biofilm, the flux of substrate into the biofilm can be substituted for the integral of substrate utilization over the entire biofilm. Therefore, the integral of the losses over the entire biofilm is simply $b'X_fL_f$. The integrated form of the steady-state mass balance on active biomass is given as (Rittmann and McCarty, 1980)

$$0 = YJ - b' X_f L_f$$

In the same manner, for an arbitrary small section within the biofilm, ΔZ, the mass flux at the left side J_z ($L_f' = Z$) and at the right side $J_{z-\Delta z}$ ($L_f' = Z-\Delta Z$) can be expressed as (Fig. 3.2),

$$0 = YJ_z - b' X_f Z \quad , L_f' = Z \tag{20}$$

$$0 = YJ_{z-\Delta z} - b' X_f (Z - \Delta Z) \quad , L_f' = Z-\Delta Z \tag{21}$$

Subtract (18) from (17), then,

$$Y(J_z - J_{z-\Delta z}) = b' X_f (\Delta Z) \tag{22}$$

$$\frac{\Delta J}{\Delta Z}\Big|_{\Delta Z=0} = \frac{dJ}{dz} = \frac{b' X_f}{Y} \tag{23}$$

Equation (23) shows that the gradient of mass flux is constant across the biofilm, which is consistent with the assumption of uniform distributed biofilm.

By integrating (23) along the boundary condition of J = 0 at Z = 0 (media surface), then the mass flux at an arbitrary point within the biofilm (Z = Z) becomes proportional to the distance from the media surface.

$$J_z = \frac{b' X_f}{Y} Z \tag{24}$$

Also, $J_f = D_s \frac{dS_f}{dZ}\Big|_{z=z} = J_z$

Then, $\frac{dS_f}{dZ}\Big|_{z=z} = \frac{J_z}{D_s} = \frac{b' X_f}{Y D_s} Z$ (25)

Integrate both sides of eq. (25) to solve for S_0,

With boundary conditions: $S_f = S_0$ at media surface, Z=0

$S_f = S_s$ at biofilm surface, $Z=L_f$

$$S_s - S_0 = \int_0^{L_f} \frac{b' X_f}{YD_s} Z dZ \tag{26}$$

$$S_0 = S_s - \frac{b' X_f}{2YD_s} L_f{}^2$$

Therefore, for TAN and COD concentrations at the media surface,

$$S_{10} = S_{1s} - \frac{b_1' X_1}{2Y_{s1} D_{s1}} L_f{}^2 \tag{27}$$

$$S_{20} = S_{2s} - \frac{b_2' X_2}{2Y_{s2} D_{2s}} L_f{}^2 \tag{28}$$

For DO concentration at the media surface,

$$C_s - C_0 = \int_0^{L_f} \frac{J_{cf}}{D_c} dZ$$

$$J_{cf} = 4.57 J_{1f} + J_{2f} = \left(\frac{4.57 b_1' X_1}{Y_{s1}} + \frac{b_2' X_2}{Y_{s2}}\right) Z \tag{29}$$

Integrate (26), then,

$$C_0 = C_s - \frac{L_f{}^2}{2D_c} \left(\frac{4.57 b_1' X_1 Y_{s2} + b_2' X_2 Y_{s1}}{Y_{s1} Y_{s2}}\right) \tag{30}$$

If any substrate concentration at the media surface (S_{10}, S_{20}, or C_0) obtained from equations (27), (28), and (30) is less than 0, then this value will be set at 0, and the biofilm will be considered as a thick biofilm.

When DO is limited to the oxidation of TAN and COD, oxygen will be used up before it reaches the bottom of the biofilm. Then, the DO penetration depth instead of the biofilm thickness should be applied as the active biofilm thickness for autotrophs and heterotrophs and no aerobic biomass activity is considered beyond the DO penetration depth.

From Eq. (30), the penetration depth of DO can be obtained,

$$L_{fO} = \sqrt{\frac{2D_c Y_{s1} Y_{s2}}{4.57 b_1' X_1 Y_{s2} + b_2' X_2 Y_{s1}} C_s} \tag{31}$$

3.3.5 Model solution

After the substrate concentrations at the media surface (C_0, S_{10}, S_{20}) and biofilm surface (Cs, S_{1s}, S_{2s}) were determined, J_c, J_1, and J_2 become the only unknown variables in the equation set of (14) through (16) since C_b, S_{1b}, S_{2b} can be measured directly (input data) and the other parameters can be obtained with experiments or literature. An integrated interaction process then can be used to solve for the mass fluxes of DO, TAN, and COD into the biofilm with Microsoft EXCEL solver and the computation process can be developed into an Microsoft EXCEL spreadsheet.

3.4. Model performance

To provide a standard procedure for the evaluation of different biofilm modeling approaches, the International Water Association (IWA) task group on biofilm modeling created a set of benchmark problems for the comparisons of analytical, pseudo-analytical, and numerical biofilm models (Noguera and Morgenroth, 2004). This set of benchmark problems consisted of three types among which the third benchmark problem (BM3) was designed to compare biofilm models with aims at simulating multi-species and multi-substrate bioiflms. The simulated domain of BM3 was described as a completely mixed reactor with a biofilm of a fixed amount of biomass composed of aerobic heterotrophs, aerobic autotrophs, and inert biomass, growing on a flat surface. The standard parameters defining the physical domain as well as kinetic and stoichiometric parameters are listed in Table 3.1 (Rittmann et al., 2004).

The IWA biofilm model research group investigated and compared different 1-D biofilm models for multi-species and multi-substrate biofilms and four numerical biofilm models were presented. The numerical models, W (by Wanner), and M1 and M2 (by Morgenroth), exploited the AQUASIM software, while model E (by Eberl) exploited a different computer code (Rittmann et al., 2004). The most important characteristic that distinguished between these models is the way in which a model represented the biomass distribution within the biofilm. Models W and M1 allowed the distribution to develop naturally according to the relative growth rates of the biomass types, while models M2 and E imposed uniform distributions throughout the biofilm. Models W and M1 are also the models that protected the slow-growing species by having them migrate to the back of

the biofilm. Three cases (Table 3.2) including a standard case, a special case with a high influent ammonia concentration, and a special case with a low influent ammonia concentration were used for the comparison of biofilm models outputs (Rittmann et al., 2004).

To verify the capability of the simplified analytical model (SAM) on predicting substrate flux into biofilms in this thesis study, the standard parameters in Table 3.1 and influent conditions in Table 3.2 were used to compute the mass fluxes of ammonia and COD. The mean density of each species of the four numerical models was used to calculate the mass fractions of autotrophs and heterotrophs for the input of the simplified model. The mass fluxes of ammonia and COD obtained with the simplified analytical model were then compared with the mean of the outputs from four numerical models from the IWA biofilm-modeling group.

From Figure 3.3, it can be seen that good agreement is obtained between the SAM (analytical) and numerical models. For the mass flux of ammonia, 9.5%, 6.5% and 60% of deviations were found between two types of models for the standard case, high ammonia influent case, and low ammonia influent case respectively. For case 3 with a low influent ammonia concentration, the difference on the ammonia mass flux between two types of models was large. However, it was noted that without protection on the slow-growing species, the nitrifiers in model M2 and E were washed out resulting in a zero flux for ammonia. If in case 3, only the mean of the ammonia mass fluxes from models W and M1 was compared with the result of the simplified model, a 3.4% deviation was observed between the two types of models. Therefore, an average deviation

of 6.4% was obtained for the ammonia mass flux between the SAM and the numerical approaches. The simplified model had an average of 12.1% lower COD flux than the numerical models for all three cases with deviations of 12.3%, 11.9%, and 12.1% for cases 1, 2, and 3 respectively. It was noted that the substrate flux obtained with the analytical method was lower for both ammonia and COD compared to that of the numerical approaches. This probably can be attributed to one of the assumptions with the simplified analytical model that the mass transfer within the biofilm is due to diffusion only resulting in an increase in the external mass transfer resistance and decrease in mass fluxes. Overall, the deviation of the simplified model from numerical models was acceptable for practical use along with benefits of time saving and computation simplicity brought by this approach.

3.5. Model application for biofilter design

3.5.1 Parameter estimation

It needs to be pointed out that the values for the standard parameters in BM3 were only for the purpose of comparison of different biofilm models. To apply a biofilm model for a design purpose in reality, the kinetic parameters need to be determined carefully to assure the prediction accuracy. Very little discussion was available on parameter estimations in biofilm modeling, which is also one of the obstacles keeping the biofilm models away from being applied to the real world. Based on numerical solutions, the AQUASIM software was developed for different biofilm processes, including bioreactors for wastewater treatment and biofilm processes in rivers and lakes, but the users have to

determine all of the input parameters on their own. This may not be difficult for researchers since they can determine some of the parameters experimentally or they can choose appropriate values from literatures specific for their requirements. However, from the perspective of a bioreactor design engineer or operator, parameter estimation can become a burden and keep them away from using biofilm models. Rittmann and Stilwell (2002) provided a brief guideline for their integrated biofilm model applied to water treatment, which made the biofilm model applicable in practice.

Estimation on the input parameters of the simplified biofilm model based on literature availability and experimental results was presented in this section to provide a parameter selection guideline for the biofilm model processes applied in recirculating aquaculture system and other wastewater treatment systems with similar characteristics.

3.5.1.1 System parameter estimation

To design a biofilm reactor, the system parameters, such as flow rate, influent characteristics and effluent requirements are presumed to be provided and serve as the input for the calculation of the substrate mass fluxes into the biofilm, which can then be used to estimate the required biofilm surface area and the reactor volume subsequently. It is important that all of the system parameters should use appropriate units, which can be referred to the units used in the standard case (Table 3.1).

3.5.1.2 Kinetic parameter estimation

The kinetic parameters for heterotrophs and autotrophs (nitrifiers) were obtained from literature and are summarized in Table 3.3 and 3.4. The values used for the biofilm model computation were based on the average of the literature results.

3.5.1.3 Estimation on biofilm thickness and biomass density

Biofilm thickness

To investigate the biofilm thickness and biofilm characteristics in common biofilter types applied in wastewater treatment and aquaculture systems, biofilm samples colleted from a chemical feed reactor series system were analyzed with a scanning electron microscopy (SEM) for floating bead filters, fluidized sand filters, and submerged bio-cube filters. These three types of biofilters were tested at different COD to TAN ratios at 10, 15 and 20 ℃. Detailed information on the reactor series system is available elsewhere (Ling and Chen, 2005). Biofilm samples for three reactors were collected and analyzed for the tests at 20 ℃, and the biofilm thickness of reactors at 10 and 15 ℃ was assumed to remain the same as that of 20 ℃.

Biofilm samples were collected directly from the three types of biofilters when they reached steady state at each C/N ratio. The collected samples were then fixed with 2.5% glutaraldehyde/2% paraformaldehyde and 0.1M phosphate buffer for overnight reaction. Subsequent treatment included a 3× 10 min buffer rinse and fixation with 1~2 % Osmium

tetroxide for 1 hr at room temperature, and a 3× 10 min buffer rinse, followed by lyophilization. The freeze-dried samples were then mounted on aluminum stubs and gold coated with a Technics Hummer V Sputtering Device. For the measurements of biofilm thickness, the collected bead samples with biofilm covered on the surface, were sliced into 1mm thick pieces with minimal disruption before gold coating. Finally, the prepared samples were viewed on a HITACHI S-570 Scanning Electron Microscope (SEM) with an accelerating voltage of 20 KW.

Figure 3.4 shows the SEM images on biofilm thickness measurement and morphology of bead biofilm samples at 3 different C/N ratios at 20 ℃. The effect of organics addition on the biofilm microorganism composition was apparent. As C/N increased, the development of filamentous heterotrophic bacteria was significant indicating faster-growing hetertrophs competing with autotrophs for more space on the biofilm. The influent C/N ratios did not have much impact on the biofilm thickness with the SEM measurement with a thickness at about 100 μm. This observation is comparable with the reports in literature. Ohashi et al. (1995) measured the biofilm thickness on clay pellets (d = 3 mm) in a biological aerated filter and found that the biofilm thickness was not affected by the addition of organic matter with a thickness of 120 μm at different C/N ratios (C/N = 0.18, 0.37, 0.52, and 1.5). The SEM measurement on the bio-cube biofilm (photos not showed) was similar to the bead biofilm and an average biofilm thickness of 100 μm was used with the bio-cube biofilm. However, it was difficult to measure the biofilm thickness of the fluidized sand filter due to the difficulty of sectioning silica sand. Therefore, estimation on the average biofilm thickness of the sand filter was based on the

observation of the morphology of the sand biofilm and an average thickness of 50 μm was estimated.

