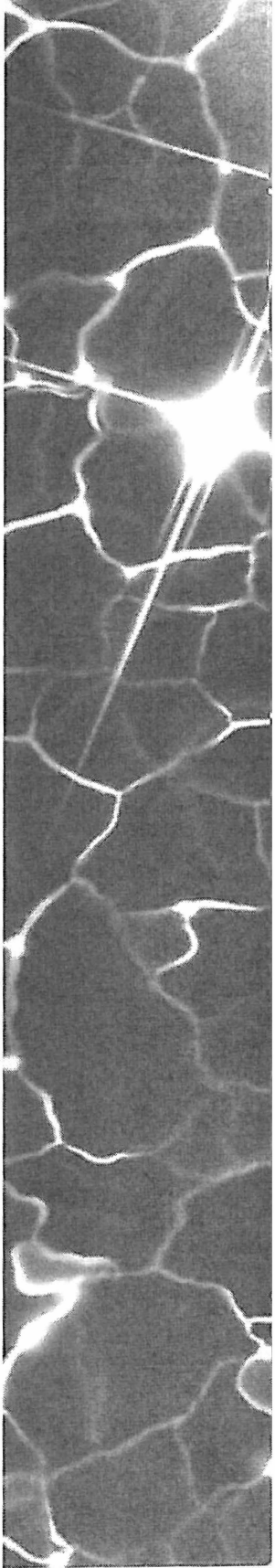
International Journal of Recirculating Aquaculture



June 2006
Volume 7

Commercial Fish and Shellfish Technologies Program
Virginia Polytechnic Institute and State University
Virginia Sea Grant
Blacksburg, VA 24061 USA

VSG-06-01

Printed in the United States of America

ISSN 1547-917X

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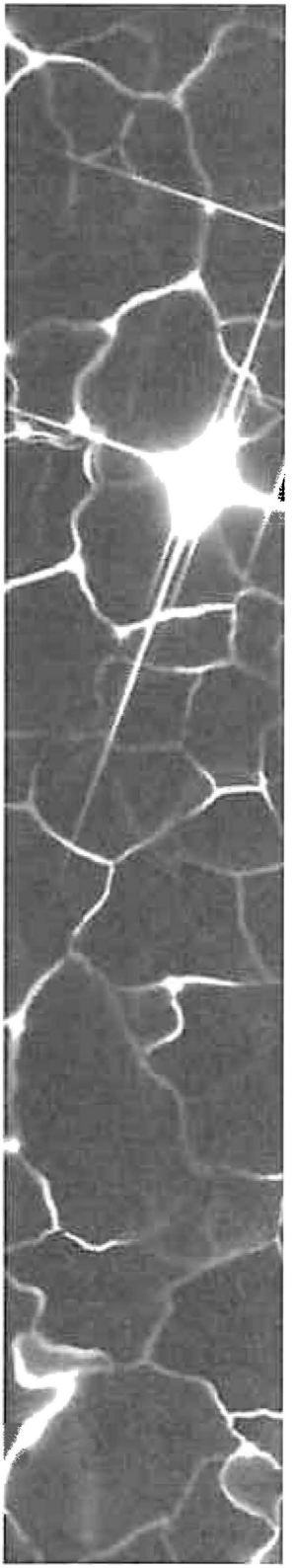


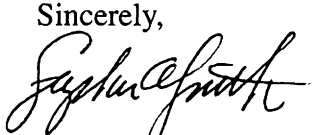
Dear Reader:

In reaching its seventh issue, the *International Journal of Recirculating Aquaculture* (IJRA) has achieved a monumental milestone. The IJRA has always been dedicated to the advancement of the science and application of recirculating aquaculture. As such, the journal provides a forum for the exchange of information and ideas on the subject of recirculating technology, including system and filter design, species selection, species evaluation, fish health and disease prevention, breeding and genetics, larval rearing and growth, fish nutrition, waste management, food and product quality, food safety, marketing, and economics. This issue has several articles on system management from practical system experience to bead filter analysis to system disinfection. There is also a review of hybrid striped bass culture techniques that our readers should find quite informative. This broad range of topics is intended to serve our diverse readership as new research and technologies are developed.

We continue to solicit high-quality manuscripts and reports on all topics relating to recirculating aquaculture. We encourage our readers to contact our production office with any comments, suggestions, or ideas they may have concerning the IJRA and its content. The journal continues to expand its stature in the world aquaculture industry, and is now listed by various U.S. and international citation and abstracting services. This means that the journal is fully searchable by author, title, and/or subject. The journal's website, www.ijra.com, contains archives of past and current abstracts of all papers and book reviews published in the journal, as well as instructions for authors. In addition, numerous university and aquaculture/agriculture libraries worldwide are now receiving issues of the journal for inclusion in their collections. Thus, the journal is available for use by aquaculturists, academicians, and researchers around the world.

We would like to thank our readers, our reviewers, and our authors for their continued support of the journal.

Sincerely,



Stephen A. Smith, Executive Editor

The Effect of Disinfection Strategies on Transmission of *Aeromonas salmonicida* and *Yersinia ruckeri* in a Recirculating Aquaculture System

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Keywords: furunculosis, enteric redmouth disease, recirculating, biofilters, disinfection

ABSTRACT

Continuous addition of *Aeromonas salmonicida* (which causes furunculosis) or *Yersinia ruckeri* (which causes enteric redmouth disease, or ERM) broth cultures to recirculating aquaculture systems, without fish, resulted in the presence of these pathogens in the fluidized sand biofilters. Disinfection of the recirculating systems, except biofilters, with 200 ppm sodium hypochlorite and flushing biofilters for 24 hours with filter-sterilized spring water (FSSW) did not prevent outbreaks of furunculosis or enteric redmouth disease after stocking Arctic char (*Salvelinus alpinus*) or rainbow trout (*Oncorhynchus mykiss*), respectively. Disinfection of the entire recirculating systems with 10 ppm Chloramine-T following outbreaks of furunculosis or ERM, or after addition of broth cultures prevented transmission of ERM in three trials and in two of three trials with furunculosis. Within 75 days of stocking Atlantic

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salmon (*Salmo salar*) with subclinical furunculosis in the recirculating system with rainbow trout, *A. salmonicida* could be cultured from the mucus of rainbow trout and from the fluidized sand biofilters. Removal of salmon and trout and disinfection of the recirculating system with 10 ppm Chloramine-T prevented a furunculosis outbreak when Arctic char were stocked into the system. However, if the recirculating system was only drained and refilled after removal of salmon and trout, furunculosis occurred within 7 days of stocking char.

INTRODUCTION

Infectious diseases can be a major cause of mortality in intensive recirculating aquaculture (Noble and Summerfelt 1996). Precautions such as use of specific-pathogen-free fish stocks, a clean water supply, proper sanitation, and other biosecurity procedures are practiced to help reduce the risk of disease outbreaks. In recirculation aquaculture systems, biofilters used for ammonia removal may be an additional reservoir of pathogens. If an infectious disease occurs in a recirculation system, the pathogen may become established in biofilters and infect newly stocked fish. In a previous unpublished study, results suggested that *Flavobacterium branchiophila* (bacterial gill disease, BGD) became established in fluidized sand biofilters of two experimental recirculating systems. When the systems were built and biofilters functioning, rainbow trout (*Oncorhynchus mykiss*) that had never experienced BGD were stocked into both systems and maintained disease free for 3 months. Rainbow trout that had recovered from a BGD outbreak were stocked with the original rainbow trout and within 7 days BGD occurred in both systems. Trout were then removed from both systems and no trout stocked for 7 days in order to allow *F. branchiophila* to be flushed from the system. However, when the system was restocked with healthy trout, BGD occurred within 21 days suggesting that the bacterium may have colonized the biofilters. The present research was initiated 1) to determine if *Aeromonas salmonicida* or *Yersinia ruckeri* would persist in fluidized sand biofilters and become a source of infection for newly stocked salmonids, and, 2) to test the efficacy of disinfection with 10 ppm Chloramine-T or 200 ppm sodium hypochlorite.

MATERIALS AND METHODS

Recirculating System

All tests were conducted in two identical recirculating systems located at the USGS National Fish Health Research Laboratory (NFHRL; Kearneysville, WV, USA) (Figure 1). Water flowed from the 1.5-m^3 culture tank into a drum filter (100 μm screen size) to remove solids, and was then pumped at 8 L/min up through each of 6 fluidized sand biofilters, each 2.5 m high, and 0.17 m in diameter. Water leaving the biofilters fell back to the culture tank through two degassing columns each 0.2 m diameter x 1.5 m length and filled with 5-cm Norpac media (NWS Corporation, Roanoke VA, USA) to remove carbon dioxide. Water temperature in the systems ranged from 14 to 17°C and oxygen levels were maintained between 9 to 12 ppm. Spring water (12°C) was added to the systems to provide two complete changes per day. Biofilters could be switched to a separate set of pumps and be isolated from the rest of the recirculating system.

