EPA is in the process of developing national effluent limitation guideline regulations for the Aquaculture industry. As part of this regulatory development work, EPA will summarize available information on aquaculture wastewater characterization, waste collection, storage, and treatment systems, and management practices. EPA will gather information on industry demographics and trends and will examine available economic data. EPA will examine environmental impacts that are associated with wastewater from aquaculture operations and existing case studies of the costs and effluent reduction benefits of controls to mitigate these impacts. This information will be used to develop a profile of the industry and to determine economically achievable aquaculture technology options.

EPA is obtaining aquaculture industry information from a variety of contributing sources for review and analysis. As part of this process, the EPA and the interagency federal Joint Subcommittee on Aquaculture (JSA) have agreed to work together to create a national forum and systematic process that can foster broad stakeholder input to generate and report information and data related to the needs of EPA. The JSA has developed the Aquaculture Effluents Study Task Force to assist EPA in these efforts. Numerous Technical Subgroups linked to the Aquaculture Effluents Study Task Force are being formed for specific production systems and practices that are often species related. One of the Technical Subgroups includes Recirculating Aquaculture Systems. Each Technical Subgroup will assist EPA in addressing their data needs and will participate in reviewing draft materials. This presentation will provide an update of EPA's activities.
Nitrification Potential and Oxygen Limitation in Biofilters

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Introduction

Nitrification rate is the most important parameter in biofilter design. Research on different aquaculture systems has suggested a wide range of nitrification rates for different types of biofilters. The maximum nitrification rate (or nitrification potential) for a given total ammonia concentration in a system, however, has not been well documented for aquacultural applications.

Nitrification is the biological oxidization of ammonia nitrogen to nitrate (Sharma and Ahlert, 1977). The following energy yielding reactions are carried out by nitrifiers and nitrafiiers, respectively:

\[
\begin{align*}
\text{NH}_4^+ + 1.5 \text{O}_2 & \rightarrow 2 \text{H}^+ + \text{H}_2\text{O} + \text{NO}_2^- + 58 \text{ to } 84 \text{ kcal} \quad (1) \\
\text{NO}_2^- + 0.5 \text{O}_2 & \rightarrow \text{NO}_3^- + 15 \text{ to } 21 \text{ kcal} \quad (2)
\end{align*}
\]

As autotrophs, nitrifying bacteria use carbon dioxide as the sole carbon source for the synthesis of cell material. Reactions for carbon dioxide assimilation into cell biomass of the respective bacteria are:

\[
\begin{align*}
15 \text{CO}_2 + 13 \text{NH}_4^+ & \Rightarrow 10 \text{NO}_2^- + 3 \text{C}_5\text{H}_7\text{NO}_2 + 23 \text{H}^+ + 4 \text{H}_2\text{O} \quad (3) \\
5 \text{CO}_2 + \text{NH}_4^+ + 10 \text{NO}_2^- + 2 \text{H}_2\text{O} & \Rightarrow 10 \text{NO}_3^- + \text{C}_5\text{H}_7\text{NO}_2 + \text{H}^+ \quad (4)
\end{align*}
\]

According to the above equations, either ammonia nitrogen or dissolved oxygen (DO) can become a limiting factor when its concentration is at a relatively low level. Based on Michaelis-Menten kinetics, the nitrification process in suspended growth is represented by Lawrence and McCarty's modified form of Monod's steady-state growth model (Srna and Baggaley, 1975): 

\[
r_g = \mu_{\text{max}} \frac{X}{K_s + S} \tag{5}
\]

where \(r_g\) = rate of bacterial growth (mass unit-volume\(^{-1}\) time\(^{-1}\)), \(\mu_{\text{max}}\) = maximum specific growth rate (time\(^{-1}\)), \(X\) = microorganism concentration (mass/unit-volume), \(K_s\) = substrate saturation constant (mass/unit-volume), and \(S\) = limiting substrate concentration (mass/unit-volume).