Biomass density

Very little information is available on biomass density in biofilm modeling. It has been reported that the average values used in aerobic biofilm models ranged from 10,000 to 50,000 g VS m^{-3} (biofilm volume) of total biomass density depending on the biofilm types and hydraulic characteristics (Rittmann and Stilwell, 2002). A total biomass density of 14,000 ~ 222,000 g VS m^{-3} was reported for fluidized bed reactors (Trinet et al., 1991) and a biomass density of 19,700 ~ 36,600 g VS m^{-3} was observed for a fixed bed filter after backwash (Ohashi and Harada, 1994). Rittmann and Stilwell (2002) suggested using values of 20,000 ~ 35,000 g VS m^{-3} for the biomass density in biofilm reactors for drinking water treatment with sand, anthracite, or GAC (granulated activated carbon) as filtration media. Based on the available information from above, the total active biomass density was estimated at 15000, 20000, and 5000 gm^{-3} for the floating bead, fluidized sand, and bio-cube filters, respectively. The density of active biomass was assumed constant at different C/N ratios based on the results from literature. Ohashi et al. (1995) reported that the influence of C/N ratios on the fraction of active biomass and total biomass density was insignificant, although the distribution of microorganism types was very sensitive to the influent C/N ratio.

The values estimated for the biofilm thickness and active biomass density for the three types of biofilters are listed in Table 3.5 for different operating conditions. The fluidized

sand filter had the highest active biomass density among the three types of reactors based on the fact that the sand biofilm encountered the largest shear stress. This hypothesis is supported from other reports, which show that the biofilm density increased with increasing shear stress on the biofilm (Vieira et al., 1993; Kwok et al., 1998; Chang et al., 1991). With a thinner biofilm thickness, the sand biofilm is also characterized by having a greater percentage of active biomass all across the biofilm. Conversely, the bio-cube filter had the least active biofilm without any backwashing or cleaning during operation and had the smallest active biomass density.

3.5.1.4 Estimation on biofilm bacteria distribution

The mass fraction for autotrophs (ϕ_1) and heterotrophs (ϕ_2) under different COD/N conditions were determined by data fitting with the biofilm model with the regression models from the reactor series system (Ling and Chen, 2005). The input bulk concentration for ammonia was fixed at 10 mg l^{-1}, and the bulk COD concentration was changed according to the change of COD/N ratios. The regression models of organic effects on nitrification rates obtained from the series reactor experiments were used to calculate the nitrification rates of biofilters, and the mass fractions of both bacteria were then determined when the results of nitrification rates from the biofilm model matched with the experimental results. For example, the nitrification rates of a floating bead filter corresponding to different COD/N ratios can be calculated with the regression model: $R = 0.67 + 2.27\ e^{(-1.38(COD/N))}$ at T= 20 ℃. If the input ϕ_1 for the mass fraction of autotrophs is adjusted corresponding to a change of COD/N ratio, the value of ϕ_1 would then be

chosen when the mass flux of ammonia from the biofilm model matches with the result from the regression model. As the summation of ϕ_1 and ϕ_2 is 1, ϕ_2 was determined thereafter. The regression models in regard to the effect of organic matter on nitrification rate for three types of biofilters are listed in Table 3.6. In the same manner, the relationship between ϕ_1 and COD/N ratio were developed in Figure 3.5 and these results were used as the input for the biofilm models of these three types of reactors.

3.5.2 Validation of biofilm model with experimental data

To further verify the simplified model for biofilter nitrification prediction, experimental data from the reactor series system with temperature at 15 ℃ were compared with the results of the simplified model based on the estimated parameters.

From Figure 3.6, the simplified model can provide satisfactory prediction on nitrification rates of biofilters under different C/N ratios with high correlation with experimental data ($R^2 > 0.5$) for most tests. The correlation of determination (R^2) between predicted and observed values was low (0.37 and 0.18) for the tests of the sand and the bio-cube filters at C/N=2. This may be due to the fact that the correlation of determination is oversensitive to extreme values. The scattered data points obtained from these two tests resulted in low R^2 correlating to model predictions although the model curves fitted within the range of observed data points. This model provided useful results for the effect of organic matter on biofilter nitrification rate. Especially for the study of nitrification kinetics at a high C/N ratio as 2, where the biofilter nitrification kinetics was difficult to determine at low substrate concentrations with the 5-reactor series system, the model

results compensated the missing data at the lower end of TAN concentration. For the test of C/N=0, the model predicted higher nitrification rates than experimental results. This can be explained by the fact of a higher Monod half saturation constant than common literature results was observed with the series reactor system study while a lower Monod half saturation constant was utilized in the biofilm model.

It was noted that the estimation of nitrifier mass fraction was based on the organic impact regression models, which was derived from the reactor series system. However, only the results from the first reactor of each series were used in the development of the regression models. Verification of the biofilm model with results from reactors of the whole series system was valid consequently.

3.5.3 Biofilter design procedure with the SAM spreadsheet

After all the input parameters were determined, the simplified analytical model (SAM) was deployed within a Microsoft EXCEL spreadsheet. The spreadsheet was divided into 3 different sections (worksheets), including section 1 for input parameters, section 2 for intermediate parameters, and section 3 for model output. The required media surface area or media volume for the designed biofilter was then determined with the spreadsheet as output data. The kinetic parameters were preset in the spreadsheet while the system parameters required input from the user. However, if any changes were required on the kinetic parameters, the user can make the adjustment easily by changing the numbers directly on the spreadsheet.

It also needs to be pointed out that the substrate fluxes (fluxes of TAN, COD and DO) predicted by this simplified model is very sensitive to input parameters such as viable biomass density and biofilm thickness. Although this research had attempt on the measurement of biofilm thickness of the three types of biofilters, estimation on biomass density was based on literature values. Operation factors, such as hydraulic conditions and concentration of dissolved oxygen, may have significant effect on biomass density as well as biofilm thickness and these effects were not evaluated in this study. Further research on the effect of operating conditions on these two parameters can assure a better application of this simplified model into biofilter design.

3.6. Conclusions

A simplified analytical model was developed to solve the mass fluxes of ammonia and COD in multi-species biofilms. Based on the concept of equilibrium mass flux at the liquid-biofilm interface from the external and internal mass balance, this simplified model can be solved easily by an integrated interaction process within an Excel spreadsheet. A comparison of the performance of the simplified model against results from complex numerical solutions resulted in deviations of less than 10%. Comparison between experimental data and model results indicated that this simplified model could provide useful information the design of biofilm processes in wastewater treatment and aquaculture systems. A complete discussion on biofilm model parameters selection was also valuable for the end users of this model.

3.7. Acknowledgements

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Table 3.1. Biological parameters, physical constants and reactor conditions for BM3 (Rittmann et al., 2004)

	Symbol	Value	Units
Physical parameters			
Volumetric flow-rate	Q	0.02	$m^3\ d^{-1}$
Biofilm surface area	A	0.1	m^2
Biofilm thickness	L_f	500	μm
Boundary layer thickness	L_w	1×10^{-8}	m
Biomass density	$X_{f,tot}$	1×10^{4}	$g\ COD_x\ m^{-3}$
Oxygen concentration in bulk liquid	C_b	10	$g\ m^{-3}$
Influent COD concentration	S_{2b}	30	$g\ COD_S\ m^{-3}$
Influent ammonium concentration	S_{1b}	6	$g\ N\ m^{-3}$
Ratio of diffusion coefficient in biofilm versus water	Dc,f/Dc	1	-
Kinetic parameters - autotrophs			
Maximum specific substrate conversion rate	$q_{max,1} = (\mu_{1max}/Y_1)$	4.17	$g\ COD_S\ g^{-1}\ COD_X\ d^{-1}$
Half saturation constant for ammonium	K_{s1}	1.5	$g\ N\ m^{-3}$
Half saturation constant for O_2, growth	K_{c1}	0.5	$g\ m^{-3}$
Yield biomass/substrate	Y_1	0.24	$g\ OD_X\ g^{-1}\ N$
Inactivation rate coefficient	$b_{ina,1}$	0.03	d^{-1}
Respiration rate coefficient	$b_{resp,1}$	0.12	d^{-1}
Diffusion coefficient in pure water	D_{s1}	0.00017	$m^2\ d^{-1}$
Diffusion coefficient for oxygen	D_c	0.0002	$m^2\ d^{-1}$
Heterotrophs			
Maximum specific substrate conversion rate	$q_{max,2} = (\mu_{2max}/Y_2)$	9.52	$g\ COD_S\ g^{-1}\ COD_X\ d^{-1}$
Half saturation constant for COD	K_{s2}	4	$g\ COD_S\ m^{-3}$
Half saturation constant for O_2, growth	K_{c2}	0.2	$g\ m^{-3}$
Yield biomass/substrate	Y_2	0.63	$g\ COD_X\ g^{-1}\ COD_S$
Inactivation rate coefficient	$b_{ina,H}$	0.08	d^{-1}
Respiration rate coefficient	$b_{resp,H}$	0.32	d^{-1}
Diffusion coefficient for substrate	D_{S2}	0.0001	$m^2\ d^{-1}$

Table 3.2. Influent concentrations of ammonia and COD for the 3 cases in model comparison

Case	Influent ammonia concentration (mg l^{-1})	Influent COD concentration (mg l^{-1})
1	6	30
2	30	30
3	1.5	30

Table 3.3. Kinetic parameters for autotrophs

Parameter	Symbol	Value used	Literature value	Unit	Reference
Maximum specific growth rate	$\mu_{1,max}^{20}$	1.1	2.2	d^{-1}	Rittmann et al., 2004
			0.14		Horn and Hempel, 1997
			0.95		Wanner and Reichert, 1996
Yield-nitrifiers	Y_1	0.12	0.063	g X g^{-1}	Rittmann et al., 2004
			0.062		Horn and Hempel, 1997
			0.22		Wanner and Reichert, 1996
Substrate half saturation constant	K_{s1}	1	1.5	g m^{-3}	Rittmann et al., 2004
			0.5		Horn and Hempel, 1997
			1		Wanner and Reichert, 1996
Oxygen half saturation constant	K_{c1}	0.5	0.5	g m^{-3}	Rittmann et al., 2004
			0.5		Horn and Hempel, 1997
			0.1		Wanner and Reichert, 1996
Decay coefficient	b_2	0.2	0.2	d^{-1}	Rittmann et al., 2004
			0.2		Wanner and Reichert, 1995
Diffusion coefficient in pure water	D_{1b}	1.67×10^{-4}	1.7×10^{-4}	m^2 d^{-1}	Rittmann et al., 2004
			1.8×10^{-4}		Horn and Hempel, 1997
			1.5×10^{-4}		Chen et al., 1995
Diffusion coefficient of O_2 in pure water	D_c	2.03×10^{-4}	2.4×10^{-4}	m^2 d^{-1}	Rittmann et al., 2004
			2.1×10^{-4}		Horn and Hempel, 1997
			1.6×10^{-4}		Chen et al., 1989
Ratio of diffusion coefficient in biofilm vs. water	D_f/D_b	0.9	1	-	Rittmann et al., 2004
			0. 8		Horn and Hempel, 1997