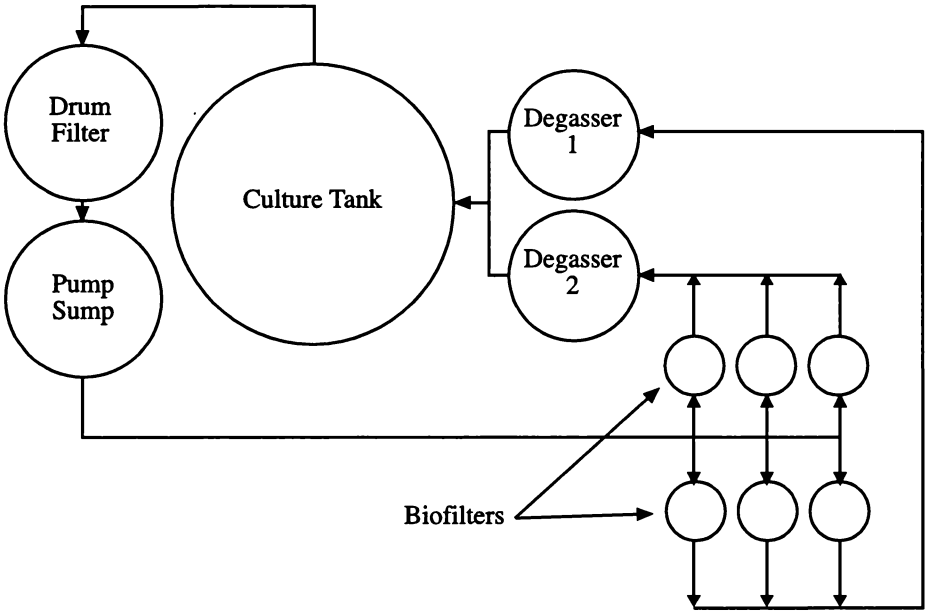

Figure 1. Schematic diagram of fish-culture recirculation system used in bacterial transmission experiments

Bacterial Strains and Media

An *A. salmonicida* strain isolated from Arctic char (*Salvelinus alpinus*) with furunculosis and a *Y. ruckeri* strain isolated from rainbow trout with ERM were used to colonize biofilters and infect stocked char or rainbow trout, respectively. Both isolates had been previously used to produce experimental infections. Isolates were grown in brain heart infusion broth (BHIB; Difco Laboratories Inc., Detroit, MI, USA) for 48 hours at 25°C, and then counted using the drop plate procedure of Miles *et al.* (1938), prior to pumping into the recirculating systems. Coomassie brilliant blue (CBB) agar (Cipriano and Bertolini 1988) was used to detect *A. salmonicida* and Shotts Waltmann (SW) differential medium (Waltman and Shotts 1984) was used to detect *Y. ruckeri.* Suspect *A. salmonicida* colonies on CBB were dark blue, 1 to 2 mm in diameter and were transferred to tryptic soy agar (TSA; Difco Laboratories Inc, Detroit MI, USA) for confirmation as *A. salmonicida salmonicida.*

Colonies on TSA slants were confirmed as *A. salmonicida* if they produced a brown water soluble pigment, were cytochrome oxidase positive, fermentative in O/F glucose (Difco Laboratories Inc, Detroit, MI, USA) and non-motile in a hanging drop. After 48 hours growth on SW, suspect *Y. ruckeri* colonies were 1-2 mm in diameter and surrounded by a zone of precipitation caused by degraded Tween 20 and calcium chloride. Colonies were confirmed as *Y. ruckeri* if they showed an acid slant in triple sugar iron agar slants (Difco Laboratories, Inc, Detroit MI, USA), and a positive slide agglutination test with type one *Y. ruckeri* antiserum (NFHRL, Kearneysville, WV, USA).

Adherence of Pathogens to Active Biofilter Sand

On two occasions, pathogen adherence to functioning (colonized) biofilter sand was determined. Pathogens were grown 24 hours in BHIB at 25°C on a shaker at 125 rpm, after which 1 mL BHIB culture was centrifuged at 10,000 x g. The pellet was washed two times in 1 mL of 0.45 μm filter-sterilized spring water (FSSW), resuspended in 1 mL FSSW, and then stained according to instructions with a Live/Dead Backlight Bacterial Viability Kit (Molecular Probes Inc., Eugene, OR, USA). After checking that most cells were viable (see below) the cell/stain preparation was centrifuged, washed 3 times with FSSW, resuspended in 1 mL of FSSW and mixed with 9 mL of a 1:10 dilution of biofilter sand. The mixture of sand and bacterial cells was inverted once a minute for 10 minutes, then

allowed to settle, and fluid aspirated from the sand. The sand/cell mixture was washed 6 times with FSSW by inverting the sample and allowing sand to settle between washings. The supernatant was then aspirated and the sand was examined under an epifluorescence microscope at 450x. Live cells fluoresced green while dead bacterial cells fluoresced red.

Salmonids

Yearling rainbow trout or Arctic char, stocked at 50 to 60 kg per system (30 to 40 kg/m^3) were used in experiments because rainbow trout are susceptible to *Y. ruckeri* and char are susceptible to *A. salmonicida*. Both salmonids were obtained from the Freshwater Institute (Shepherdstown, WV, USA), which has neither furunculosis nor ERM. However, both species were sampled to ensure they were not infected by these pathogens. Mucus from 25 char were cultured onto CBB agar to detect *A. salmonicida* (Cipriano *et al.* 1992, 1994) and feces from 25 rainbow trout were cultured onto SW agar to detect *Y. ruckeri* (Bullock 2004). All samples were negative.

Pathogen Transmission and Disinfection Trials

Three trials were carried out to determine whether *Y. ruckeri* or *A. salmonicida* could become established in biofilter sand, and to test disinfection methods prior to stocking the systems with either rainbow trout or Arctic char. One gram samples of sand were collected from each biofilter (Bullock *et al.* 1993) and assayed to ensure neither pathogen was present. In trials one and two, broth cultures of test pathogens were added to the systems for 5 days to colonize biofilters. In trial one, sodium hypochlorite (Fisher Scientific, Hampton, NH, USA) disinfection of the system (except for biofilters), and 24-hour flushing of biofilters with FSSW were used to attempt removal of added pathogens. If disease occurred when the system was restocked, fish were removed and a 1-hour 10-ppm Chloramine-T (N-chloro-p-toluene sulfonamide sodium salt, Sigma Chemical Co., St. Louis, MO, USA) treatment of the entire recirculating system was carried out and the system restocked with char or rainbow trout. A concentration of 8.5 ppm Chloramine-T has been shown to be effective in controlling BGD (From 1980) and isolates of *A. salmonicida* were found to be inhibited by 9.0 ppm Chloramine-T (Cipriano *et al.* 1996b). However, efficacy of Chloramine-T for disinfection of *Yersinia ruckeri* has not been reported. In trial two, only the Chloramine-T treatment of the entire system was used to attempt pathogen removal. In

trial three, it was determined whether low numbers of *A. salmonicida* shed from Atlantic salmon (*Salmo salar*), with subclinical furunculosis would result in establishing the pathogen in biofilters, and whether a 1-hour Chloramine-T treatment would remove the *A. salmonicida.*

Trial one – Using a peristaltic pump, 48-hour BHIB broth cultures of either *A. salmonicida* (2.5 x 10^6 to 2.2 x 10^8 cfu/mL) or *Y. ruckeri* (2.8 x 10^8 cfu/mL) were added continuously, at 1 mL/min for 5 days, to the pump intake of the recirculating system (without fish). Once per day for 5 days, a 1-gram sand sample from each of the 6 biofilters was weighed, diluted 1:10 with sterile phosphate buffered saline (PBS), sonicated using the procedure of Bullock *et al.* (1993), and streaked onto culture plates. Each day for 5 days, a 10 μL sample of tank water was also streaked onto culture plates. On day 6, the biofilters were taken off-line. The recirculating system was then disinfected by adding 200 ppm sodium hypochlorite to the culture tank and pumping it throughout the system for 2 hours. The sodium hypochlorite was neutralized with 250 ppm sodium thiosulfate (Univar, Middletown, PA, USA), the system drained, refilled with spring water, and checked with a sodium hypochlorite test strip (Hach Company, Loveland, CO, USA). The biofilters were then continuously flushed for 24 hours with FSSW to attempt removal of added pathogens, after which they were returned on-line to the disinfected system. The day after sodium hypochlorite disinfection of the system and FSSW flushing of biofilters, Arctic char or rainbow trout were added and the system was monitored for an outbreak of furunculosis or ERM. An outbreak was defined by lethargy, loss of equilibrium, mortality, and a bacteriological confirmation of infection. If an outbreak occurred within 6 weeks or sooner after stocking, fish were removed and the system, including biofilters, was disinfected for 1 hour with 10 ppm Chloramine-T. The system was then restocked and monitored for 6 weeks for furunculosis or ERM. During Chloramine-T treatment, total chlorine was measured every 30 minutes in fish-tank water and water entering and leaving biofilters using the DPT method (N,N-dimethyl-p-phenylenediamine; Hach Chemical Co., Loveland, CO, USA). Two trials were carried out with each pathogen.

Trial two – Broth cultures of the test pathogen were again pumped into the system, without trout or char, for 5 days and the pathogen's presence confirmed by culture of biofilter sand and fish-tank water. The entire recirculating system was then disinfected for 1 hour with 10 ppm

Chloramine-T as described, char or trout added, and the system monitored for disease outbreaks for 6 weeks. A single trial was carried out with each pathogen using choramine-T disinfection to remove pathogens.

Trial three – In trials one and two the recirculating systems were subjected to high concentrations of pathogens via high-density cultures. To determine if biofilters could be colonized by a bacterial pathogen from infected fish, two systems were stocked with 50 kg specific-pathogen-free rainbow trout and 8 Atlantic salmon, subclinically infected with *A. salmonicida*. Once per week, 1 sand sample from each of the 6 biofilters and 1 mucus sample from each of 25 rainbow trout from each system were cultured for presence of *A. salmonicida* (Bullock *et al.* 1993; Cipriano *et al.* 1996a). When the pathogen was isolated from biofilter sand and trout mucus, all fish were removed from the systems. One system was disinfected for 1 hour with 10 ppm Chloramine-T and the other was only drained and refilled with FSSW. Char were then stocked in both systems and monitored for furunculosis for 6 weeks or until the disease occurred. A single trial was carried out.

RESULTS

Adherence of *A. salmonicida* and *Y. ruckeri* to Biofilter Sand

On each occasion, color and fluorescence were used to identify the bacteria and indicate whether either bacterial pathogen would attach to colonized biofilter sand. Green fluorescing (live) *A. salmonicida* cells were found attached to sand particles. Only red (dead) scattered *Y. ruckeri* cells were seen and were not attached to sand particles.