Equation 5, developed for suspended growth, does not address diffusion problems encountered in the fixed-film processes. In a fixed film biofilter, the supply of essential nutrients to the nitrifier bacteria is through a diffusion process. Thus, the diffusion rates of ammonia nitrogen and oxygen are critical in determining the nitrification rate. Since nitrification reactions occur in the biofilm and not in the bulk liquid (Moreau et. al., 1994), the substrate utilization rate is dependent on local substrate concentrations within
the biofilm. At the local reaction sites, reactant concentrations are depressed; and product concentrations are elevated (Boller et al., 1994). Horn (1994) observed that nitrifying populations deep within the biofilm are maintained by endogenous respiration during oxygen limitations and that nitrifying populations on the surface are the only survivors during ammonia limitations.

Tanaka and Dunn (1982) developed penetration parameters to represent the diffusion distance of oxygen relative to ammonia within the biofilm. The substrate penetration is proportional to the oxygen to ammonia concentration ratio in the bulk liquid, and the relative penetration depends only on the stoichiometric ratio divided by the diffusion coefficient ratio \(3.4/[D_{O2}/D_{NH4}]\). Assuming biofilm diffusivities are 80% of pure water diffusivities (Tanaka and Dunn 1982 and Wanner and Gujer 1985), the diffusion coefficient ratio equals 1.25; and the relative penetration criterion becomes 2.72. Therefore, oxygen is the limiting substrate if the oxygen to ammonia concentration ratio in the bulk liquid is less than 2.72. For instance, McHarness et al. (1975) stated that nitrification rates were lower than normal for oxygen to ammonia ratios less than required by stoichiometry. In addition, Horn (1994) showed that oxygen concentrations reach zero within the biofilm when oxygen to ammonia ratios are 1.75. Zhang et al. (1994) observed decreased nitrification activity when DO concentrations were lowered in feed solutions with oxygen to ammonia ratios below 1.8.

Variations in maximum nitrification rates among biofilters are typically caused by differences in the bulk DO concentration. When ammonia concentration becomes relatively high, maximum nitrification rates are often dependent on oxygen concentration and independent of ammonia concentration. Experimental results showed that the first-order relationship between TAN removal rate and TAN concentration could reach 100 mg/l of ammonia nitrogen (Liu and Capdeville, 1994), and that oxygen to ammonia ratios greater than 3 mg O2/ mg NH4-N are necessary before nitrification rate stops increasing (Tanaka and Dunn, 1982). Hagopian and Riley (1998) report that an up-flow, packed-bed biofilter can remove ammonia nitrogen at rates as high as 34,800 mg m\(^{-2}\)d\(^{-1}\) when TAN and oxygen maintained at 50 and 36 mg/l, respectively.

The objective of this paper is to present the results of a study examining nitrification potential and oxygen limitation of biofilters in different substrate concentrations and surface hydraulic conditions.

**Methods and Material**

**Reactor Series System**

A reactor series system (Zhu and Chen 1999, Figure 1) was set up in this study to evaluate the relationship between removal rate and concentration of total ammonia nitrogen (TAN) in salt water. Biocube (Keeton Industries, Inc., Colorado, USA) was used as biofilter media. The reactor vessels were placed in a water bath with a chiller (Aqua Logic, Inc.) and heater (Clepco) for temperature control. An ammonia nitrogen solution resulted from mixing the stock solution and the water from the bath was fed to the system. The ammonia nitrogen feed solution consisted of ammonium chloride, sodium bicarbonate, and micronutrients. Oxygen was supplied to each reactor with
diffused aeration. The experiments were conducted at 10 and 20 °C, respectively. Once
the ammonia nitrogen levels were stabilized, effluent samples from each reactor were
collected for a week to be analyzed for ammonia concentration at the Water Quality and
Waste Analysis Laboratory at Washington State University, a certified laboratory by the
Washington Department of Ecology. Other related parameters such as pH (Oakton pH10
Series Meter), ammonia loading rate, and NaHCO₃ were recorded daily. Temperature
was maintained within ± 2 °C for each experiment. DO was kept above 5 mg/l and pH
between 7.6 and 8.2. Salinity averaged 30±4 ‰. Alkalinity, nitrate, and nitrite were
monitored twice a week.