Table 3.4. Kinetic parameters for heterotrophs

Parameter	Symbol	Value used	Literature value	Unit	Reference
Maximum specific growth rate	$\mu_{2,max}^{20}$	6.42	9.52	d^{-1}	Rittmann et al., 2004
			5.5		Horn and Hempel, 1997
			5		Saez and Rittmann, 1992
			7.3		Furumai and Rittmann, 1994
			4.8		Chen et al., 1989
True yield coefficient	Y_2	0.57	0.63	g X g^{-1}	Rittmann et al., 2004
			0.92		Horn and Hempel, 1997
			0.4		Saez and Rittmann, 1992
			0.5		Furumai and Rittmann, 1994
			0.4		Wanner and Reichert, 1996
Substrate half saturation constant	K_{2s}	9.75	4	g m^{-3}	Rittmann et al., 2004
			5		Wanner and Reichert, 1996
			10		Furumai and Rittmann, 1994
			20		Chen et al., 1989
Oxygen half saturation constant	K_{c2}	0.1	0.2	g m^{-3}	Rittmann et al., 2004
			0.1		Furumai and Rittmann, 1994
			0.1		Wanner and Reichert, 1996
Decay coefficient	b'	0.4	0.4	d^{-1}	Rittmann et al., 2004
Diffusion coefficient in pure water	D_{2b}	0.89×10^{-4}	1×10^{-4}	m^2 d^{-1}	Rittmann et al., 2004
			0.58×10^{-4}		Horn and Hempel, 1997
			1.09×10^{-4}		Chen et al., 1989
Diffusion coefficient of O_2 in pure water	D_c	2.03×10^{-4}	2.4×10^{-4}	m^2 d^{-1}	Rittmann et al., 2004
			2.1×10^{-4}		Horn and Hempel, 1997
			1.6×10^{-4}		Chen et al., 1989
Ratio of diffusion coefficient in biofilm vs. water	D_f/D_b	0.9	1	-	Rittmann et al., 2004
			0. 8		Horn and Hempel, 1997

Table 3.5. Biomass density and biofilm thickness for biofilm model of three biofilters at 10, 15, and 20 ℃

Temperature (℃)	Biofilter type	$X_{tot,a}$ (g m^{-3})	L_f (μm)
20	Floating bead	15000	100
	Fluidized sand	20000	50
	Submerged bio-cube	5000	100

Table 3.6. Expressions of the COD/N effect on nitrification rate (g m^{-2} d^{-1})

Biofilter type	Temperature (℃)		
	20	15	10
Bead filter	$R=0.67+2.27e^{(-1.38(COD/N))}$	$R=0.85+1.88e^{(-1.36(COD/N))}$	$R=0.52+0.47e^{(-0.84(COD/N))}$
Sand filter	$R=1.0+1.16e^{(-0.92(COD/N))}$	$R=0.90+0.93e^{(-1.21(COD/N))}$	$R=0.48+0.40e^{(-1.75(COD/N))}$
Bio-cube filter	$R=0.33+0.65e^{(-1.38(COD/N))}$	$R=0.51+0.62e^{(-2.22(COD/N))}$	$R=0.40+0.38e^{(-1.14(COD/N))}$

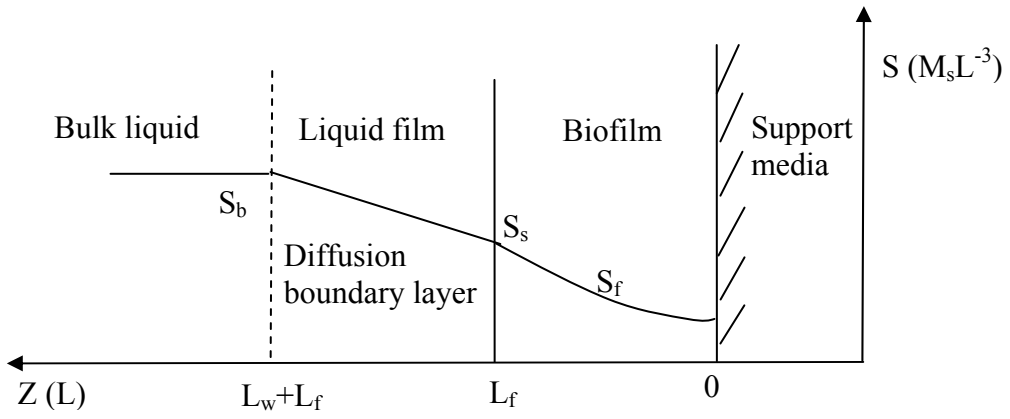


Figure 3.1. Schematic on substrate transfer from the bulk liquid into a biofilm

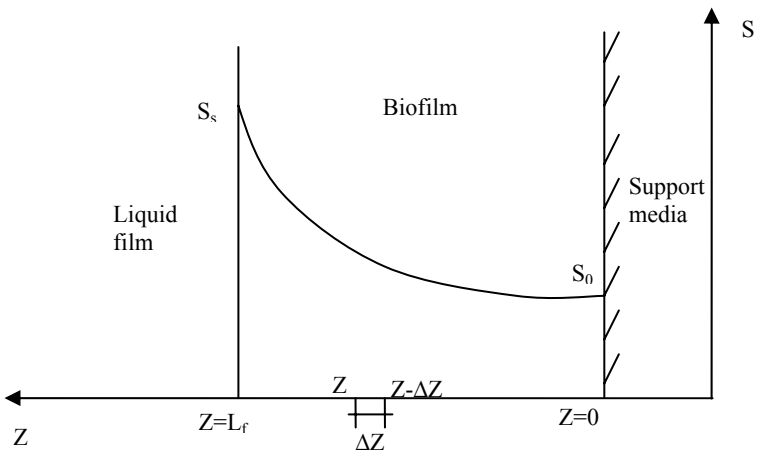
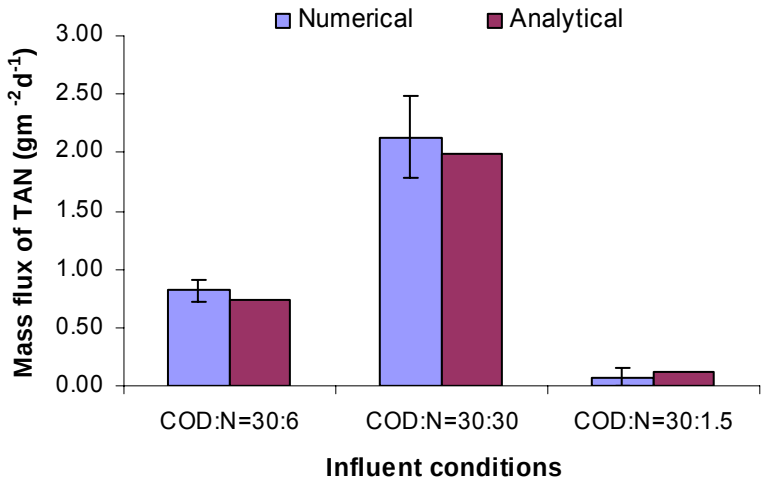
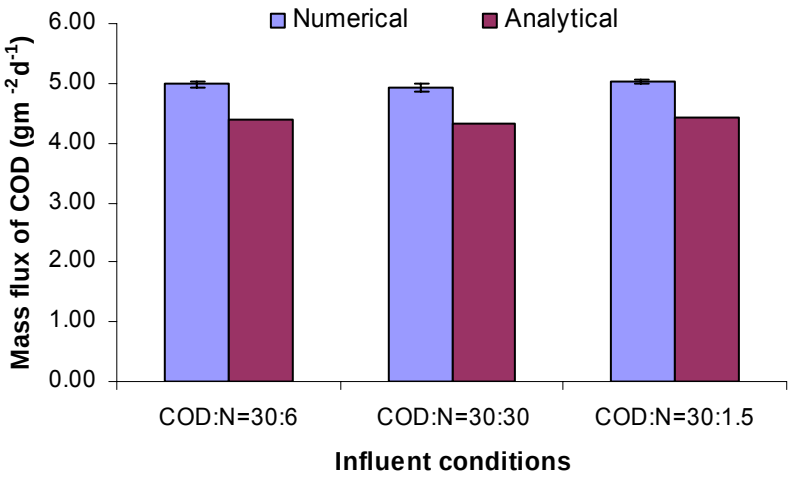


Figure 3.2. Schematic for the substrate profile within a biofilm

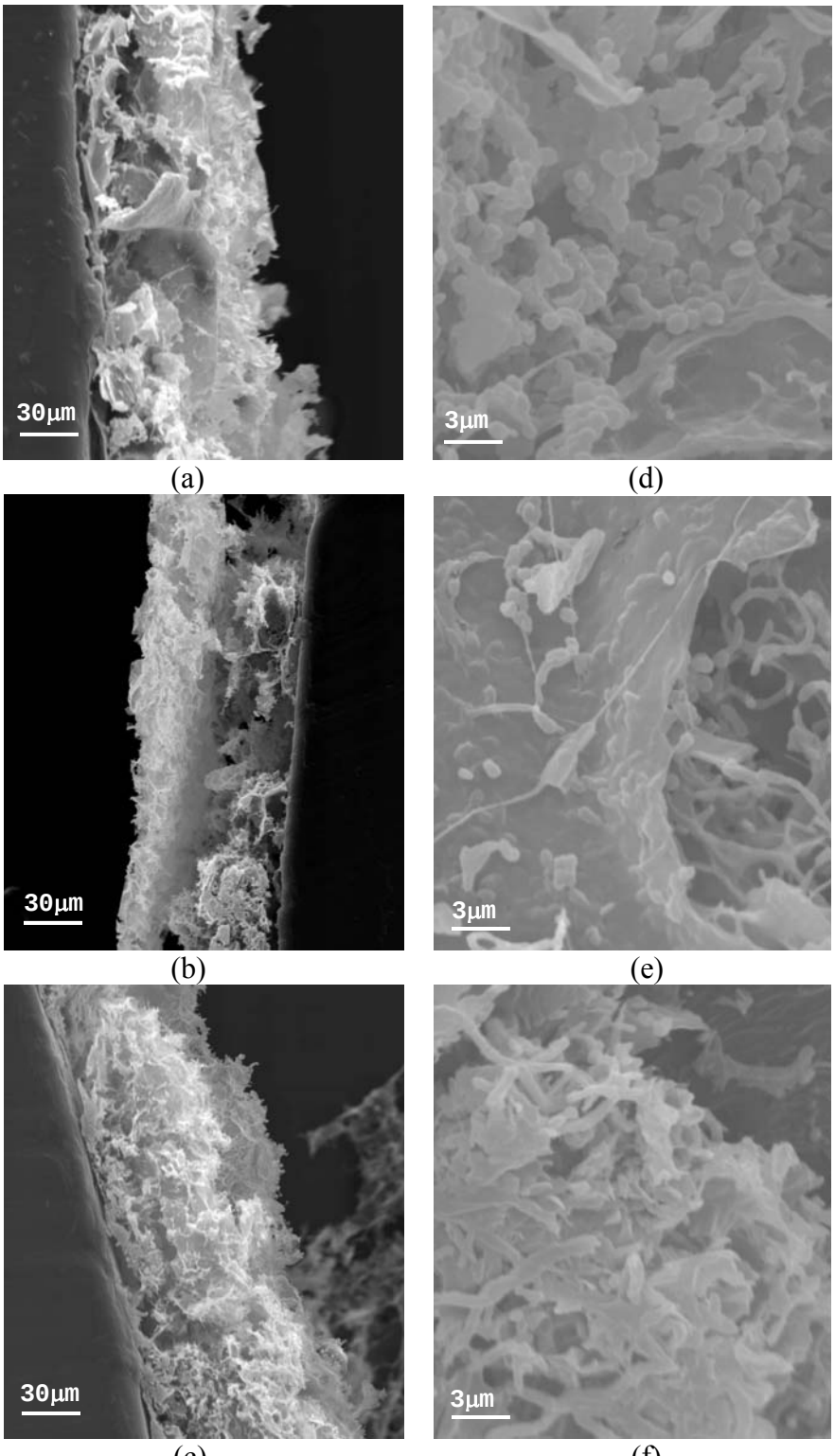


a)



b)

Figure 3.3. Comparison on mass fluxes between the simplified analytical model and numerical models; a) mass flux of TAN; b) mass flux of COD. The numerical results are the means of the outputs from four numerical solutions.



(a) (d)

(b) (e)

(c) (f)

Figure 3.4. Scanning electron microphotographs demonstrating biofilm thickness and biofilm morphology under different loading conditions for floating bead filters at T=20℃. a), b), c): measurement of bead filter biofilm thickness with C/N= 0, C/N= 0.5, C/N= 2 (500x); d), e), f): bead filter biofilm morphology with C/N= 0, C/N= 0.5, C/N= 2 (5000x).