Trial One

Arctic char and A. salmonicida – Five-day continuous pumping of *A. salmonicida* resulted in the establishment of the pathogen in all 6 biofilters and tank water both times the trial was carried out. In both tests with *A. salmonicida*, biofilters were still positive for the bacterium after the 24-hour flushing and furunculosis occurred within 3 weeks after char were stocked. When the entire system was treated with 10 ppm Chloramine-T, biofilters were negative for the pathogen after disinfection in the first test and furunculosis did not occur within 6 weeks after char were stocked. However, in the second test, biofilters were also negative after Chloramine-T disinfection, but furunculosis occurred in char within

6 weeks after stocking. The total chlorine concentration in fish-tank water and water entering and leaving biofilters was 9.0 ppm at 30 and 60 minutes of chloramine treatment.

Rainbow trout and Y. ruckeri – The 2.0 x 10^9 cfu/mL *Yersinia ruckeri* cultures that were added to the system also became established in all 6 biofilters of both recirculating systems each time the trial was done. An ERM outbreak did not occur after the biofilters were flushed during the first test, but did occur in the second test. Chloramine-T disinfection of the entire recirculating systems, after disease outbreaks, did prevent ERM outbreaks in both of the two tests, but 1 of 6 biofilters was still positive for *Y. ruckeri* after disinfection in test one. The total chlorine concentration in fish tank water and water entering and leaving biofilters was 9.0 ppm at 30 and 60 minutes of chloramine treatment.

Trial Two

In the single test with each pathogen when the recirculating systems were disinfected immediately after addition of cultures, both pathogens were cultured from biofilters after disinfection but neither furunculosis nor ERM occurred within 6 weeks after char or rainbow trout were stocked. The chlorine concentration in water entering and leaving biofilters was 9.0 ppm during disinfection.

Trial Three

When Atlantic salmon, subclinically infected with *A. salmonicida*, were stocked into both recirculating systems containing rainbow trout, the pathogen could be cultured from biofilters and mucus from rainbow trout (with weekly sampling), but required at least 75 days after stocking infected salmon. Disinfection of one system with 10 ppm Chloramine-T, following removal of trout and salmon, prevented transmission to newly stocked char. Simply draining and refilling the second system after removal of fish resulted in an outbreak of furunculosis to newly stocked char within 8 days.

DISCUSSION

While these studies are not definitive, they show that sodium hypochlorite disinfection of the recirculating systems, except for biofilters, can destroy added *A. salmonicida* and *Y. ruckeri*. However, 24-hour flushing of

biofilters with FSSW after 5-day addition of pathogens does not remove the pathogens and stocking of susceptible salmonids after flushing can result in disease. Furunculosis occurred in both tests of trial one and ERM occurred in one of two tests in trial one.

Cipriano *et al.* (1996b) reported that 9.0 ppm Chloramine-T inhibited *A. salmonicida*, but in this study the efficacy of Chloramine-T disinfection for either pathogen was not consistent. Although ERM did not occur following Chloramine-T disinfection, the pathogen was isolated from one biofilter after disinfection. Conversely, furunculosis did occur after Chloramine-T treatment in test two of trial one even though *A. salmonicida* could not be isolated from biofilters. Failure to isolate *A. salmonicida* from biofilters after Chloramine-T disinfection and the subsequent outbreak of furunculosis is not unexpected. Cipriano *et al.* (1996a) were unable to culture *A. salmonicida* from biofilters during an active furunculosis epizootic. Furunculosis may have occurred after flushing biofilters in both tests of trial one and in one test after Chloramine-T disinfection of the entire system because *A. salmonicida* was protected by biofilm attachment to sand particles and other surfaces (Carballo *et al.* 2000). Cells of *Y. ruckeri* did not attach to sand particles and ERM occurred in only one of two trials after biofilter washing and no outbreaks occurred after disinfection of the systems with Chloramine-T. However, this pathogen may have also been protected by biofilms on system surfaces (Coquet *et al.* 2002). In any case, it is evident that a single 1-hour 10-ppm Chloramine-T treatment will not reliably remove either pathogen.

These studies clearly show that washing biofilters with FSSW or a single 10-ppm Chloramine-T treatment is not reliable in removing two bacterial pathogens of salmonids from a small-scale recirculating system. Using a higher concentration of Chloramine-T or multiple treatments are alternative approaches. The use of an appropriate detergent before Chloramine-T and paying special attention to dead spots such as crevices would likely be more effective. Disinfection with 200 ppm sodium hypochlorite was an effective method, and should still be considered reliable (Piper *et al.* 1982). Efficacy of sodium hypochlorite would also be increased by first using an appropriate detergent. Regardless of the method used, the entire recirculating system, including biofilters, should be disinfected, with subsequent reestablishment of functioning biofilters.

ACKNOWLEDGMENTS

The authors thank Dr. Julie Bebak-Williams for critical review and revision of the manuscript. The experimental protocol and methods described are in compliance with the Animal Welfare Act (9CFR) requirements and were approved by the Freshwater Institute's Institutional Animal Care and Use Committee. This material is based upon work supported by the U.S. Department of Agriculture, Agriculture Research Service, under Agreement No. 59-1930-1-130.

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In-situ Determination of Nitrification Kinetics and Performance Characteristics for a Bubble-washed Bead Filter

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Keywords: nitrification, kinetics, bubble-washed bead filter, Monod kinetics model, performance evaluation

ABSTRACT

Intensive recirculating aquaculture systems rely almost exclusively on some form of fixed-film biofilter for nitrification. Currently there is no standardized way to determine and report biofilter performance to facilitate user selection among the numerous options. This type of information is critical for the end user, and also important for both the design engineer and the manufacturer. In an attempt to address this issue, a simple procedure for estimating nitrification reaction rate kinetics is described and applied to a bubble-washed bead filter. Reaction rate kinetics were determined through a series of batch reaction rate experiments with a commercially available 0.06-m^3 (2.0-ft^3) bubble-washed bead filter. Empirical mathematical models for the nitrification of ammonia-nitrogen to nitrate-nitrogen were developed. The kinetics of nitrification were found to fit a simple first-order reaction model, when the ammonia-nitrogen concentration was less than 1 mg NH_4-N/L, and a zero-order reaction when the ammonia-nitrogen concentration was

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greater. The exact breakpoint between first- and zero-order reaction kinetics was found to be a function of the flow rate. In addition, the first-order kinetic reaction rate constants were also a function of the flow rate, reflecting the influence of high nutrient gradients and associated higher nutrient gradient across the biofilm. No effect of flow rate was found for the zero-order reaction rate constants. Kinetic reaction rate parameters, maximum reaction rates, and half-saturation constants were determined for the Monod kinetics model as functions of hydraulic loading rate. Based on these results, an evaluation tool was proposed to help characterize bead filter performance based on reaction rate kinetics. A series of performance characteristic curves were developed to show maximum removal rates as a function of ammonia-nitrogen concentration and flow rates through the bubble-washed bead filter.

INTRODUCTION

All recirculation systems require basic unit operations to remove particulate solid wastes, biological filters to oxidize toxic ammonia and nitrite-nitrogen to nitrate-nitrogen, and aeration or oxygenation of the water to remove carbon dioxide and increase oxygen concentrations (Timmons *et al.* 2002). Additional unit processes can be added depending on the scale of production and the unique water-quality parameters required for each species, such as pH control, foam fractionation, ozone, and disinfection systems (Timmons *et al.* 2002). Over the past few years, numerous solutions have been proposed and developed to handle each one of these unit operations and processes. At the same time, entire recirculation systems and individual components have become available commercially for almost any scale production facility.

This segment of the aquaculture industry relies almost exclusively on some form of fixed film biofilter for nitrification, such as those found in trickling towers, fluidized-bed, floating bead, and rotating biological contactors. The advantages of these forms of biofilter include resistance to short-term toxic loads, ability to perform at low influent concentrations, and high volumetric biomass concentrations (Riefler *et al.* 1998). In addition, the high cell-residence time of a fixed-film biofilter is needed for the low growth rates of both ammonia oxidizing bacteria and nitrite oxidizing bacteria. In November 2004, the Oceanic Institute sponsored a workshop entitled: Design and Selection of Biological Filters for Freshwater and

Marine Applications. During the four-day workshop, numerous papers were presented, reviewing the many types and applications of biological filters in aquaculture. One of the problems discussed was the lack of a standardized way to determine and report biofilter performance to facilitate user selection among the numerous types of biofilters. One entire afternoon was spent discussing standardized evaluation rating of biofilters from the design approach, and the manufacturer's and user's perspectives in relationship to their capital and operational costs. Malone (2004) recommended using a set of standardized conditions for rating biofilter performance consisting of: chemical feed of ammonia-nitrogen, excess dissolved oxygen concentration, alkalinity greater than 150 mg/L $CaCO_3$, pH of approximately 7.5, and temperature of 20°C. In addition, Malone recommended that specialized conditions for low-temperature performance evaluation could be conducted at 10°C. Malone also suggested that biofilter performance be evaluated at several levels of ammonia-nitrogen concentration reflecting his categorization of aquaculture systems as shown in Table 1.

In the past, the selection of the most applicable biofilters for any given species, production level or economic consideration has for the most part been by "rules of thumb" and operating experience based on existing systems. Today, with the commercial availability of standardized families of biofilters, there exists the potential to fully characterize their operating parameters and develop sets of characteristic curves, reflecting ammonia-nitrogen removal rates as a function of operating parameters such as hydraulic loading rates and ammonia-nitrogen concentrations. The overall objective of this study was to develop a simple biofilter evaluation process that could be used to characterize the nitrification removal rate as a function of several simple operating parameters for a bubble-washed bead filter, most importantly, hydraulic loading rate of the biofilter and the operating level of ammonia-nitrogen.

Table 1. Aquaculture systems classification and corresponding ammonia-nitrogen level.