Figure 1  Series Reactor Schematic

Hydraulic controlled system

A second experimental system (Figure 2) was assembled for evaluating nitrification
performances at different hydraulic conditions (Reynolds numbers). A flexible plastic
tubing (30.5 m long and 0.038 m in diameter) was used as biofilm support media for the
nitrifying bacteria to grow on the surface of the inner wall. The bulk solution was
pumped from a sump through a one-way valve, and then returned to the sump by two
paths: the short-circuit path for overflow and regular path through the flexible tubing.
Two ball valves were used in both of the two paths to regulate water flow rates. The
tubing was placed in a large water bath where water temperature was controlled by a
computer-interfaced data acquisition and control board (ADC-1, Remote Measurement
Systems, Inc., Washington, USA). An air diffuser was placed in the water bath to mix
the water and keep the temperature homogeneous. The sump was also aerated with a
diffuser, so that the dissolved oxygen concentrations were maintained 6.75±0.67 mg/l for
sump water, and 5.41±0.89 mg/l for the effluent of the tubing. A synthetic substrate
solution containing ammonia chloride, sodium bicarbonate and other necessary nutrients
was continuously fed into the sump by a peristaltic pump. The resultant wastewater was
continuously pumped through the tubing until a steady-state was established. This
required about six weeks of acclimation. In the steady state, TAN removal rates by the
reactor equaled the feeding rates. It was assumed that for a fixed TAN feeding rate, a
biofilm had a fixed thickness. Thus, a series of TAN concentrations can be obtained for
different feeding rates and water flow rates to the tubing. The pH value in the reactors was kept in the range of 7.3-8.6, which was optimal for nitrification bacteria growth (Winkler, 1981). The temperature of the reactor was maintained at 20.8±0.4°C.

Figure 2 Schematic of the hydraulic controlled experimental system.

**Results and Discussion**

**Nitrification kinetics**

Figure 3 illustrates the typical Michaelis–Menten type response obtained from the reactor series system at 20ºC. The relationship between TAN removal rate and TAN concentration started as first-order reaction at low concentrations and approached zero-order above approximately 4 mg/l of TAN. Curve fitting the experimental data resulted in the following expression:

\[
\mu_{20} = 2020 \cdot \left( \frac{S}{1.8 + S} \right)
\]

Equation (6) implies that the maximum nitrification potential under the experimental conditions is 2,020 mg TAN-N/m²-day. The nitrification rate reaches the 50% potential at a TAN concentration around 1.8 mg/l.

Both Figure 3 and equation (6) provide additional insights for understanding nitrification processes. At low concentrations, TAN is assumed to be the limiting substrate since the dissolved oxygen concentration was maintained above 5 mg/l. As TAN concentration increased, nitrification rates limitation was switched to DO (Bovendeur et. al. 1990 and Zhang et. al. (1994). According to Tanaka and Dunn (1982), oxygen becomes the limiting substrate when the bulk liquid concentration ratio of oxygen to ammonia drops below 2.72 based on the relative amounts of the two nutrients penetrated to the biofilm. For a DO concentration in bulk solution of 5.5 mg/l, oxygen becomes the limiting substrate when the bulk ammonia concentration reaches 2 mg/l. Therefore, the zero-order response in Figure 3 is due to a second limiting substrate, oxygen.
It needs to be pointed out that the Michaelis-Menten model is only valid for steady-state biofilms. In order to evaluate a zero-order reaction due to substrate saturation, the microorganism concentration must be constant throughout the range of substrates. Since separate reactors cannot contain equal active biomass concentrations under different substrate concentrations, rate data must be normalized using the maximum rate for each reactor (Tanaka and Dunn, 1982).

Figure 3 Relationship between nitrogen removal rate and TAN concentration obtained from the reactor series at 20°C. Diamond points and error bars indicate mean values and standard deviations (n=18).