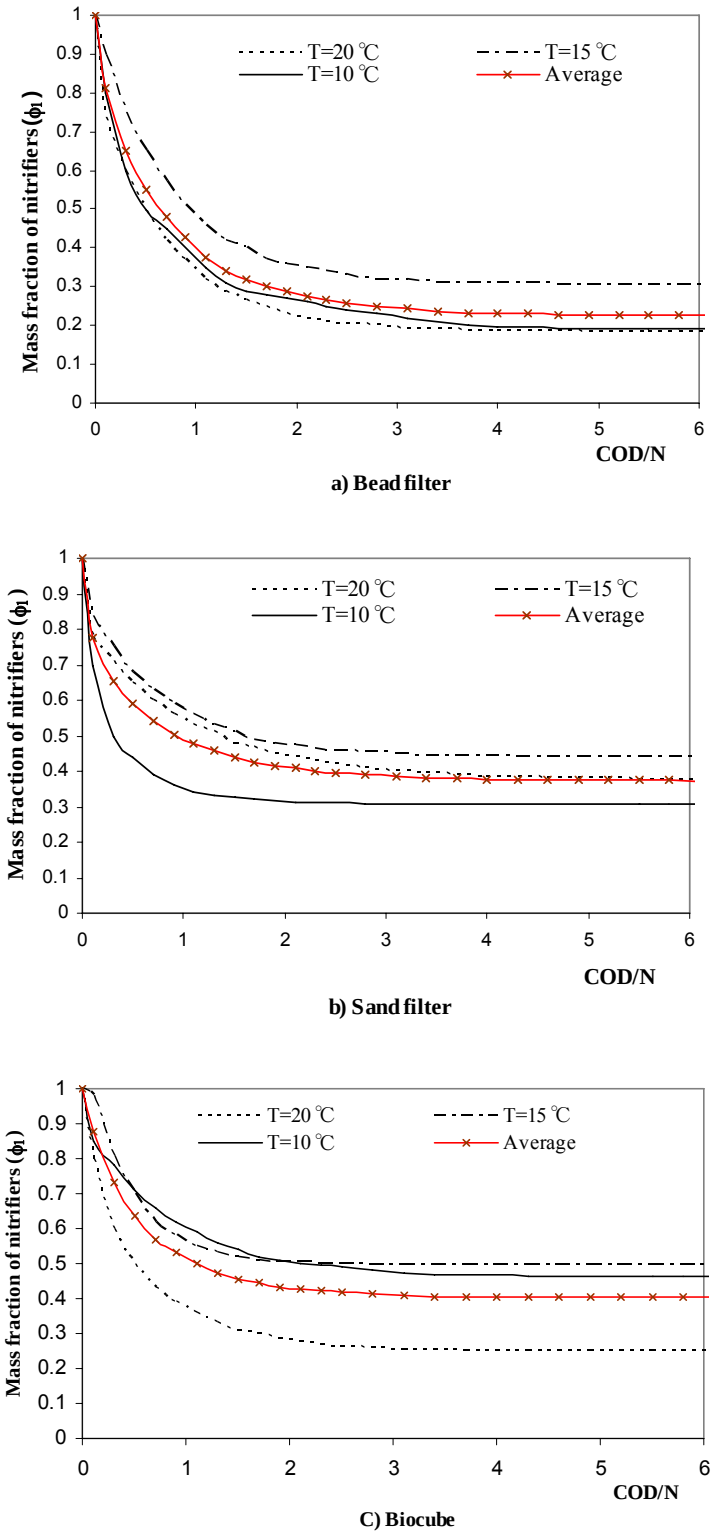
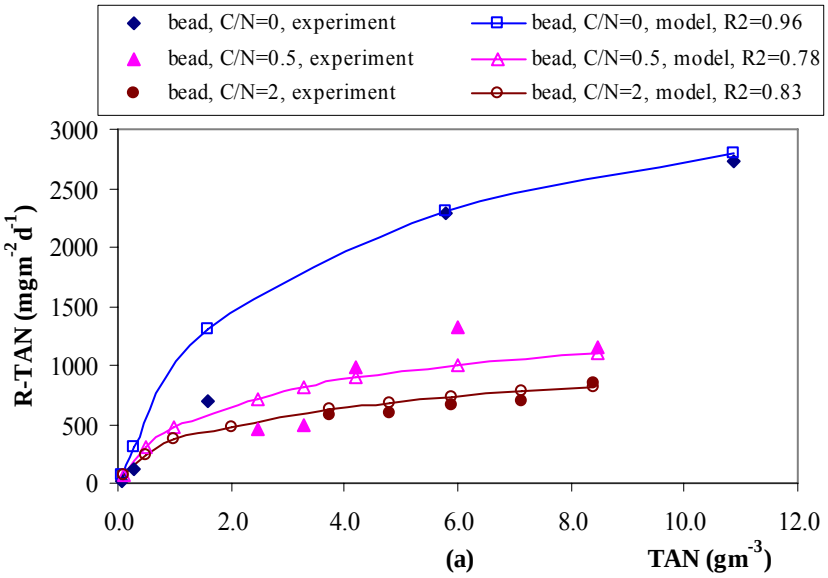
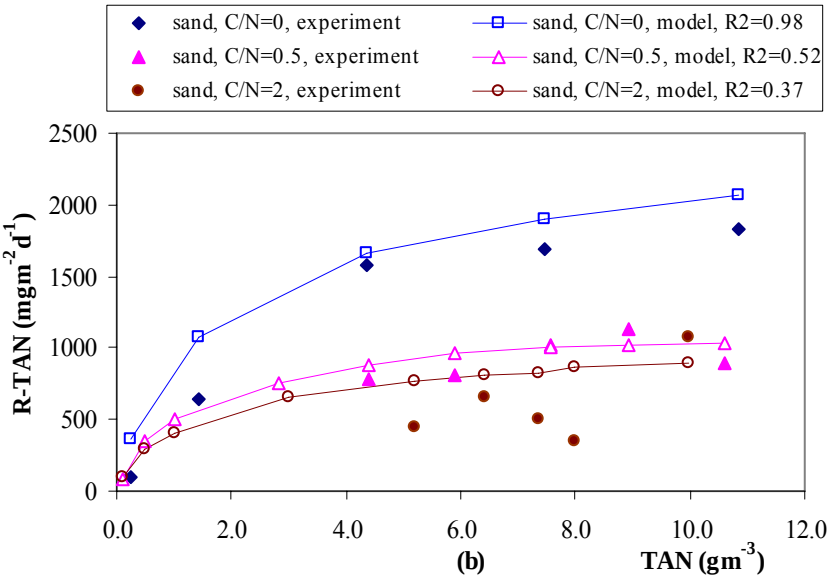


Figure 3.5. Mass fraction of nitrifiers (ϕ_1) in biofilm under different COD/N conditions: a) floating bead filter; b) fluidized sand filter; c) submerged bio-cube filter.


bead, C/N=0, experiment
bead, C/N=0, model, R2=0.96
bead, C/N=0.5, experiment
bead, C/N=0.5, model, R2=0.78
bead, C/N=2, experiment
bead, C/N=2, model, R2=0.83
R-TAN (mgm-2d-1)
TAN (gm-3)
(a)


sand, C/N=0, experiment
sand, C/N=0, model, R2=0.98
sand, C/N=0.5, experiment
sand, C/N=0.5, model, R2=0.52
sand, C/N=2, experiment
sand, C/N=2, model, R2=0.37
R-TAN (mgm-2d-1)
TAN (gm-3)
(b)

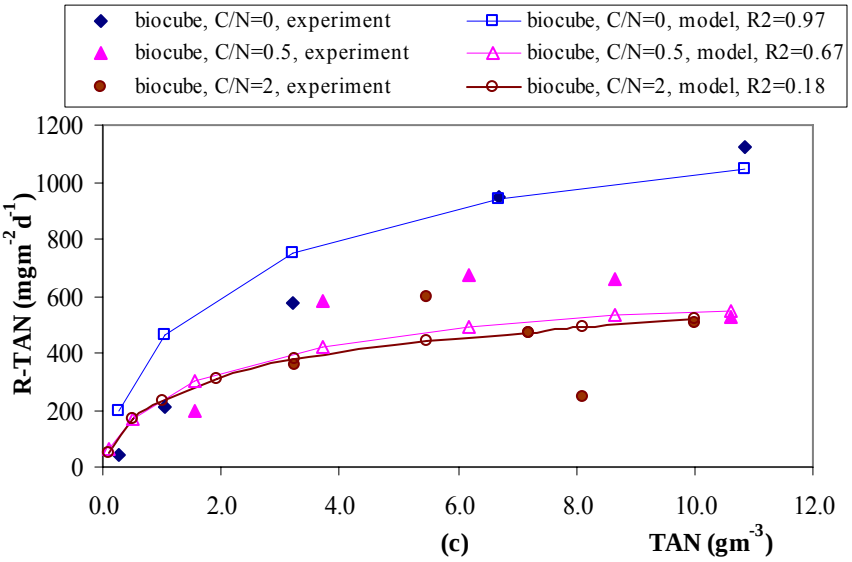


Figure 3.6. Comparison between experimental results and biofilm model prediction for the effect of C/N ration on nitrification rates of: a) floating bead filter; b) fluidized sand filter; c) submerged bio-cube filter.

CHAPTER FOUR

NITRIFICATION DESIGN RECOMMENDATIONS TO COLD WATER RECIRCULATING AQUACULTURE SYSTEMS

4.1. Abstract

With rapid growth of the seafood market, there is a need for developing cost-effective recirculating aquaculture systems (RAS), which serve as an important alternative to traditional pond/raceway systems. Development of a set of appropriate design criteria for nitrification biofilters, the key component in RAS, is crucial for the improvement of cost -effectiveness and performance reliability of RAS. To provide new information on nitrification biofilters for cold water systems, this research combined the nitrification kinetic results obtained from a chemical-feed, lab-scale system and a simplified biofilm model with the results from pilot and commercial scale systems to develop nitrification design recommendations. Design recommendations were provided for several types of biofilters commonly used in cold water systems. Correction factors accounting for the effect of organic matter in RAS and the deficiencies of system scale-up were suggested for biofilter design.

Keywords: Nitrification; Recirculating; Aquaculture; Biofilter; Recommendations

4.2 Introduction

With the increase in demand for fish production, the aquaculture industry has grown dramatically in the last two decades and this growth is expected to continue with the increase in population and the per capita consumption of seafood (Gutierrez-Wing and Malone, in press). The seafood consumption per capita has accelerated, with an increase of 24% from 1970 to 1998 compared to a decrease in the consumption per capita for eggs and meat during the same period (Gutierrez-Wing and Malone, in press).

Among the different types of aquaculture systems, recirculating aquaculture systems (RAS), or water reuse systems, have evolved most rapidly to meet the growing demand. Although RAS may not be comparable to flow-through or pond systems in terms of market share within the current food fish industry, they are very competitive in the high-priced fish market because of their advantage in maintaining a well-controlled growth environment for high quality products. In addition, RAS also feature other advantages such as water conservation, pollution reduction, small footprints, and location independence compared to conventional culture systems. In the past few years, RAS have been identified as one of the two main research areas in aquaculture (the other one is open ocean aquaculture, NOAA, 2001) in the United States and is one of the proposed research areas for the European Union (Gutierrez-Wing and Malone, in press). Updated information and technologies have been available on the design and operation of RAS with an emphasis on engineering and management aspects (Timmons et al., 2001;

Wedemeyer, 2001). The purpose of this paper, though, is focused on the design concerns of a specific and fundamental component of RAS, the biological treatment process.

Biological treatment systems have been used in RAS for ammonia removal and other wastewater purification treatments since the 1960s (Wedemeyer, 2001). In a recirculating system, the waste produced in the culture tanks must be removed at a sufficient rate to guarantee sufficient water quality in the system to prevent stress on the cultured species (Wheaton et al., 1994). Biological nitrification processes mainly consist of two types of systems: attached-growth and suspended-growth. The aerobic attached-growth system, also called the fixed-film process, dominates the nitrification processes in wastewater treatment and aquaculture systems due to its advantage for favoring the slow-growing nitrifying bacteria. Conventional fixed-film biofilters applied in aquaculture systems include: fluidized bed reactor, biological rotating contactor, trickling filter, submerged filter, and floating packed-bed reactor. New biofilter types being recently introduced to RAS include: moving bed reactor, three-phase fluidized filter, and hybrid biofilter.