Classification	**System**	**TAN (mg/L)**
Ultra Oligatrophic	Larval rearing system	< 0.1
Oligatrophic	Broodstock holding system	< 0.3
Mesotrophic	Fingerling production system	< 0.5
Eutrophic	Growout systems	< 1.0
Hypertrophic	Hardy species growout	< 5.0

BACKGROUND

The concept of using a floating plastic media as biofilter media dates back to the mid-1970s, when they were first used at the Dworshak National Fish Hatchery (Cooley 1979) for the rearing of food and game fish. Although successful, the air-washed bead filter design did not find wide acceptance. In the late 1980s, a hydraulically washed bead filter, which combined both solids capture and biofiltration, was developed at Louisiana State University (Wimberly 1990). Later development of the mechanically washed bead filter (Malone 1992, 1993, 1995) overcame many of the operational difficulties of earlier designs and it proved to be compact and simple to operate (Malone *et al.* 1998, 2000). Malone *et al.* (1993) developed the bubble-washed bead filter initially for the outdoor ornamental or garden-pond market. Since then, the bubble-washed bead filter has found wide application for small aquaculture systems, combining clarification and biofiltration in a single unit. Most recently, an air-driven recirculating system employing a bubble-washed bead filter has been designed and tested by DeLosReyes *et al.* (1997), to minimize the complexity and energy requirements of commercial recirculation systems.

Bead filters are classified as expandable granular biofilters (EGB), which include upflow and downflow sand filters. EGB biofilters offer the competitive advantage of using smaller media with corresponding higher specific surface areas per unit volume when compared to other treatment devices such as trickling filters and RBCs. The higher specific surface area translates into smaller biofilter size. The application of sand filters in aquaculture is limited by the inherent constraint on ammonia conversion due to oxygen limitations in the bed, the high pressure required for fluidization, and the excessively high water use for back flushing. These shortcomings were overcome with low-density plastic beads, which float. Filtration of suspended solids is accomplished by settling, straining, and interception within the granular bead matrix (Malone *et al.* 1993). The plastic beads themselves act as a fixed-bed bioreactor for the growth of nitrifying bacteria on the surface and in the pore spaces between the beads. As the solids and bacterial biomass accumulate, the head loss across the filter bed increases and the hydraulic conductivity decreases. The transfer of oxygen and nutrients to the bacteria is reduced, reducing the nitrification capacity of the filter. During the backwashing cycle, the beads are agitated and homogenized, dislodging trapped solids and shearing off excess biofloc from the beads.

When the floating-bead filter is operated under low solids loading, or frequent backwashing, it should behave like a classical fixed-bed biofilm reactor. Under these conditions, the exchange of soluble substrate between the recirculated water and the attached biofilm is relatively unimpeded and the nitrification process can be described by a simple Monod expression. Malone and Beecher (2000) summarized the performance of floating-bead filters based on the three application categories: broodstock, fingerling, and growout, and listed criteria for the sizing of filters based on feed application rates with the primary method for sizing based on volumetric organic loading rates. Table 2 lists typical values for several performance parameters based on operational filters (Wimberly 1990, Sastry *et al.* 1999). Table 3 presents interim guidelines for the design of systems using floating bead biofilters for both clarification and biofiltration filters (Malone and Beecher 2000).

Table 2. Some typical values for performance parameters for floating-bead biofilters (Malone et al. 1998)

Performance parameter	**Broodstock**	**Fingerling**	**Growout**
Feed loading (kg feed /m^3 media day)	<4	<8	<16
Design TAN (mg/L)	0.3	0.5	1.0
VTR* (g TAN/m^3 media)	35 - 105	70 - 180	140 - 350
O_2 consumption (g O_2/m^3 media day)	0.7 - 2.5	1.4 - 2.5	2.5 - 3.0
Temperature (°C)	20 - 30	20 - 30	20 - 30
pH	6.5 - 8.0	6.8 - 7.0	7.0 - 8.0
Alkalinity (mg/L $CaCO_3$)	>50	>80	>100

**VTR = volumetric TAN removal rate*

Table 3. Interim guidelines for the design of systems utilizing floating bead

Design parameter	**Broodstock**	**Fingerling**	**Growout**
Bead volume (m^3 media /kg of feed day)	0.250	0.125	0.062
Circulation rate (Lpm /kg feed day)	208	83	50
Fish density (kg/m^3)	15	10	60
TAN loading (g/m^3 media day)	84	168	339
Hydraulic loading (Lpm /m^3 media)	832	664	806
HRT (days)	11	16	25
Tank turnover rate (min)	32	40	33

These guidelines were developed by examining a wide range of operating systems of various sizes, species selection, and operation management protocols. In an attempt to standardize the characterization of biofilter performance and in particular, the bubble-washed floating bead filter, a series of batch performance evaluation tests were conducted to characterize the nitrification reaction rates as a function of ammonia-nitrogen concentration and flow rate through the filter. Several nitrification models including simple zero-order and first-order kinetic reaction rates and Monod kinetics were examined to determine how well they fit the experimental data and the corresponding kinetic reaction rate constants were estimated.

MATERIALS AND METHODS

Two commercially available 57-L (2.0-ft^3) bubble-washed bead filters (Model BBF-2P, Aquaculture Systems Technologies, LLC, New Orleans, LA, USA) were employed (Figure 1) for the evaluation trials. The two biofilters were part of a research program, characterizing over time the physical and chemical properties of the solids, dissolved nutrient, and organic substances found in four separate recirculation system designs (Ebeling *et al.* 1998a, Ebeling *et al.* 1998b, Singh *et al.* 1999). Each of the four systems consisted of a fiberglass 2.0-m^3 circular culture tank combined with either a settling basin or a rotating microscreen drum filter with a 60-μm screen and either a trickling tower or a bubble-washed bead filter, forming a 2x2 factorial experimental design. Total volume of each system was estimated at 2.13 m^3. Each system had been initially stocked with 320 hybrid striped bass (average weight 100 g) which were fed a commercial diet at 1.5 to 2 percent of body weight once per day. At the time of the kinetic reaction rate experiments, the filters had been in continuous operation for over 24 months and had a well-established biofilm.

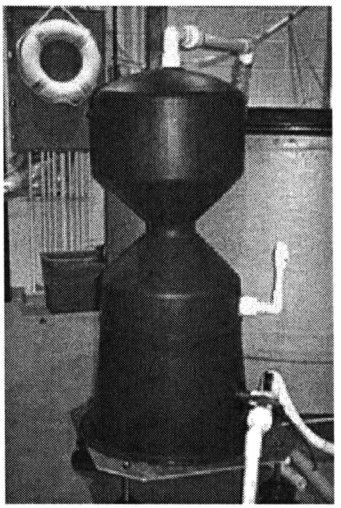

Figure 1. 57 L (2 ft^3) bubble-washed bead filters (Model BBF-2P, Aquaculture Systems Technologies, LLC, New Orleans, LA, USA)

The bubble-washed bead filters have an "hourglass" shaped internal geometry with a constricted washing throat. During continuous filtration, water from the production tank enters from the bottom through a slotted inlet pipe, flows upward through the bed of floating polyethylene beads, and exits through a slotted discharge pipe at the top. The inlet pipe also serves as a sludge discharge line during backwashing. Backwashing consists of completely draining all the water from the filter, causing the beads to be sucked through the washing throat, where they are vigorously scrubbed by cavitation and bubbles from the air inlet valve. The solids-laden water is discharged and the filter refilled, and placed back into operation. Each biofilter contained approximately 57 L of food-grade polyethylene beads, with a mean diameter of 4.4 mm, porosity of 35 percent and a specific surface area of 1050 m^2/m^3 (Sastry *et al.* 1999).

At the conclusion of the above mentioned research project, the fish were removed and the research tanks cleaned and refilled with tap water. The four recirculation systems were then operated for a period of time (approximately 3 weeks) with inorganic ammonia-nitrogen (ammonium chloride) as the sole source of ammonia by a daily addition of approximately 20 to 25 g of NH_4Cl, bringing the ammonia-nitrogen concentration in the tanks to between 2.5 and 3.0 mg-N/L. In addition, each bubble-washed bead filter was backwashed every other day to remove excess biofloc from the system. Heterotrophic bacterial growth was assumed minimal in the biofilters due to the removal of the fish, the backwashing of the systems, and the extended length of time (3 weeks) with little available carbon for their growth.

Each batch nitrification reaction rate trial consisted of spiking each tank with 20 g NH_4Cl and then monitoring water quality in the tanks and the influent and effluent of the individual bead filters at 30-minute intervals until the ammonia-nitrogen concentrations were too low to accurately measure or for a maximum of 8 hours. A range of flow rates through the biofilters was investigated from approximately 10 Lpm to 100 Lpm. These flow rates bracket the design loading rates for the bubble-washed bead filter suggested by Malone and Beecher (2000) from 400 to 800 Lpm/m^3 of beads. All experiments were conducted at room temperature, which varied from 20 to 22°C. Each trial's flow rate was randomly selected from a low flow rate followed by a high flow rate.

The following water quality parameters for the influent and effluent of the biofilter were measured at 30-minute intervals by withdrawing a sample into a 250-mL Erlenmeyer glass flask:

- ammonia-nitrogen (Hach Nessler Method No. 8038 adapted from Standard Methods: 4500-NH_3, APHA 1995) using a HACH DREL/2000 spectrophotometer,
- pH using a Fisher-Scientific Accumet pH Meter 25 (calibrated daily at 4, 7.02, and 10 pH),
- dissolved oxygen and temperature using a YSI Model 58 DO meter (air calibrated method daily),
- alkalinity following standard methods, 2320 B/Titration Method (APHA 1995).

Flow rates through the biofilters were determined by weighing a 20-L bucket of filter discharge water collected over a known time period.

The kinetic reaction rate for the removal of ammonia-nitrogen, r_a, was evaluated based on the change in concentration of ammonia-nitrogen across the filter divided by the hydraulic retention time in the filter, or:

$$r_a = \frac{dC_F}{dt} = \frac{(C_i - C_e)}{V_F / Q} * 1440 \, \text{min/day} \qquad (1)$$

where: r_a = kinetic reaction rate (g/m^3 day)
dC_F = change in ammonia-nitrogen across biofilter [mg/L]
C_i = concentration in influent to biofilter [mg/L]
C_e = concentration in effluent from biofilter [mg/L]
V_F = volume of biofilter [L]
Q = flow rate through biofilter [Lpm]

Figure 2 shows an example of kinetic reaction rate for the removal of ammonia-nitrogen with respect to influent ammonia-nitrogen concentration for several flow rates through the bubble-washed bead filter.