A similar relationship was demonstrated in the 10°C experiment (Figure 4), and a similar equation can be obtained (equation 7).

\[
\mu_{10} = 1948 \times \left( \frac{S}{2.1 + S} \right)
\]

Equation (7)

Apparently, the temperature effects on nitrification rates did not produce a pronounced difference between the kinetic constants in equations 6 and 7. Experimental error and dissolved oxygen limitations could explain why the temperature effect on substrate utilization was unsubstantial. Increased percentage error in analysis below 2 mg/l subjected removal rate calculations to more error than at high concentrations. Therefore, temperature effects were difficult to distinguish below 2 mg/l when ammonia is the limiting substrate.

When ammonia removal rate calculations became more accurate at high ammonia concentrations, oxygen became the limiting substrate. The removal rate of ammonia at 20 °C might be almost twice the rate at 10 °C, but the ammonia removal rates appear equal because of the limited oxygen supply.

Equations (6) and (7) can be used only for estimating nitrification potentials. The actual nitrification rate in a commercial facility is often much lower than that predicted using...
these equations. Concentrations of organics, total suspended solids and variations in operating conditions contribute to the lower nitrification rates in these facilities.

Fig. 4  Relationship between nitrogen removal rate and TAN concentration for the reactor series operated at 10°C. Diamond points and error bars indicate mean values and standard deviations (n=13).

**BOD Impact**

In order to explore the effects of BOD$_5$ on ammonia removal rate, sucrose was added to the ammonia feed solution at a carbon-to-nitrogen ratio (C/N) of 2. The impact of BOD$_5$ competition on nitrification is clearly demonstrated in Figure 5. Reactor 1 had the highest organic concentrations that allowed heterotrophic growth to dominate the biofilm surface. Due to the quick growth of heterotrophic bacteria, the biofilm growth in the first two reactors was visually distinguishable from that in the remaining reactors. The

Figure 5  TAN Removal Rates in Series Reactor with adding sucrose
ammonia removal rate was low in the first reactor, peaked in the second reactor, and then dropped in the remaining reactors. The influence of BOD$_5$ was assumed to be less significant after the first reactor since nitrification rates increased with decreased organic concentrations. Similar results were observed in a full-scale, six-stage biological reactor where ammonia removal rate increased through the first four stages and then decreased in the final two stages as BOD$_5$ removal dropped through all six stages (Wanner and Gujer, 1985).

**Hydraulic impact**

The maximum nitrification rate can be significantly affected by the hydraulic conditions as represented by Reynolds number at the biofilter surface due to the nature of the diffusion transport processes. The relationship between nitrification rate and TAN concentration resulted from the second experimental system was plotted in Figure 6. According to the classic fluid theory, laminar flow occurs when Reynolds number Re < 2,000. There is a critical zone of Reynolds number from 2,000 to 4,000. Figure 6 indicates that there was no significant difference of the maximum TAN removal rates between Re = 1,668 and 4,003. TAN removal rates corresponding to the two Reynolds numbers were much lower than those at higher Reynolds number. For a fixed TAN concentration, TAN removal rate at Re=66,710 was about 5 times of that at Re=1,668. This demonstrates that hydraulic condition was a major factor affecting TAN removal rate. A higher Reynolds number corresponds to a thinner liquid layer on the biofilm surface and a more rapid mass transfer of nitrogen and oxygen into the biofilm. This is
important for the design and optimal operation of biofilters. When dealing with a shock load, for example, nitrification rate of a biofilter can be improved through increasing Reynolds number, by raising rotation speed of a RBC, or increasing turbulence of the flow over a trickling filter.

**Conclusions**

(1) Nitrification potential was a function of substrate concentration. The relationship between nitrification rate of a biofilm reactor and substrate concentration followed Michaelis-Menten type equation. The maximum ammonia removal rate was above 2000 mg-N/m$^2$-day.

(2) At steady-state, temperature impact to nitrification rate was not significant, especially under high substrate concentration condition when oxygen limitations became apparent.