A great deal of research has been conducted on the design and operation of major biofilters in aquaculture systems. In addition to the general reviews on characteristic advantages and simple engineering methods for commonly used biofilters (Wheaton et al., 1994; Timmons et al., 2001), researchers have provided valuable information in terms of system design, operation and performance evaluations on fluidized bed reactors (Summerfelt et al., 1996; Summerfelt, in press; Sandu et al., 2002), floating bead filters (Malone et al., 1998; Golz et al., 1999; Sastry et al., 1999; Malone and Beecher, 2000),

trickling filters (Miller and Libey, 1984; Kamstra et al., 1998), RBCs (Brazil, in press), and moving bed filters (Greiner and Timmons, 1998; Yossi Tal et al., 2003) for their applications in aquacultural systems. The biofilter design workshop held by the Oceanic Institute in Hawaii in November 2004 provided a stage for researchers to explore improving biofilter efficiency and their application in aquaculture systems. Most of the critical issues confronting RAS were discussed at the meeting, including the difference in water quality requirements for freshwater and saltwater systems, current available nitrification technologies, future trends on biofilter development, categories of RAS, as well as the development of denitrification systems. State-of-the-art knowledge on the design and operation of specific types of biofilters, such as fluidized sand filters (Summerfelt, in press), floating bead filters (Pfeiffer and Malone, in press), RBCs (Brazil, in press), as well as moving bed filters (Rusten, in press; Timmons et al., in press) were summarized. Standardized methods on reporting biofilter performance were also proposed from the perspective of both the academic researcher and manufacturer (Colt et al., in press; Malone and Pfeiffer, in press; Drennan et al., in press). The outcome from this workshop provided a great deal of information that can be used to propel the improvement of RAS. In particular, the effort on the standardization of biofilter evaluation allowed for an important step forward and will be very useful in regulating and improving the RAS market.

Although great efforts have been made on the investigation of nitrification biofilters for aquaculture applications, most research has concentrated on the performance of an individual component under specific operating conditions, and an average ammonia

removal rate was often used to describe the biofilter nitrification performance. Table 4.1 summarizes nitrification design information for various types of biofilters from different systems. The reported nitrification rates of biofilters varied among systems depending on operating conditions and ammonia loadings. From Table 4.1, the volumetric TAN conversion rate (VTR, kg TAN m^{-3} d^{-1}) of biofilters commonly used in RAS are: floating bead filter, 0.07-0.35; fluidized sand filter, 0.1-2.7; trickling filter, 0.02-0.64; moving bed filter, 0.51-2.22; RBC, 0.10-0.13; and submerged filter, 0.01. Based on over ten years of floating bead filter research, Malone and his research team (Malone et al., 1998; Malone and Beecher, 2000) recommended the use of a VTR (kg TAN m^{-3} d^{-1}) of 0.035-0.105, 0.07-0.18, and 0.14-0.35, for the design of floating bead filters in brookstock, ornamental, and growout systems, respectively, for warm water systems. Backwash frequency has a significant effect on the bead filter nitrification rate and Golz et al. (1999) determined that a high VTR (kg TAN m^{-3} d^{-1}) of 0.37 could be achieved by a bubble-washed bead filter with an 8 hr backwash interval and an optimal VTR (kg TAN m^{-3} d^{-1}) of 0.39 for an aggressively-washed bead filter at a 48 hr backwash interval. Recommended nitrification rates for fluidized bed filters were 0.7 kg TAN m^{-3} d^{-1} for applications in cold water systems and 1.0 kg TAN m^{-3} d^{-1} for warm water systems based on a series of pilot scale tests (Timmons et al., 2001). However, the nitrification performance of a commercial fluidized sand filter system reported much lower nitrification rates with 0.35-0.49 kg m^{-3} d^{-1} in a cold water system (Summerfelt et al., 2004) and 0.1 kg m^{-3} d^{-1} in a warm water system (Pfeiffer and Malone, in press). Nitrification rates for trickling filters also varied significantly in different systems.

Design criteria based on an average nitrification rate may be suitable for the design of biofilters operated at higher TAN concentrations where a zero order reaction could be applied. However, for low TAN concentrations, such as cold water systems (TAN < 1 mg l^{-1}), nitrification rate is affected greatly by TAN concentrations and consequently, the use of an average nitrification rate may not be appropriate for biofilter design. The resulting small variations in the design criteria because of the divergence from the use of an average nitrification rate could have a significant effect on the cost of large-scale filters, although the design of small biofilters may not be affected as much.

As biofilm nitrification process could be affected by various parameters, a kinetic study on major impact parameters is very important, especially for the effect of substrate loadings, which is a key factor in categorizing different systems. Very limited work, however, has focused on the nitrification kinetics of aquaculture biofilters. Bovendeur et al. (1990) investigated nitrification kinetics of a trickling filter in a warm water system (African catfish, 25℃) and found that the biofilter nitrification rate followed half-order kinetics for a TAN concentration of less than 2 mg l^{-1}, while zero-order kinetics was applied to a TAN concentration of 2 to 10 mg l^{-1}. A reduction of 0.015 g m^{-2} d^{-1} in nitrification per g m^{-2} d^{-1} of COD removal was also reported by the same authors. In a comparison study of nitrification performance between a micro-bead and a trickling filter in a tilapia culturing system (26.4℃), Greiner and Timmons (1998) reported that nitrification rates of both reactors increased linearly with influent TAN concentrations up to 2.5 mg l^{-1}. A similar linear relationship between TAN loading rate and nitrification rate at low TAN concentrations was also reported for floating bead filter (Malone et al., in

press), RBC (Brazil, in press), and Kaldnes moving bed reactors (Rusten et al., in press). With a reactor series system, Zhu and Chen (1999, 2000, 2001, and 2002) conducted a series of experiments to quantify the effects of TAN concentration, organic matter, temperature, as well as hydraulic loadings (Reynolds number) on nitrification of submerged biofilters. Tseng and Wu (2004) studied the effects of temperature, ammonia, and suspended solids on biofilter ammonia removal efficiency and developed a regression model to provide operating guidelines for biofilter backwash frequency. However, most of these limited studies were from warm water systems, and scarce information is available for cold water systems. The water quality requirement is much higher in a cold water system than a warm water system, especially for ammonia and dissolved oxygen concentrations (Table 4.2). Therefore, simply applying data obtained from a warm water system to cold water system is not appropriate.

The purpose of this paper is to summarize the author's recent research on nitrification kinetics with laboratory studies and its validation with pilot or commercial scale systems, so as to provide nitrification design recommendations for cold water RAS. This paper will also serve as a research example for the linkage between fundamental research and the use of nitrification biofilters in aquaculture industries.

4.3. Nitrification design information regarding different biofilter types

4.3.1. Biofilter design equations without organic impact

Biofilter nitrification design equations were developed with a chemical feed and reactor series system for three different types of biofilters: floating bead filter, fluidized sand filter, and submerged bio-cube filter. A reactor series system is an effective experimental apparatus that can create and maintain a stable substrate concentration in each of the reactors, thus demonstrating their convenience for use in the quantification of nitrification rates under different substrate concentrations (Zhu and Chen, 1999). A 5-reactor series system was employed for the measurements of each type of biofilter in this study. Detailed experimental material and methods were presented in a previous study (Ling and Chen, 2005). This research included tests for three biofilter types with three different organic loadings under three temperature levels, which represent cold water conditions (20 ℃, 15 ℃ and 10 ℃), and the results at 20 ℃ and 15 ℃ were reported in Ling and Chen (2005).

As cold water RAS are usually operated under low TAN concentrations (TAN< 1 mg l^{-1}), the biofilter nitrification rate can be considered to follow a 1st order reaction rate (Ling and Chen, 2005),

$$R = \frac{R_{max}}{K_s}(S - 0.07) \quad (1)$$

Table 4.3 shows the nitrification kinetics parameters and the 1st order nitrification reaction rates at low substrate concentrations for the three biofilters derived from the reactor series system. In terms of the areal nitrification rate (mg m^{-2} d^{-1}), the bead filter and sand filter had better performance than the submerged filter at 15 ℃ and 20 ℃, and it is the author's speculation that this was due to the difference in hydraulic conditions among the different biofilters. However, the difference between the three filters decreased and became insignificant as the temperature dropped to 10 ℃. This was probably because the bacteria had significantly lower growth rates at 10 ℃ and the nitrification rates of biofilters were limited by bacterial growth instead of substrate transfer rate. As a result, the difference in mass transfer through the biofilm, created by the different flow regimes of biofilters became insignificant.

4.3.2. Effect of organic matter on biofilter nitrification performance

With the results from the reactor series system, the impact of organic matter on the nitrification performance of the biofilters was evaluated for the three temperatures tested and compared to the results without organic interaction.

An exponential decrease of the nitrification rate with an increase COD/N ratio was observed for all three types of biofilters at 20 and 15 ℃ (Ling and Chen, 2005). Similar to the results at 20 and 15 ℃, exponential regression models were also developed for these biofilters at 10 ℃ (Table 4.4). In addition, the relationship between COD/N and

nitrification rate in this study was very similar to the results obtained from an activated sludge system (Carrera et al., 2004).

The results in Table 4.4 provide useful information for the design of nitrification biofilters, which will be operated in mixed culture conditions like that in aquaculture systems. In order to clarify the negative effect of COD/N ratio on biofilter nitrification rates, the percentage TAN reduction rates of biofilters compared to the increase of COD/N ratio were calculated and plotted in Figure 4.1 for each temperature level. The percentage nitrification reduction rate was calculated with,

$$\text{R-TAN reduction rate} = \left(\frac{R_0 - R_{COD}}{R_0}\right) \times 100\% \qquad (2)$$

where R_0 is the biofilter nitrification rate with the absence of organic carbon (mg m^{-2} d^{-1}), and R_{COD} is the biofilter nitrification rate with organic carbon addition (mg m^{-2} d^{-1}).

From Figure 4.1, it can be seen that the inhibition of organic matter on nitrification rates of the different biofilters was apparent. The reduction of nitrification rates of the three types of biofilters were about 50~80% under the tested conditions when the COD/N ratio increased from 0 to 5.4 (C/N=2). It is also obvious that the degree of organic impact on nitrification decreased as the temperature decreased with a TAN removal rate reduction of 50~80%, 50~70%, and 45~50% at 20 ℃, 15 ℃, and 10 ℃ respectively. As temperature decreased, both of the heterotrophic and autotrophic bacteria reduced their activity resulting in less competition for space and oxygen between the two species.

Among the three biofilters, the bead filter showed a greater reduction on nitrification with the presence of organic matter at 15 and 20 ℃ while the organic effect was similar in all three biofilters at 10 ℃. A possible reason for the higher organic effect on the bead filter nitrification rate was the packed bed filter could effectively capture the organic particles. The decay of these particles consumed oxygen thus leaving less oxygen available for nitrification. The accumulation of filamentous heterotrophs could also clog the packed bed of the bead filter easily at high organic loadings. As a result, a higher backwashing frequency was required for bead filters at high COD/N ratios to decrease the negative effect of organic matter.

The fluidized sand filter encountered less inhibition with the addition of organic carbon, compared to the other two types of biofilters with a reduction of 46~54% in nitrification at the three temperatures. The fluidized bed of the sand filter contributed to the self-cleaning of sand media and provided more resistance to the negative effect of organic matter. This may also imply that the sand filter had the advantage for systems operated at high organic loadings given sufficient abrasion within the filter to maintain adequate self-cleaning. Siphoning the biosolids layer at the top of the expanded bed is often used to control the growth of biofilm in commercial scale fluidized sand filters with relatively large sand (i.e., with a D_{10} of 0.6 mm or larger) as the expansion depth of these sands remains fairly constant and the biosolids can be removed easily from sand (Summerfelt, in press). However, for finer sand, siphoning of the biosolids usually caused sand loss and sand replacement was typically required within 1~2 years of operation (Summerfelt, in press).

Due to the significant effect of COD/N ratio on biofilter nitrification capacity, a nitrification reduction coefficient needs to be taken into account when biofilters are installed in an aquaculture system. The nitrification capability of aquacultural biofilters may be overestimated if test data from a pure nitrification condition were used in the design of biofilters.