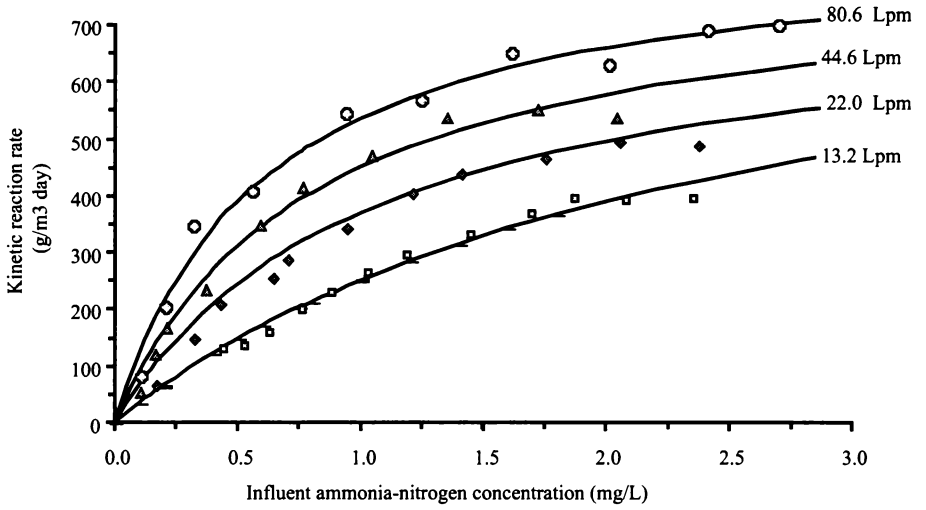

Figure 2. Kinetic reaction rate for the removal of ammonia-nitrogen with respect to influent ammonia-nitrogen concentration for several flow rates after initial spiking with 25 g of NH_4Cl.

RESULTS

Two general approaches to the development of design equations for biological filters have been developed over the past years. The first was to approach the problem from an empirical viewpoint and develop models relating the inlet and outlet concentrations as functions of relevant physical variables such as flow rate, media size, and configuration, dissolved oxygen concentration, pH, and temperature (Wheaton 1985, Metcalf and Eddy 1991). The second approach is to examine the individual processes involved in nitrification, including external mass transfer of ammonia-nitrogen to the biofilm, internal diffusion within the biofilm, and the actual nitrification kinetics (Williamson and McCarthy 1976a,b; Gujer and Boller 1986). Both methods have distinct advantages and disadvantages and are useful in both the design and development of biofilters.

As the aquaculture industry has matured over the past decade, distinct biofilter designs and media are becoming "standards." These include trickling towers, fluidized sand beds, and floating-bead filters. Although a purely theoretical analysis of these filters is useful from an academic research viewpoint, it does little to assist the aquaculture engineer attempting to specify a particular filter design for a given biomass load,

system configuration, and economic constraint. Thus, a purely empirical approach is taken here to describe the bead filter's nitrification kinetics as a function of ammonia-nitrogen concentration and flow rate through the filter. From this analysis a series of design curves very similar to pump design curves can be developed that will help the design engineer select the most appropriate filter size and flow rates based on ammonia-nitrogen concentrations desired within the system.

Empirical Model – Reaction Rate Order

The approach used to develop design equations for the biological filters was based on the assumption that the rate of reaction was proportional to the n^{th} power of the concentration:

$$r_a = \frac{dC_a}{dt} = k \times C_a^{\,n} \tag{2}$$

where k is the reaction rate constant, C_a is ammonia-nitrogen concentration, and n is the reaction rate order. The reaction rate order can then be obtained by plotting the log of both sides, or:

$$\log (r_a) = \log(k) + n \log (C_a) \tag{3}$$

Thus, a log-log plot of the experimental data should yield a straight line whose slope corresponds to the order of the reaction rate, n. An example of the resulting plot for the bubble-washed bead filter is shown in Figure 3. This plot and others suggested that the design equation for the rate of reaction could be divided into simple first- and zero-order equations, i.e. n = 1 and n = 0.

The first- and zero-order data range for these plots was determined by starting at the lowest and highest values of r_a, and then sequentially adding data points one at a time, until there was a significant change in the R^2 value for the two regression lines. Figure 3 demonstrates that near the breakpoint value, the data no longer conform to the simple interpretation outlined above. As Figure 3 shows, at this flow rate and for low concentrations of ammonia-nitrogen, less than 1.0 mg-N/L, the reaction rate order is approximately 1.0. Moreover, for higher concentrations (greater than 1.0 mg-N/L), the reaction rate order appears to be approximately zero. For the purposes of aquaculture system design,

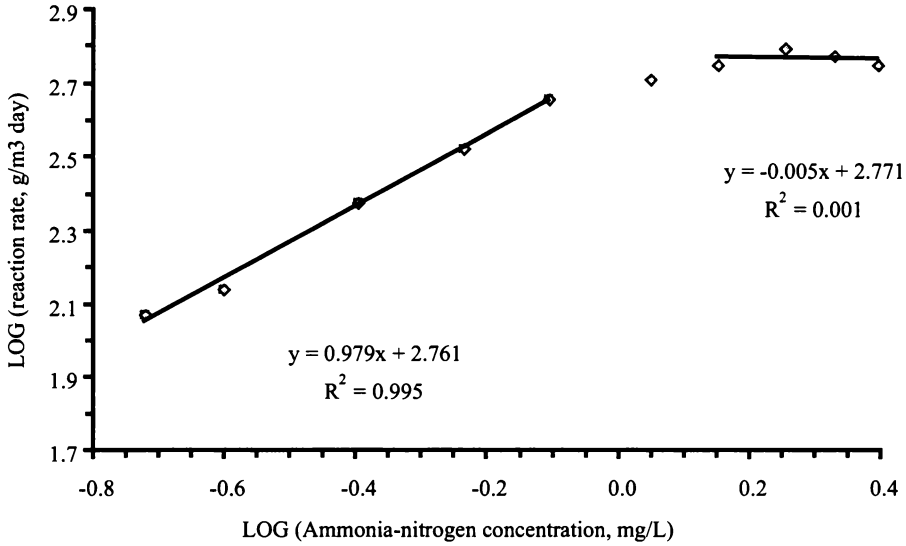

Figure 3. Example of a kinetic reaction rate order analysis for bubble-washed bead filter #1, flow rate of 39.3 Lpm.

this demarcation between first- and zero-order reaction rate corresponds approximately to the two ranges of ammonia-nitrogen concentrations usually encountered in commercial intensive recirculating aquaculture systems. Alternatively, using the classification system proposed by Malone (2004), biofilters designed for larval rearing, fingerling, and broodstock systems would be based on first-order reaction rates, whereas systems designed for growout could be based on either first- or zero-order reaction rates, depending upon species ammonia-nitrogen tolerance.

By extrapolating the linear regression lines for the two rate equations, a breakpoint concentration can be found that corresponds to the concentration where the overall reaction rate shifts from a first-order relationship to a zero-order relationship. The exact value can be found by equating the two regression equations, and solving for the ammonia-nitrogen concentration. Table 4 lists these values as a function of both flow rates through the filters and the corresponding hydraulic retention time. Figure 4 shows the values of the break point as a function of the flow rate through the bubble-washed bead filters.

Table 4. Ammonia-nitrogen concentration break point between first- and zero-order reaction kinetics for the two bubble-washed bead filters.

HRT[1] (min)	Flow (Lpm)	Break point (mg-N/L)
4.32	13.2	1.47
3.80	15.0	1.48
3.63	15.7	1.18
2.60	21.9	1.07
2.59	22.0	1.19
1.87	30.5	0.89
1.82	31.3	0.92
1.45	39.3	1.02
1.28	44.6	0.86
1.05	54.2	0.98
0.88	59.0	0.88
0.95	60.0	0.47
0.77	74.1	0.65
0.71	80.6	0.65

[1]*HRT = hydraulic retention time*

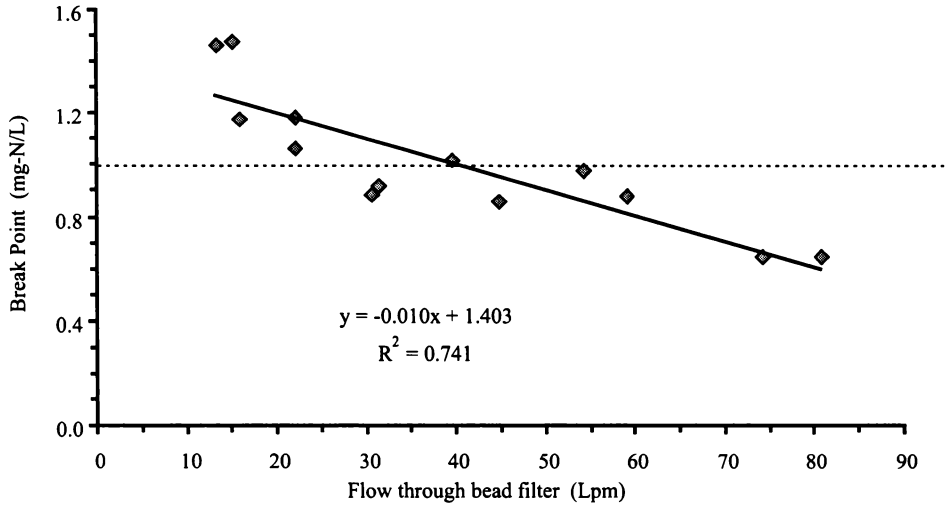

Figure 4. Ammonia-nitrogen break point concentrations between first- and zero-order kinetic reaction rates for the bubble-washed bead filters as flow rate through the biofilter.