(3) Oxygen limitation became more pronounced when organic substance was present. The availability of organic matter stimulated the growth of heterotrophic bacteria, which in turn competed with nitrifiers for limited available oxygen.

(4) Hydraulic condition was an important factor affecting oxygen and TAN mass transfer from bulk solution to biofilms, and thus limited TAN removal rate for a nitrification biofilm. For the same TAN concentration level, TAN removal rate at Re=66710 was about 5 times that at Re=1668.

**References**


Nitrification Performance of Nitrifiers Immobilized in PVA (Polyvinyl alcohol) for Marine Recirculating Aquarium System

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Introduction

Nitrification is one of the most important processes in recirculating aquarium system for the storage of high density of fishes, because ammonia is the major excretory production of fish and has toxicity to them (Huguenin et al., 1989). In the biological ammonia removal system of seawater, the nitrifying activity of bacteria have been reported to be extremely low due to the slow growth rate of nitrifying bacteria, the inhibition of nitrification by nitrite ion and high salt concentration (Bower et al., 1981, Furukawa et al., 1993). However, immobilization techniques can be used to overcome in these problems. Immobilization of nitrifier microorganism in support gel is one way to maintain high cell density and prevent washout of the slow-growing autotrophic nitrifiers under low temperature or high flow rate condition.

In this study, characteristics of nitrification processes with immobilized nitrifier consortium in PVA were studied for the development application of marine recirculating aquarium system for raw fish restaurant.

Immobilization of nitrifiers and activity recovery of immobilized nitrifiers in PVA

One portion of concentrated nitrifiers (4.5% dw/w) was mixed thoroughly with one portion of PVA-HC aqueous solution (30% w/v). This mixture was then dropped into gently stirred saturated boric acid solution to form spherical beads (Susumu et al., 1987). In order to complete gelation inside beads, these beads were kept in a saturated boric acid solution for 24h under gentle stirring. Then the beads were taken out and washed with distilled water. The beads containing nitrifiers were reactivated by feeding ammonia in the 50L continuous reactor.
Ammonia removal activity was increased with operating times, and ammonia removal rate reached to 67 ammonia g/m$^3$/day after 22 days of operation. The activity of recuperated nitrifiers beads were higher than that of free cell. The PVA-immobilized nitrifiers were used for the application seawater system.

**Conversion of freshwater to seawater system**

Four bioreactors were used to determine nitrification activities with various salt concentrations. Nitrification characteristics in seawater system were evaluated and the results are shown in Fig. 1. Thirty ppt of salt concentration (seawater), 15 ppt, 7.5 ppt and freshwater were used for reactors, respectively. Influent average ammonia concentration was 10 mg/L and HRT of the reactor was controlled to 12 hours. Salt added reactors were showed a similar pattern on ammonia removal and the initial effluent ammonia concentrations were high due to the incomplete nitrification. The initial ammonia removal activity was low when the salt concentration was high because of the damage to the nitrifier by salt.

Influent ammonia was completely removed after 25 days of operation by addition salt of 3 reactors. However, nitrite concentration reached up to 7 mg/L until 18 days of operation for the reactors with salt, and decreased to lower than 0.1 mg/L after 30-40 days of operation. The salt concentration affected the activity of nitrification. Especially, *Nitrobacter* was damaged by the salt. Consequently, reactor with high salt concentration took long period for the stabilization of the system without the formation of nitrite. Therefore, initial nitrite accumulation occurred due to more damage to *Nitrobacter* rather than *Nitrosomonas* by the addition of salt. After obtaining stable system with seawater, optimum HRT was obtained by the increase of flow rate. The HRT was set in the range of 6.12-0.7 hours as shown in Fig. 2. The ammonia removal efficiency of the system operated with 6.12 h of HTR was 83 % and it dropped to 12 % with 0.7 h of HRT.