4.3.3. Evaluation of fluidized sand filter with pilot and commercial scale systems

4.3.3.1 Performance of the pilot scale cold water system

A pilot scale cold water RAS was constructed and operated for evaluation of the nitrification performance of a fluidized sand filter in aquaculture systems at the Aquaculture Research and Education Laboratory of Washington State University. The WSU cold water system consisted of the following components (Figure 4.2): (1) “Cornell Dual-Drain” culture system; (2) radial flow clarifier; (3) fluidized sand filter; (4) floating bead filter; (5) cone oxygenator; (6) ultraviolet sterilization (UV); and (7) CO_2 stripping column. A “Cornell Dual-Drain” culture system was used with 5~10% of the water going to a radial flow clarifier for solids removal and 90~95% of the water going to the nitrification biofilter for nitrogen removal. Both flows returned to the culture tank after passing through treatment units. A radial flow clarifier has proven to be very efficient for settable solids removal and was utilized in this system as a result. A fluidized sand filter utilizing fine sand with high specific surface area was selected for ammonia removal because of its high nitrification rate with a compact size. The bead filter was used mainly

for solids capture in this study and its nitrification performance was not evaluated. The other components in the system, cone oxygenator, ultraviolet (UV), and CO_2 stripping column, were selected based on the best available and economical technology for the purpose of aeration, disinfection and CO_2 removal, respectively. Specifications of the system components are also listed in Table 4.5.

The pilot scale cold water RAS was stocked with about 10,000 rainbow trout (size: 1.8 g/fish) in January 2005. The system was stabilized for at least two months before water samples were collected for the evaluation on the nitrification performance of the fluidized sand filter. The system was monitored for DO, pH, temperature, alkalinity and fish mortalities on a daily basis. Water samples were collected for the measurements of TAN, NO_2-N, BOD_5, and COD starting from the middle of March until the end of July (Table 4.6). The pH and water temperature in the culture tank was maintained at 7.46 ± 0.21 and 16.1 ± 0.85 ℃ respectively, and alkalinity was 162 ± 42 mg l^{-1} ($CaCO_3$).

Data collected for the months of June and July of 2005 were used to evaluate the system performance in terms of fish growth. During this period, the average fish weight increased from 58.4 g/fish to 79.6 g/fish with a feeding rate 2.0~2.5%. The total biomass increased from 449 to 598 kg. The system was successful in raising rainbow trout at a high density of 84~108 kg m^{-3} (0.7~0.9 lbs/gallon) with a low mortality (about 0.079% mortality per day). The feed conversion ratio was about 1.47 during this testing period.

4.3.3.2 Nitrification performance of the pilot scale sand filter

Water samples were collected for the influent and effluent of the fluidized sand filter and analyzed for TAN, NO_2^-, BOD_5, and COD. The nitrification performance of the full scale fluidized sand filter was then evaluated with the data collected for the month of July when the feeding rate of the system was stable at 2.5% and the biofilter was considered at a steady state. The nitrification rate of the sand filter can be obtained with the mass balance equation,

$$R_A = 1440Q(S_{in} - S_{out})/A \quad (3)$$

where, R_A, is the nitrification rate of the sand filter (mg m^{-2} d^{-1}); Q, is the flow rate, l min^{-1}; A, is the total biofilm surface area of the sand filter, m^2; S_{in}, S_{out}, are the influent and effluent TAN concentration for the sand filter, mg l^{-1}.

To compare the results of the laboratory scale sand filter with the pilot scale system, the input parameters from the pilot scale system were entered into the regression model derived from the reactor series system. Based on the high C/N ratio in the influent to the biofilter (C/N>>2), the sand filter was assumed to be encountering the maximum organic inhibition with a reduction in nitrification rate of 50%. As a result, the design equation of the sand filter in Table 4.3 was adjusted for a 50% reduction in nitrification rate and the design equation for sand filters at 15 ℃ turned into:

Laboratory scale sand filter nitrification rate (mg m^{-2} d^{-1}): R=278S-19 (4)

Due to the large volume of a production scale fluidized sand filter, complete mixing is difficult to achieve and a concentration gradient is unavoidable across the depth of the reactor. Therefore, a log mean concentration instead of effluent concentration was used to stand for the mean concentration of the reactor, representing the fact that the concentration gradient across the reactor is non-linear (Rittmann and Manem, 1992). The log-mean concentration was obtained with,

$$\bar{S} = \frac{S_i - S_e}{\ln(\frac{S_i}{S_e})}$$

$\bar{S}$ = log-mean concentration, mg l^{-1};

S_i = the concentration at the inlet of the reactor, mg l^{-1};

S_e = the effluent concentration, mg l^{-1}.

With recycle flow, $S_i = S_e + \frac{S_f - S_i}{1+R}$.

S_f = feed concentration, mg l^{-1}.

R = recirculation ratio.

With an average influent and effluent concentration of 0.65 and 0.05 mg l^{-1}, a log-mean TAN concentration of 0.22 mg l^{-1} was obtained for the reactor. The nitrification rate of the fluidized sand filter obtained from the regression model (Eq. 4) was 45 mg m^{-2} d^{-1} compared to the removal rate of 30 mg m^{-2} d^{-1} calculated directly from the system (Eq. 3). The nitrification rate of the commercial scale sand filter showed a 33% reduction as compared to the lab scale sand filter. A volumetric nitrification rate for the commercial

sand filter was also calculated over the experimental period and the results were comparable to the results from other commercial systems (Timmons et al., 2001) with a TAN removal rate of 0.46~1.4 kg N per m^3 of media per day and 0.15~0.47 kg N per m^3 of expanded media per day (based on a maximum expansion of 300%, actual expansion 150~300%).

Several possible reasons may be an answer to the discrepancy between the biofilter nitrification rates for the pilot scale and the laboratory scale systems. Firstly, the media used in the two systems were different. Sand with an average size (D_{50}) of 0.74mm was used in the bench scale sand filter (specific surface area (SSA) = 6070 m^2 m^{-3}), but very fine sand (D_{50}= 0.18 mm, SSA=23800 m^2 m^{-3}) was utilized in the commercial scale sand filter. As a result, problems experienced with the fine sand fluidized bed reactor such as sand blowing out and bio-fouling could be an explanation for the discrepancy in results and lower performance in the commercial system. Secondly, the lower performance in pilot scale biofilters could be due to the factor of scale-up. Biofilters' nitrification performance typically reduced when they were enlarged to larger scales due to the increase of difficulty to maintain optimal operations. For example, it was found at the end of this study that about 2 inches of sand accumulated at the bottom of the filter when the filter was operating. Ester et al. (1994) reported that the nitrification of a commercial scale RBC was reduced about 3 times compared to a laboratory scale reactor although the authors had no explanations for this fact. Summerfelt et al. (2004) suggested that in the design of a full-scale CycloBio^{TM} fluidized sand biofilter (FSB) a 10~40% reduction in expansion should be expected during its test column evaluation with the same hydraulic

loading rate. They explained that the lower expansion in the full-scale CycloBio^{TM} was due to water spouting along the wall of the vessel and to increased formation of transient sand mounds especially when the overall bed expansion was low. Therefore, the deficiencies associated with system scale-up, such as insufficient mixing or expansion can contribute to the lower nitrification rates of biofilters. Finally, the difference in the influent wastewater composition was also a possible reason for the occurrence of the discrepancy. The lab scale sand filter was fed with synthetic chemical solution while the pilot scale sand filter confronted more complex fish culture wastewater in the commercial RAS. Trace elements in aquaculture wastewater can also have inhibitions on the nitrification of biofilters.

4.3.3.3 Comparison of results in lab scale, pilot scale, and commercial scales

In order to extend the validation of the design equations drawn from the reactor series system, results from other pilot scale or commercial scale systems were compared for the fluidized sand filter and listed in Table 4.7. All data were collected from cold water systems (14 - 17 ℃), and different size sand (reflecting to the variation in media specific surface area) were used for fluidized bed filters except in the pilot-scale test of Skjolstrup et al. (1998), where glass beads were used as the biofiltration media.

A normalized nitrification rate, which is calculated by contrasting the results drawn from pilot or commercial scale systems with that from the lab scale measurement, was used for the purpose of comparison.

$$R_N = \frac{R_A}{R_M} \times 100\%$$

Where, R_N= the normalized nitrification rate to lab scale;

R_A = the actual TAN removal rate of sand filters in pilot or commercial scale RAS, mg TAN m^{-2} d^{-1};

R_M = the TAN removal rate calculated with design equation (Eq. 4), mg TAN m^{-2} d^{-1}.

From Table 4.7, it can be seen that fluidized sand filters encountered a reduction of 10~80% on nitrification rate when they were enlarged to either pilot or full production scales except for the pilot scale test with a sand size of 40/70. To eliminate the discrepancy due to different media size, the lab scale results were compared to the tests with similar sand size (30/50 and 20/40) from the pilot study of Tsukuda et al. (1997). About 50% reduction on nitrification rate of pilot scale fluidized sand filters was then observed compared to a lab scale filter given that the same size of media were used in both systems.

Based on the comparison of biofilter nitrification performance with lab scale, pilot scale, and commercial scale systems, it was concluded that the nitrification design equations drawn from the study of the laboratory scale system was valuable in terms of providing kinetics models and design guidelines for cold water RAS. However, in addition to the consideration of a reduction coefficient factor caused by organic impact, a supplementary coefficient corresponding to the difference between a commercial system and the bench

scale system should be addressed before applying the design equations in Table 4.3. Further discussion was provided in the following section.

4.4. Biofilter nitrification design for cold water RAS－the ultimate message

As the addition of organic matter can cause 0~80% reduction in the nitrification rate of biofilters with the deficiencies associated with system scale-up causing another 10~80% reduction of biofilters nitrification rates, the ammonia nitrification rate in a commercial scale production system therefore can be determined as,

R_A (actual) = $\alpha*\beta*R_L$ (α = 0.2~1.0, β = 0.2~0.9)

Where,

R_L = the TAN removal rate from a pure culture laboratory scale biofilter, mg m^{-2} d^{-1};

α = reduction coefficient due to the effect of organic matter;

β = reduction coefficient due to scale-up deficiency.

For the design of floating bead, fluidized sand, and submerged bio-cube filters, α can be determined by Figure 4.1, while β has to be determined by comparing commercial scale data with laboratory scale data. Table 4.8 shows the necessary steps for the calculation of biofilter nitrification rates in a production scale system based on the design equations developed from the lab-scale series reactor system. The nitrification design equation was first selected from Table 4.3 after the biofilter type and operating temperature were

determined. Then, the selected design equation was corrected according to α and β. Recommendations on the other operating parameters based on literature results (Chen et al., in press) are also presented in the same table. The effect of DO concentration on biofilters' nitrification rates at different bulk TAN concentrations was developed in Figure 4.3 with the simplified biofilm model from Chapter 3. The effect of DO/TAN ratio on nitrification rates was similar to all three types of biofilters. Based on the simulation results with the biofilm model, the DO concentration in bulk liquid of biofilters was recommended with a DO/TAN ratio above 2 for TAN = 1~2 mg l^{-1} or DO>2 mg l^{-1} for TAN concentrations lower than 1 mg l^{-1} in order to achieve over 80% nitrification compared to an ideal nitrification without DO limitation. Similar results were also reported in literature that oxygen limitation occurred when the oxygen to ammonia concentration ratio was below 1.5-2 g O_2/g TAN in a circulating bed biofilm reactor for both lab and pilot scale studies (Nogueira et al., 1998). Considering stratification of alkalinity and pH in a biofilm, an alkalinity concentration of 200 mg l^{-1} and pH of 7.5~9 were recommended in the bulk liquid of biofilters, especially for the applications where the water exchange rate is minimal. In addition, if the results from this study were applied to a salt water system, another 30~40 % reduction on biofilter nitrification rate would be recommended (Chen et al., in press; Rusten et al., in press).

Nitrification reduction of biofilters due to problems with system scale-up could be significant. Methods used for the studies of biofilters either in a production scale or lab scale system therefore played an important role for the determination of β in the design equations. It has to be pointed out that the evaluation methods of biofilters in commercial

production systems as well as laboratory studies varied from system to system resulting in discrepancies of reporting data from the real biofilter performance. This also can cause considerable confusion for the final customers when they select biofilters from a large variety.

Fortunately, researchers and engineers from the aquacultural engineering community have started to pay attention to this issue and significant progress has also been made in this area. In the biofilter design workshop held by the Oceanic Institute in Hawaii Nov. 2005, experts, Colt et al. (in press), Malone and Pfeiffer (in press), and Drennan et al. (in press) together with other experts proposed establishing an evaluation standard for nitrification biofilters used in RAS from the perspectives of academic researchers and biofilters manufacturers as well as that of customers.