Empirical Model – First- and Zero-Order Reaction Rate Constants

Based on the above results, the design equations for the biological filters were divided into either a first- or a zero-order kinetic reaction rate, depending upon the influent ammonia-nitrogen concentration and the break point concentration. Thus where the influent ammonia-nitrogen concentration is relatively low (< 1 mg/L NH_4-N), the reaction rate can be modeled as a first order reaction using Equation 4:

$$\frac{dC_a}{dt} = -k_1 \times C_a \tag{4}$$

where: C_a = ammonia-nitrogen concentration [mg/L]
k_1 = first-order reaction rate constant [day^{-1}]

When the above differential equation is integrated once, a plot of *ln* C_a versus time should yield a straight line with slope equal to the first-order reaction rate constant, k_1. Figure 5 shows several plots at various flow rates through the bead filter. A simple regression analysis of the resulting straight line (Figure 5) less than the break point concentration should correspond to the first-order reaction rate coefficient, k_1. This slope was estimated by starting at the break point between first- and zero-order reactions previously calculated and successively deleting data points to the regression analysis to maximize the R^2 value.

Correspondingly, for higher influent ammonia-nitrogen concentrations (> 1 mg/L NH_4-N), the reaction rate kinetics can be modeled as a zero order reaction rate using Equation 5:

$$\frac{dC_a}{dt} = -k_0 \tag{5}$$

where: k_0 = zero-order reaction rate constant [g/m^3 day]

The zero-order reaction rate coefficient can be estimated by a simple regression analysis of the slope of the straight line found by plotting ammonia-nitrogen concentration versus time, Figure 6, or a mean value and standard deviation could be estimated by averaging the removal reaction rates at ammonia-nitrogen concentrations greater than the break point concentration. Table 5 presents summaries of the first-order and zero-order reaction rate coefficients for the bubble-washed bead filter.

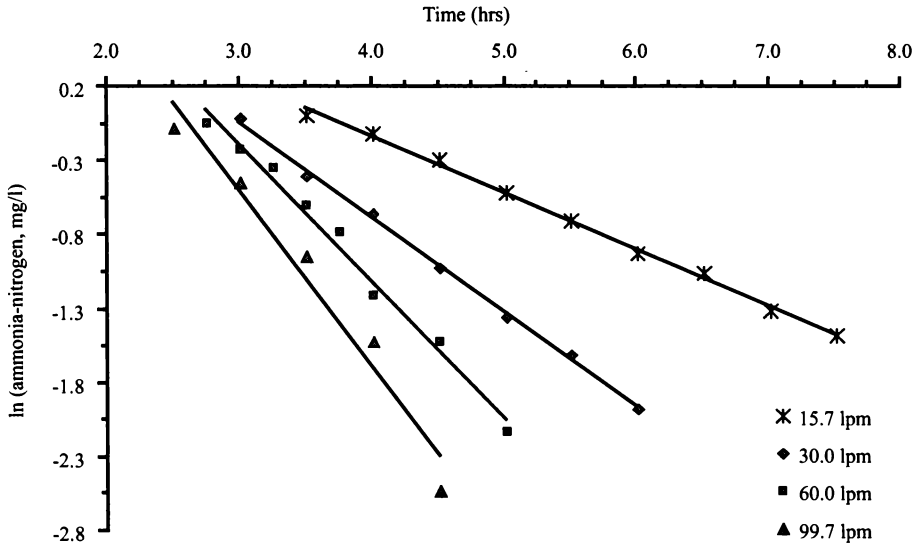

Figure 5. Plot of the graphical solution to determine the first-order reaction rate coefficient for the bubble-washed bead filter #1.

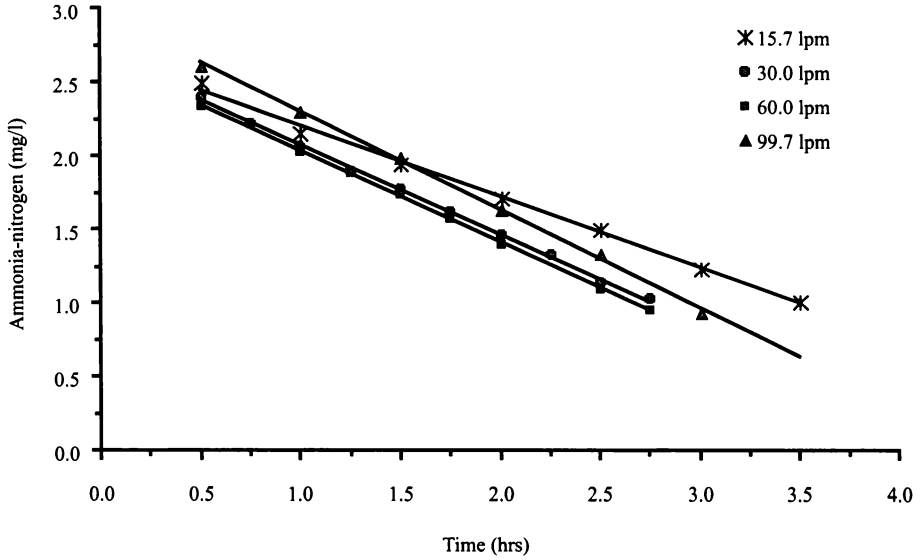

Figure 6. Ammonia-nitrogen concentration as a function of time showing the zero-order reaction rate coefficient.

Table 5. First- and zero-order kinetic reaction-rate coefficients, bubble-washed bead filter

	first-order rate constant		zero-order rate constant	
Flow Rate (Lpm)	**k_1 (day^{-1})**	**Regression R-squared**	**k_0 (g/m^3 day)**	**StDev (g/m^3 day)**
Bubble-washed bead filter #1				
13.2	256	0.99	396	87
15.7	283	0.98	433	19
22.0	424	0.96	478	16
30.5	482	0.98	499	12
39.3	586	0.99	588	29
54.2	634	0.98	609	18
60.0	712	0.47	500	48
66.3	681	0.76	427	39
80.6	827	0.83	611	74
99.7	1014	0.99	544	38
Bubble-washed bead filter #4				
15.0	275	0.99	380	22
21.9	332	0.98	439	44
31.3	437	0.99	403	16
44.6	588	0.95	535	46
59.0	681	0.99	525	39
74.1	712	0.89	618	63
92.5	905	—	432	81

Empirical Model – Monod Reaction Rate Parameters

Hagopian and Riley (1998), Williamson and McCarthy (1976a), Srna (1975), and other researchers suggested the use of a single- or double-saturation equation, where either the influent ammonia-nitrogen or dissolved oxygen concentration or both may limit the reaction rate. The overall kinetic reaction rate then becomes:

$$\frac{dC_a}{dt} = -r \tag{6}$$

where:

$$r = r_{max}\left[\frac{TAN}{TAN + K_{1/2}}\right]\left[\frac{DO}{DO + K'_{1/2}}\right] \quad (7)$$

and: r = reaction rate [g/m^3 day]
r_{max} = maximum reaction rate [g/m^3 day]
$K_{1/2}$ = half-saturation coefficient for TAN [mg NH_4-N /L]
$K'_{1/2}$ = half-saturation coefficient for DO [mg oxygen /L]
TAN = total ammonia-nitrogen [mg NH_4-N /L]
DO = dissolved oxygen [mg/L]

When $K_{1/2}$ is much smaller than the ammonia-nitrogen or dissolved oxygen concentration, the saturation-rate function appears to be a zero-order reaction and when $K_{1/2}$ is much greater than ammonia-nitrogen or dissolved oxygen, the saturation-rate function appears to be a first-order reaction. In the past, the saturation equations were solved from a Lineweaver-Burke plot of the inverse of the reaction rate versus ammonia-nitrogen concentration. Today, several software programs include solutions to this equation, either as a single- or two-site saturation coefficient. Sigma Plot 2002 (http://www.systat.com) for Windows Version 8.02 graphics software program includes a regression algorithm called Ligand Binding, which allows for the solution of the one- or two-site saturation equation and determination of multiple statistical parameters, including standard error of the measurement. In this study, the single-site saturation equation was used since dissolved oxygen concentrations were maintained above 6 mg/L and was not a rate limiting factor. Table 5 lists the coefficients for the two bead filters and their standard error. It should be noted that for flow rates less than 20 Lpm through the biofilters significant discrepancies were seen. This can be attributed to the difficulty in obtaining accurate measurements of the difference in ammonia-nitrogen across the biofilter at these low flow rates, since the magnitude of the difference is so small.

DISCUSSION

It needs to be pointed out that this analysis of reaction coefficients for nitrification is not the same as is often reported in the literature (Zhu and Chen 1999, 2000, 2002). Rather than analyze a pure strain of *Nitrosomonas*

and *Nitrobacter* acclimated to a narrow range of ammonia-nitrogen concentration, this analysis looks at a "real-world" biofilter *in-situ*, with all the confounding factors that affect commercial production biofilters. These include the impact of heterotrophic bacteria, a wide range of influent or system ammonia-nitrogen concentrations due to varying feed rates and times, system upsets, stress and disease of the cultured animals, and numerous other factors. It is the authors' opinion that measurements made on these types of systems will better represent actual "real-world" biofilters. It is interesting to note that the reaction rates determined by pure laboratory systems usually present maximum nitrification rates significantly higher than those seen in "real-world" production systems. This difference is then explained as being due to the impact of total organic carbon, temperature, salinity, or some other mitigating factor.