Considering HRT and amount of ammonia removed, the ammonia removal rate and ammonia removal efficiency were calculated and shown in Fig. 3. The removal rate becomes highest when HRT was 1 h. The rate increased to 63 g/m$^3$/day when HRT decreased to 1 h. However, the removal rate decreased with a further decrease in HRT. The decrease in HRT lower than 1 h caused the removal efficiency to decrease drastically which indicated the limitation of external diffusion and wash out of ammonia. This indicated that the maximum capacity of the immobilized nitrifiers to convert NH$_4^+$-N to NO$_3^-$ was obtained when the HRT was 1 h. At a higher flow rate (low HRT), the NH$_4^+$-N did not have sufficient contact time to be converted to NO$_3^-$.

The actual setup for the nitrifier reactor also needs much shorter HRT to reduce the size of reactor. Therefore, this study is still in progress.
Figure 1. Nitrification activity of immobilized nitrifiers in continuous reactor with various salt concentration
Figure 2. Ammonia removal profile in seawater nitrification process with immobilized nitrifier by changing HRT

Figure 3. Change of ammonia removal rate and efficiency of immobilized nitrifier in seawater nitrification process with airlift bioreactor by changing HRT

Reference


Recirculating Systems for Maintenance and Propagation of Freshwater Mussels (Unionidae)


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Air driven recirculating systems, an automated feeding system for delivery of algae, and water conditioning and air delivery systems have been developed and tested for the maintenance and propagation of freshwater mussels at the Fisheries and Wildlife Department, Virginia Tech. The twelve-350 L, one-200 L, and twelve-145 L air-driven recirculating trough systems are powered by a 1 hp air blower creating water outputs of 44.3 L/min. Over an 8-week period, water quality virtually did not change in 350 L experimental units that contained 200 mussels (160 mussels/m²). The means and standard deviations of concentrations of total ammonia nitrogen (TAN), NH₃, NO₂, and NO₃ in the experimental units over the 8-week period were 0.10 mg/L (0.07), 0.02 mg/L (0.01), 0.01 mg/L (0.005), and 2.7 mg/L (0.7), respectively. The means and standard deviations of dissolved oxygen (DO) and percentage saturation DO in these treatment units over the same period were 7.8 mg/L (0.6) and 83.4% (4.5), respectively. All systems have been successful in long-term holding of adult and juvenile mussels. Over 2,000 animals of 25 species of adult freshwater mussels have been held over a 3 year period with an approximate mortality of 4%. In addition, fertilization and hermaphroditic shifts in mussels have been observed in these systems. Algae is cultured in Kalwall tubes and pumped to treatment units via PVC pipes and timed solenoid valves. Designs for water conditioning and air delivery systems are also discussed in the presentation.
Abstract

Conventional sensor technology to continuously monitor water quality parameters is outdated. In general, when sensor technologies are placed in water for extended periods, their measurements drift due to a phenomenon known as biofouling. Biofouling, or contamination from algae, bacteria, or a combination of both, restricts the useful timeframe that conventional sensors can be placed in-situ for continuous readings to less than a week, and often less than 24 hours. Before today, the only way to address the effects of biofouling was to physically clean and recalibrate the sensor and support system – an extremely labor intensive and costly maintenance requirement that most industries have been reluctant to adopt. Airak, Inc. is developing proprietary, patent-pending technologies for its state-of-the-art fiber optic water quality sensors that address and mitigate the biofouling problem. This enabling technology will open the door for continuous, long-term monitoring of water quality parameters, specifically those in demanding applications, such as recirculating systems for aquaculture, differential monitoring of industrial effluent points, and the monitoring of ecosystems containing endangered species.

Airak, in conjunction with the Virginia Tech Aquaculture Center (VTAC), is preparing to validate these newly developed sensors in demanding fish culture settings, starting in August 2000. Specifically, the sensor technologies will be subjected to extremes concerning temperature, high-suspended solids, and high algal, bacterial, and fungal content, and their performance documented.

This paper presents an overview of the sensor and system technology, highlights of the planned research, expected outcomes based upon existing research, and a synopsis of the impact this technology could have on increasing aquaculture production.