By classifying biofilter studies into three categories (Table 4.9): kinetics, pilot-scale, and system (production) scale, Colt et al. (in press) proposed detailed standardization on parameters required by each type of study. As proposed, a complete evaluation of a biofilter required study in 6 categories: 1) media characteristics; 2) filter characteristics; 3) general influent waste characteristics (culture system supply); 4) general influent waste characteristics (synthetic waste supply); 5) filter performance; and 6) system performance (culture system). The requirement for the study parameters in each category was also specified, including the basis or source of the parameters, units, symbols, and priority for the three types of study. The priority of study was set as mandatory (MAN), optional (OPT), or not applicable (N/A). Colt et al. (in press) did not specify under what

circumstance a certain type of study was required. It was the author's understanding that a complete set of three types of studies would be included for the evaluation of a new type of biofilter by the manufacturer while the evaluation of a conventional filter would rely on empirical results and only require pilot or full-scale studies. Malone and Pfeiffer (in press) classified biofilters into 7 categories (Table 4.10) according to requirements of different applications (systems) based on the trophic levels (TAN and nitrite concentrations). They also presented a detailed procedure to set up experiments for the evaluation of biofilters' nitrification capacities. The manufacturers of biofilters from the United Sates also provided information on how different manufacturers sized their own bioflters so that the similarities and differences between these methods can be recognized. The information is useful for the establishment of a uniform sizing criteria (Drennan et al., in press).

These proposed reporting standards and procedures for biofilter evaluation will be very beneficial to the application of biofilters in the aquaculture industries as well as other wastewater treatment industries by providing a valuable research platform. The manufacturers suggested that either an areal or volumetric TAN conversion rate (ATR or VTR) based on the standard method should be provided for each type of biofilter at three trophic levels (oligotrophic, mesotrophic and eutrophic) with appropriate correction factors for water temperature and salinity (Drennan et al., in press). Therefore, system designers would be able to select biofilters with appropriate type and size for their requirement. It was also anticipated that biofilter studies based on an updated version of

these standards would become mandatory for future manuscripts submitted to aquaculture and fisheries journals (Colt et al., in press).

4.5. Summary

Nitrification design recommendations were provided for several common types of biofilters used in cold water recirculating systems. Nitrification design equations were developed with a lab scale reactor series system for floating bead, fluidized sand, and submerged bio-cube filters operating at 10 ℃, 15 ℃, and 20 ℃. Although the kinetic studies with the lab scale system provided valuable information for biofilter design, significant reduction on the nitrification rates of biofilters were observed in pilot or commercial scale systems compared to the results from laboratory experiments. A correction factor of 0.2~1.0 for taking into account the effect of organics and another factor of 0.2~0.9 for the effect of system scale-up were recommended when the nitrification design equations resulting from a pure culture measurement were applied in the design of commercial scale biofilters. Recommendations on other operating parameters were also provided for nitrification processes in cold water RAS.

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Table 4.1. Nitrification rates for different types of biofilters in freshwater systems

Biofilter type	System	Temperature, ℃	pH	DO, mg l^{-1}	Influent TAN, mg l^{-1}	Nitrification rate, kg m^{-3} d^{-1}	Reference
Floating bead filter	Brookstock	20-30	6.5-8.0	>3.0 effluent	0.3	0.04-0.11	Malone and Beecher, 2000
Floating bead filter	Ornamental	20-30	6.8-7.0	>3.0 effluent	0.5	0.07-0.18	Malone and Beecher, 2000
Floating bead filter	Growout	20-30	7.0-8.0	>3.0 effluent	1	0.14-0.35	Malone and Beecher, 2000
Floating bead filter	Tilapia, commercial	28	7.2	4.3 influent	0.72	0.10	Pfeiffer and Malone, in press
Floating bead filter	Tilapia, commercial	28-30	7.2-7.5	5-7 influent	0.42-1.09	0.12-0.16	Westerman et al., 1996
Fluidized sand filter	Tilapia, commercial	27.9	7.2	<4.3 influent	<0.72	0.10	Pfeiffer and Malone, in press
Fluidized sand filter	Tilapia, commercial	28-30	7.2-7.5	5-7 influent	0.42-1.09	0.25-0.29	Westerman et al., 1996
Fluidized bed filter	Warm water, pilot	26	7.3	6-7 influent	0.6-0.7	2.70	Timmons and Summerfelt, 1998
Fluidized bed filter	Cold water, pilot	15	7.3-7.5	10-11 influent	0.5-0.6	0.50-1.50	Timmons and Summerfelt, 1998
Fluidized sand filter	Rainbow trout, commercial	15	-	9.4 -10.9 influent	1.07-1.68	0.35-0.49	Summerfelt et al., 2004
Trickling filter	Eel, pilot	25	7.0-7.5	7.4 - 8.2 influent	0.5-5	0.02-0.16	Nijhof, 1995
Trickling filter	Tilapia, commercial	28	-	8.2 influent	0.62	0.14	Twarowska et al., 1997
Trickling filter	Eel, commercial	20-24	6.1-7.9	4.2-10.7 influent	0.3-0.5, effluent	0.02-0.08	Kamstra et al., 1998

Table 4.1. Nitrification rates for different types of biofilters in freshwater systems (cont.)

Biofilter type	System	Temperature, ℃	pH	DO, mg l^{-1}	Influent TAN, mg l^{-1}	Nitrification rate, kg m^{-3} d^{-1}	Reference
Trickling filter	Cold water, lab scale	15	7.7	10.7 influent	1.3	0.03-0.06	Lekang and Kleppe, 2000
Trickling fitler	Tilapia, pilot	26	6.7	>5.0 influent	0.8-4.6	0.15-0.64	Greiner and Timmons, 1998
Moving-bed, microbead	Tilapia, pilot	26	6.7	>5.0 influent	0.8-4.6	0.51-2.22	Greiner and Timmons, 1998
Moving-bed, microbead	Warm water	-	-	-	2-3	1.20	Timmons et al., in press
RBC	Tilapia, commercial	28-30	7.2-7.5	5-7 influent	0.42-1.09	0.10	Westerman et al., 1996
RBC	Tilapia, commercial	28	6.9	-	1.0-1.7	0.13	Brazil, 1996
Submerged filter, static	Eel, commercial	25-32	7.3-7.6	7.1-7.8 influent	0.22-0.25	0.01	Tseng and Wu, 2004

Table 4.2. Water quality criteria of cold and warm water species (Timmons, et al., 2001)

Parameter	Concentration (mg l^{-1})	
	Warm water	Cold water
Temperature, ℃	25~35	8~20
Alkalinity	50~300	50~300
Ammonia (NH_3-N)	<0.6	<0.0125
TAN	<3.0	<1.0
Dissolved Oxygen	4~6	6~8
pH	6.5~8.5	6.5~8.5
TSS	<15	<10

Table 4.3. Biofilters nitrification kinetic constants and first order reaction rates at low TAN concentration (C/N=0, TAN <1 mg l^{-1})

Temperature (℃)	Biofilter type	R_{max} (mg m^{-2} d^{-1})	K_s (mg l^{-1})	R (mg m^{-2} d^{-1})
20	Floating bead	5000	8.5	R=588*S-41
	Fluidized sand	3330	5.3	R=625*S-44
	Submerged bio-cube	1670	5.5	R=345*S-24
15	Floating bead	5000	9.5	R=526*S-37
	Fluidized sand	3330	6	R=556*S-39
	Submerged bio-cube	1670	6	R=278*S-19
10	Floating bead	1000	2.4	R=417*S-29
	Fluidized sand	1429	7.1	R=201*S-14
	Submerged bio-cube	1250	4	R=312*S-22

Table 4.4. Expressions of the COD/N effect on nitrification rate (g m^{-2} d^{-1})

Biofilter type	Temperature (℃)		
	20	15	10
Bead filter	$R=0.67+2.27e^{(-1.38(COD/N))}$	$R=0.85+1.88e^{(-1.36(COD/N))}$	$R=0.52+0.47e^{(-0.84(COD/N))}$
Sand filter	$R=1.0+1.16e^{(-0.92(COD/N))}$	$R=0.90+0.93e^{(-1.21(COD/N))}$	$R=0.48+0.40e^{(-1.75(COD/N))}$
Bio-cube filter	$R=0.33+0.65e^{(-1.38(COD/N))}$	$R=0.51+0.62e^{(-2.22(COD/N))}$	$R=0.40+0.38e^{(-1.14(COD/N))}$

Table 4.5. Specifications of WSU cold water RAS components

System Components	Volume (gal)	V (m^3)	Media SSA (m^2 m^{-3})	Media volume (m^3)	Comments
Culture tank	1450	5.48			-
Fluidized sand filter	238	0.90	23800	0.32	Model: FBB-50, Aquaneering Inc.
Floating bead filter	127	0.48	1310	0.17	Model: PBF-10S, Aquaculture Systems Technologies, LLC
Clarifier	250	0.95	-	-	15^o cone bottom tank
Sump 1	375	1.42	-	-	-
Sump 2	220	0.83	-	-	-
Cone oxygenator	30	0.11	-	-	Aquatic-Eco Systems, Inc.
CO_2 stripping column	40	0.15	160	0.11	-

Table 4.6. Water quality within RAS

Location	Flow rate ($lmin^{-1}$)	TAN ($mg\ l^{-1}$)	NO_2^-N ($mg\ l^{-1}$)	DO ($mg\ l^{-1}$)	BOD ($mg\ l^{-1}$)	COD ($mg\ l^{-1}$)
Culture Tank	190~270	0.61 ± 0.20	0.04 ± 0.03	5.04 ± 0.88	14.7 ± 3.4	53.5 ± 10.2
Bead filter influent	300 ± 32*	0.48 ± 0.13	0.12 ± 0.06	12.4 ± 0.72	12.4 ±1.5	46 ± 5.9
Bead filter effluent/Sand filter influent	-	0.65 ± 0.34	0.29 ± 0.27	11.4 ± 0.78	13.6 ± 0.6	50 ± 3.9
Sand filter effluent	268 ± 30*	0.05 ± 0.02	0.08 ± 0.04	6.01 ± 1.27	9.1 ± 2.1	46.1 ± 4.4

*: Flow rate through components.

Table 4.7. Validate laboratory results in pilot and commercial scale RAS

System	SSA ($m^2 m^{-3}$)	Expansion (%)	TAN-in ($mg\ l^{-1}$)	TAN-out ($mg\ l^{-1}$)	TAN (Log-mean, $mg\ l^{-1}$)	R_A (VTR) ($kg\ m^{-3}\ d^{-1}$)	R_A ($mg\ m^{-2}\ d^{-1}$)	R_M ($mg\ m^{-2}\ d^{-1}$)	Normalized Nitrification rate, R_N (%)
Laboratory Scale	6070	50%	-	-	-	-	R=278S-19	R=278S-19	100
Pilot Scale (Skjolstrup et al., 1998)	1000	50%	2.2 ±0.5	1.1 ±0.4	1.59	0.27	270	423	64
Pilot scale evaluation on fluidized sand filter of four sand sizes (Tsukuda et al., 1997)									
40/70	11700	50%	0.62	0.07	0.25	1.5	128	51	254
30/50	7400	50%	0.62	0.55	0.58	0.48	65	142	46
20/40	5500	50%	0.62	0.57	0.59	0.45	82	145	56
18/30	4400	50%	0.62	0.57	0.59	0.56	127	145	88
Pilot Scale (WSU)	23800	150~300%	0.65 ± 0.34	0.05 ± 0.02	0.23	0.71	30	45	67
Commercial-1 (Summerfelt, 2004)	15700	116%	1.18	0.09	0.42	0.35	22	98	23
Commercial-2 (Summerfelt, 2004)	13800	190%	1.07	0.38	0.67	0.49	36	167	21

Table 4.8. Nitrification design recommendations to cold water RAS

Steps	Impact factors	Response
1	Determine biofilter type and operating temperature	Design equations for C/N=0 (Table 4.3)
2	Determine organic lever (C/N)	Correction for organic effect: α=0.2~1.0 (Figure 4.1)
3	Scale-up due to hydraulic deficiencies and other undesirable operating conditions	β = 0.2~0.9 depending on system design
Recommendations for operating parameters		
4	Effect of DO concentration	Suggest DO/TAN > 2 or DO> 2 mg l^{-1}, correction for DO effect (Figure 4.3)
5	Effect of pH	7.5< pH <9.0 * $r = r_{max}[1+10^{(6.5-pH)}]^{-1}$
6	Alkalinity	> 200 mg l^{-1}
7	Salinity	Suggest for 30~40% reduction with salt water system