Empirical Model – Reaction Rate Order

In the application of a first- and zero-order kinetic reaction rate model, one of the parameters of interest in design and sizing of biofilters is the value of ammonia-nitrogen corresponding to the break point between the two models. First-order kinetic reaction rates are directly dependent on the influent ammonia-nitrogen concentration, whereas zero-order rates are independent of influent ammonia-nitrogen concentration. The break point concentration would reflect the change from a diffusion rate limit on nitrification to a reaction rate limit. Experimentally determined break point values for bead filters are plotted versus the flow rate through the biofilter in Figure 4. Two things are of interest, first the almost linear relationship with flow rate, and the range of values from 1.5 mg/L at the lowest flow rates to approximately 0.5 mg/L at the highest rates (R^2 value of 0.74). Second, the decrease in the break point ammonia-nitrogen concentration as the flow rate increases. Based on the guidelines for the design of systems utilizing floating-bead filters, Table 3, (Malone and Beecher 2000), the design hydraulic loading (Lpm/m^3 media) for broodstock and growout would correspond to approximately 47 Lpm. From Figure 4, this would correspond to a break point between first- and zero-order reaction rates at an ammonia-nitrogen concentration of about 0.9 mg-N/L. This would support the concept that for systems requiring ammonia-nitrogen concentrations less than 1.0 mg/L, the bubble-bead filter should be designed based on a first-order reaction rate constant and for growout of hardy species at ammonia-nitrogen concentrations above 1 mg/l with a zero-order reaction rate constant.

Since the external mass transfer of ammonia-nitrogen by diffusion to the biofilm is directly dependent upon the thickness of the stagnant liquid layer surrounding the beads, and that thickness depends on the velocity of the water passing over the beads, it follows that the reaction rate coefficient should be affected by the water flow rate through the filter. Figure 7 shows the first-order reaction rate parameter as a function of the flow rate through the biofilter. It demonstrates nicely the effect of flow rate, in that at low flow rates the reaction rate is significantly lower than that at the highest flow rate. Thus, the first-order reaction rate coefficient at a flow rate, Q (Lpm) for the bubble-washed bead filter can be expressed as:

$$k_1 = (7.9 * Q + 197) \tag{8}$$

and the first-order reaction rate or removal rate becomes:

$$\frac{dC_a}{dt} = -(7.9 * Q + 197) \times C_a \tag{9}$$

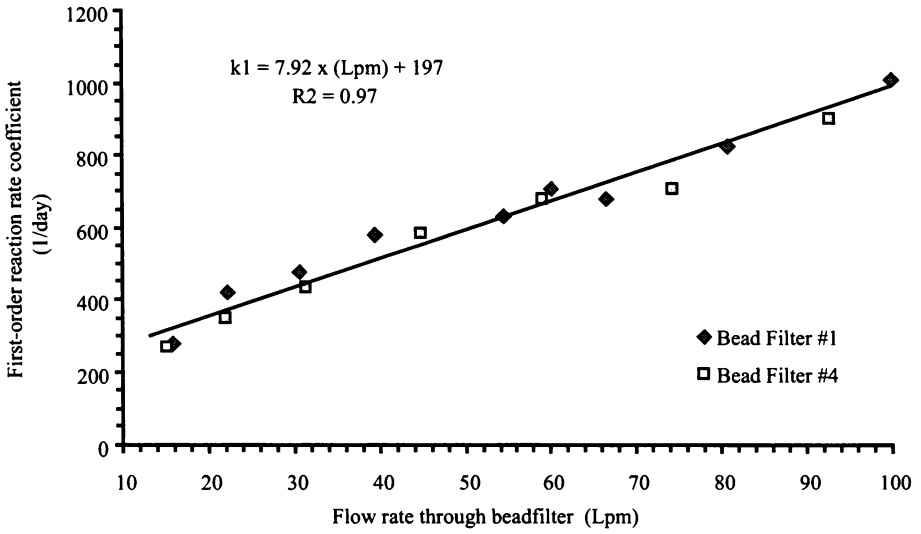

Figure 7. First-order reaction rate coefficient as a function of the flow rate through the bubble-washed bead filter.

Empirical Model – Application of First- and Zero-Order Empirical Model Results

In order to apply the results of this study to the sizing of biofilters, a series of performance characteristic curves were developed, similar to what is commonly used in characterizing pump performance. In this case, the reaction or removal rate (g/m^3 day) is plotted against either flow rate through the biofilters at several values of ammonia-nitrogen or plotted against ammonia-nitrogen concentration for several different flow rates. An example of these performance curves for the bubble-washed bead filter is shown in Figure 8. For this graph, the experimentally derived values for the first-order reaction rate constant as a function of flow rate through the biofilter were used (Eq. 8) and the first-order reaction rate model, solved for the removal rate of ammonia-nitrogen as a function of media volume per day (g/m^3 day). In addition, the experimentally determined reaction rates plotted demonstrate the validity of this model, at least at low ammonia-nitrogen concentrations.

Figure 8 shows clearly the effect of flow rate and ammonia-nitrogen concentration on the performance of the bead filter. The first observation is that at low ammonia-nitrogen concentrations, the impact of flow rate is not as significant as at the higher concentrations. Although the increased

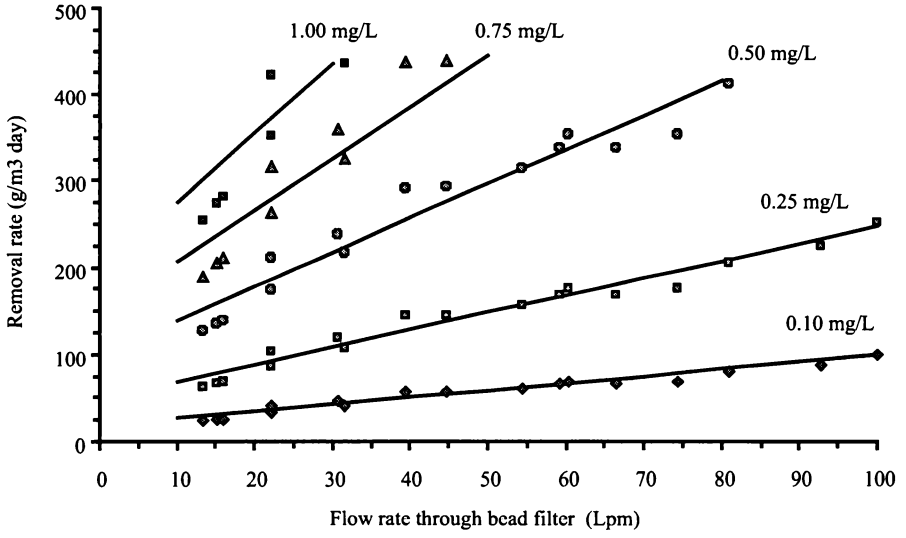

Figure 8. First-order performance characteristic curves for the bubble-washed bead filter as a function of flow rate through the filter and influent ammonia-nitrogen concentration.

flow rate would theoretically reduce the boundary layer between the bulk liquid and the biofilm, increasing external mass transfer, the diffusion rate is also a factor in the concentration gradient. Thus, the high concentrations yield higher gradients, which in turn yield a higher nitrification rate.

The second observation from the results is that as the ammonia-nitrogen concentration increases and, especially at low flow rates through the biofilter, the reaction moves quickly towards a zero-order reaction rate. Under zero-order, the ammonia-nitrogen removal rate is constant and the removal rate is not influenced by the flow rate (Fig. 9). From a design standpoint, this is important since it suggests that the only way to increase the first-order biofilter removal rate is either by increasing the ammonia-nitrogen concentration in the production tanks or, to a limited extent, by increasing the flow rate through the biofilter. The first choice is limited by the species being produced and the second by the hydraulic characteristics of the biofilter, i.e. bursting pressure and the economic cost of pumping.

Figure 9 shows the zero-order reaction rate coefficient as a function of the flow rate through the biofilter. It shows that there appears to be no significant effect of flow rate. This is consistent with the concept that the reaction is kinetic-reaction-rate limited and not a function of the diffusion rate. The mean value for the zero-order reaction rate coefficient is 495 g/m^3 day or assuming a specific surface area of 1050 m^2/m^3, 0.47 g/m^2 day.

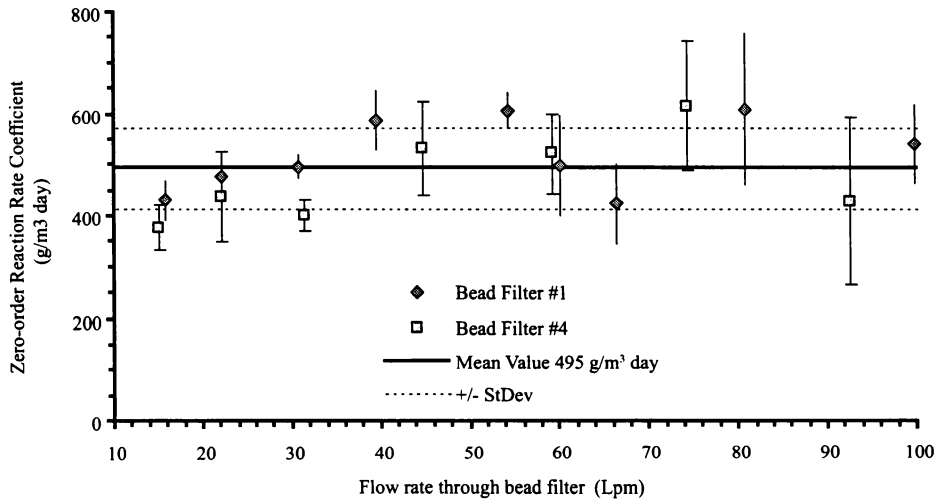

Figure 9. Effect of flow rate on the zero-order reaction rate coefficient, showing the mean value of 495 g/m^3 day ± standard deviation.

Monod Model

The simplified first- and zero-order reaction rate model can be useful in understanding and characterizing biofilter performance for either very low or very high ammonia-nitrogen concentrations. Its major drawback is characterizing the biofilter performance near the break-point between the two models, around 1.0 mg/L ammonia-nitrogen. To overcome this difficulty, most models of biofilms use some form of saturation equation such as the Monod relationship, Equation 6 and 7.

It can be shown that the break point concentration, $C_{bp,}$ determined for the simple empirical kinetic-rate model is approximately equal to the Monod half-saturation coefficient. This is accomplished by equating the Monod equation for high and low values of C in relation to $K_{1/2}$.