*: Siegrist and Gujer (1987)

Table 4.9. Classification of the three basic types of biofilter studies (Colt et al., in press)

Type of study	Typical biofilter size (l)	Typical objectives	"Wastewater" characteristics
Kinetic	1-50	Determination of the kinetics of ammonia removal under very controlled conditions of temperature, substrate, etc.	Typically use a synthetic "wastewater" of defined composition, commonly without significant BOD component
Pilot-scale	5-100	Evaluation of new media or filter configuration under controlled conditions	May use synthetic wastewater or waste from an experimental system
System	100-10000	Evaluation of new media or filter configuration under production conditions	Use waste from large production systems

Table 4.10. Biofilter classification suggested to the needs of the recirculating aquaculture community (Malone and Pfeiffer, in press)

Class	Application	TAN/nitrite performance range (mg l^{-1})
Ultra-oligotrophic	Larval	0.0-0.1
Acidic-oligotrophic	Ornamental	0.1-0.3
Oligotrophic	Broodstock	0.1-0.3
Mesotrophic	Fingerling	0.3-0.5
Eutrophic	Growout	0.5-1.0
Hypereutrophic	Hardy growout	1.0-5.0
Acidic-hypereutrophic	Hardy growout	1.0-2.0

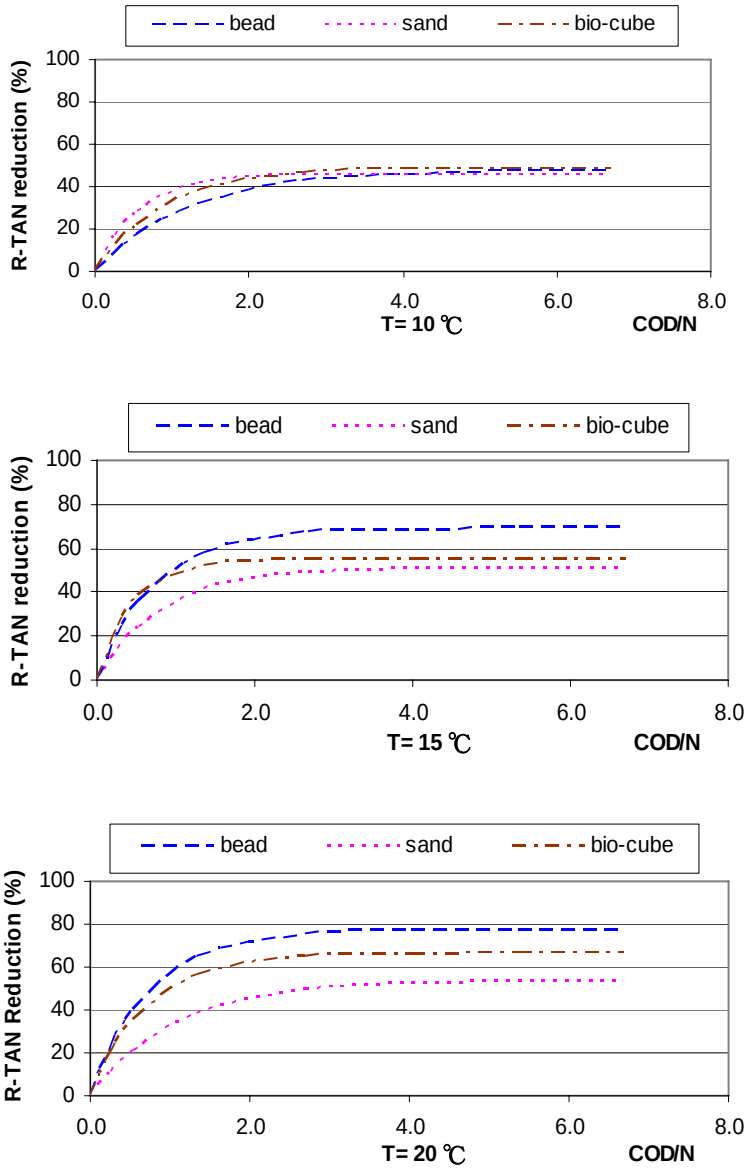

Figure 4.1. Effects of COD/N ratio on biofilters nitrification reduction rates: (a) T=20 ℃; (b) T=15 ℃; (c) T=10 ℃.

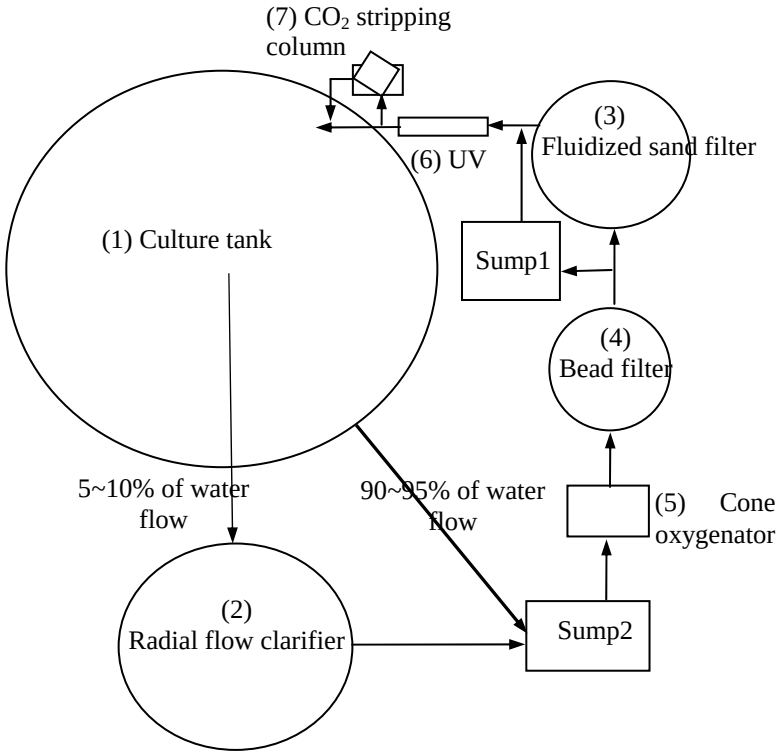

Figure 4.2. A schematic of the WSU pilot scale cold water RAS

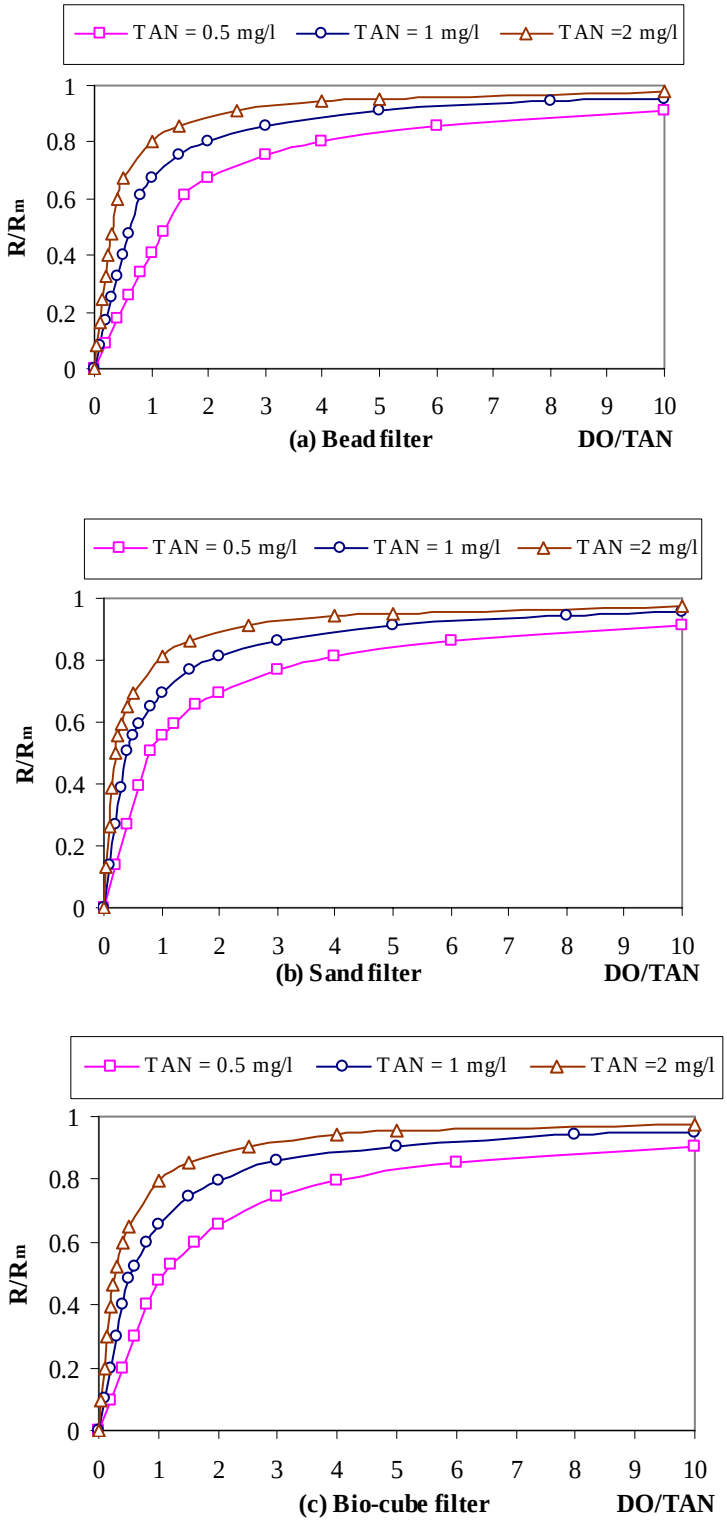

Figure 4.3. Nitrification rates (R) of biofilters relative to maximum rates (R_m, defined as nitrification rates without DO limitation) as affected by DO/TAN ratio.

CHAPTER FIVE

SUMMARY

This research focused on fixed-film nitrification process as affected by the interaction of low TAN and relatively high organic concentrations, which are important characteristics in RAS that differentiates it from other wastewater treatment systems. New information was obtained for the design and operation of nitrification biofilters for RAS, especially for cold water RAS. Main research efforts included biofilter kinetic studies with lab-scale experimental systems and the development of a simplified mathematical model, and nitrification design recommendations to cold water RAS. The contributions from this research are summarized as follows.

1. A lab-scale reactor series system was constructed to study the impact of organic matter on the nitrification rates of three types of biofilters commonly used in RAS at a temperature range of 10-20 ℃ thatreflects cold water culture conditions. The results from this study were:

1) The negative effect of organic matter on biofilter nitrification rates could be quantified with exponential regression models between nitrification rate and influent COD/N ratio.

2) The organic impact on biofilter nitrification rates increased with an increase in temperature from 10 ℃ to 15 ℃ and 20 ℃. Among the three types of biofilters, bead filters were the most affected by the increase of COD/N while sand filters were the least affected by the addition of organic matter.

3) Temperatures of 15 ℃ and 20 ℃ had no significant effect on the nitrification rates of the three types of biofilters. However, all three types of biofilters encountered significantly lower nitrification rates when temperature decreased from 15 ℃ to 10 ℃.

2. To extend the results from the lab-scale reactor series system into a better understanding of the effect of organic matter on nitrification, a simplified analytical model was developed to simulate the mass fluxes of TAN and COD into multi-species biofilms. Based on the concept of equilibrium mass flux at the liquid-biofilm interface from the external and internal mass balance, this simplified model was solved by an integrated interaction process with Excel spreadsheet. By comparing the performance of the simplified model with results from complex numerical solutions, satisfactory results were obtained for the prediction on biofilter nitrification rates with a deviation less than 10%. Thus, this simplified model could provide useful information for the design of biofilm processes in wastewater treatment and aquaculture systems.

3. To provide recommendations to the design and operation of nitrification biofilters in commercial cold water RAS, the results from the lab-scale reactor series system was verified with pilot and commercial systems. A correction factor of 0.2~1.0 for taking into

account the effect of organic matter and another factor of 0.2~0.9 for the effect of system scale-up were recommended when the nitrification design equations resulting from a pure culture measurement were applied in the design of commercial scale biofilters. Recommendations on other operating parameters based on the results from the biofilm model and literature were also provided for nitrification processes in cold water RAS.