Thus, at high values of ammonia-concentration and Equation 7:

$$C_a >> K_{1/2} \qquad \frac{dC_a}{dt} \cong r_{max} \tag{10}$$

And at low values of ammonia-nitrogen concentration:

$$C_a << K_{1/2} \qquad \frac{dC_a}{dt} \cong r_{max} * \frac{C_a}{K_{1/2}} \tag{11}$$

Equating the two models at the break-point concentration, C_{bp}, yields:

$$C_{bp} \cong K_{1/2} \tag{12}$$

Thus it becomes possible to estimate the break point between first- and zero-order reaction rates from the Monod reaction rate coefficient. This would suggest that the half-saturation coefficient also would correspond approximately to the break point between kinetics controlled by diffusion across the stagnant layer next to the biofilm and kinetics controlled by the kinetic reaction rates of the bacterial film.

The half-saturation coefficient and the maximum reaction rate coefficient are shown in Figure 10 and 11 in relationship to the flow rate through the biofilter. It is interesting that there appears to be a relationship between

the half-saturation coefficient and the flow rate through the biofilter, similar to what was seen for the first-order reaction rate coefficient, although in this case the relationship is reflected in a decrease in value rather than an increase. Similarly with the zero-order reaction rate

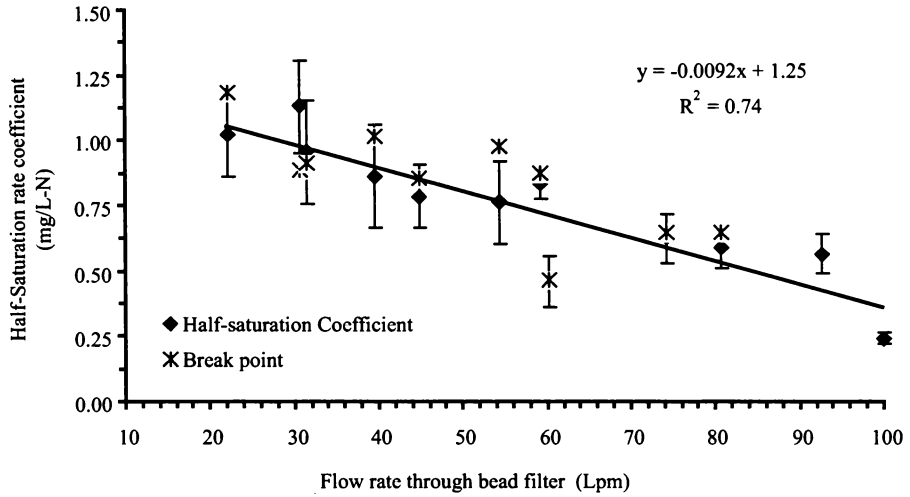

Figure 10. The relationship between the half-saturation coefficient and the flow rate through the bead filter, along with the break point values determined experimentally.

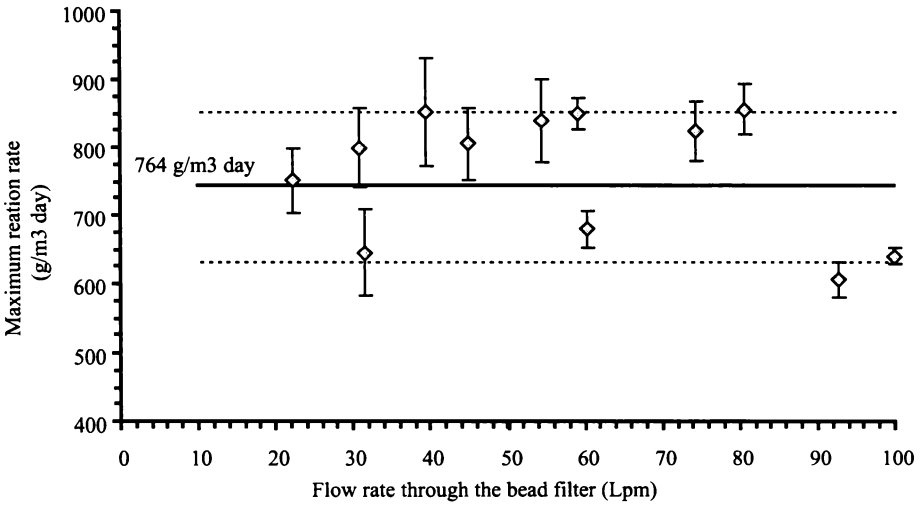

Figure 11. The relationship between the maximum reaction rate coefficient and the flow rate through the bead filter, showing a mean value of 764 g/m³ day and ± one standard deviation (94 g/m³ day).

coefficient, there appears to be no significant impact of flow rate through the biofilter on the maximum reaction rate coefficient, with a mean value of 764 ± 94 g/m^3 day or assuming a specific surface area of 1050 m^2/m^3, 0.73 ± 0.09 g/m^2 day.

Monod Model – Application of the model

The experimentally-derived values for the Monod reaction rate coefficients (Table 6) were used along with Equations 6 and 7 to develop a series of performance characteristic curves as a function of the flow rate through the biofilter and the ammonia-nitrogen concentration, shown in Figures 12 and 13. The chief advantage of these curves is that they

Table 6. Monod kinetic reaction-rate coefficients for the two bubble-washed bead filters for ammonia-nitrogen concentration up to 3.0 mg-N/L.

	Monod Reaction Rate Coefficients			
Flow Rate (Lpm)	**r_{max} (g/m^3 day)**	**Std Error**	**$K_{1/2}$ (mg/L)**	**Std Error**
		Bead filter #1		
13.2	867	90.4	2.44	0.41
15.7	915	73.0	2.30	0.31
22.0	739	40.3	1.10	0.14
30.5	801	58.5	1.14	0.18
39.3	853	79.6	0.87	0.20
54.2	840	61.6	0.77	0.16
60.0	681	27.6	0.47	0.10
80.6	858	37.4	0.60	0.08
99.7	641	11.7	0.25	0.02
		Bead filter #4		
15.0	1095	121	3.23	0.50
21.9	1349	113	3.18	0.37
31.3	647	62.7	0.96	0.20
44.6	807	52.5	0.79	0.12
59.0	851	23.3	0.84	0.05
74.1	825	42.9	0.63	0.09
92.5	608	24.8	0.57	0.07

are applicable over the entire range of ammonia-nitrogen concentrations. The end product of this evaluation technique is a set of design curves that can be used by engineers to properly size a biofilter for a given intensive recirculation system design and production species. In addition, existing systems can be evaluated to determine if they are operating at maximum removal rate for a given flow rate and operating ammonia-nitrogen concentration. From the performance curves, suggestions can be made on how to improve overall removal rate or filter efficiency by modifying the flow rate through the biofilter or adjusting the ammonia-nitrogen concentrations in the production system. However, both modifications have limitations due to the increased cost of pumping either water or species-specific ammonia-nitrogen tolerances.

Figure 12 displays the ammonia-nitrogen removal rate as a function of ammonia-nitrogen concentration based on the Monod relationship for four flow rates. Starting with the loading regime corresponding to broodstock holding or a very light feeding regime, the experimentally determined removal rates span almost exactly the range of volumetric nitrification rates reported by Malone *et al.* (1998). At the recommended flow rate of 11 Lpm, the removal rate at the highest recommended ammonia-

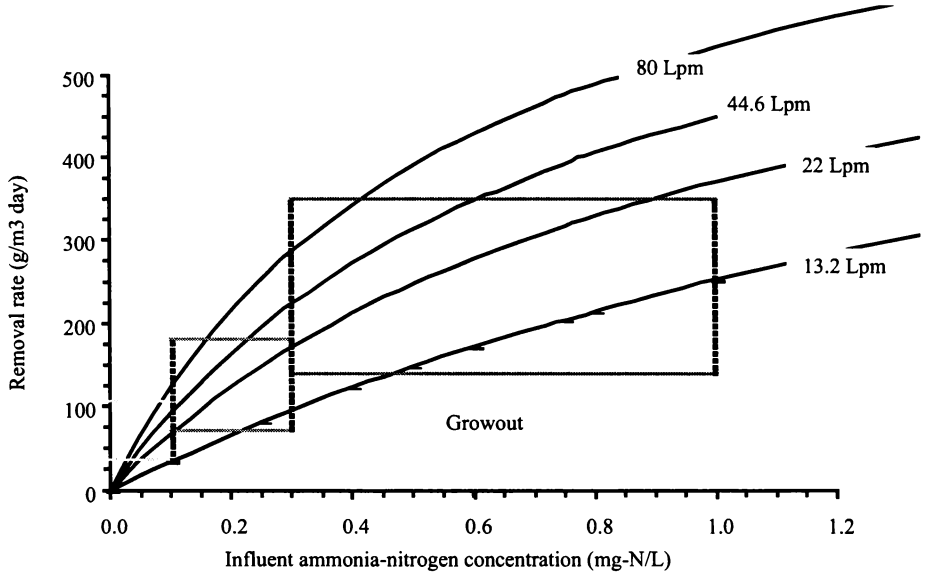

Figure 12. Performance characteristic curves for the bubble-washed bead filters, based on the experimentally determined Monod coefficients as a function of the ammonia-nitrogen concentration, showing the three fish life-stage application levels of ammonia-nitrogen concentration.

nitrogen level is equal to the lower value suggested by Malone *et al.* (1998). For the moderate loading regime of ornamentals, the removal rates corresponding to the recommended flow rate of 22 Lpm curve, bisecting the range of recommended removal rates. Finally, for the growout loading regime or the heavy loading rate, the removal rates corresponding to the recommended flow rate of 45 Lpm covers the full range of reported removal rates from the low end to the high end of 450 g/m^3 day. Malone, *et al.* (1998) reported that, based on their group's experimental data, an ammonia-nitrogen removal rate of 350 g/m^3 day would be expected under normal operation conditions for a production tank TAN concentration of 0.75 mg/L. This is similar to what the experimentally-based performance curves developed in this research suggest as the removal rate for a flow rate of approximately 45 Lpm and TAN concentration of 0.75 mg/L, shown in Figure 13. This graph also shows the recommended flow rates for the three production classifications and the corresponding ammonia-nitrogen removal rates.

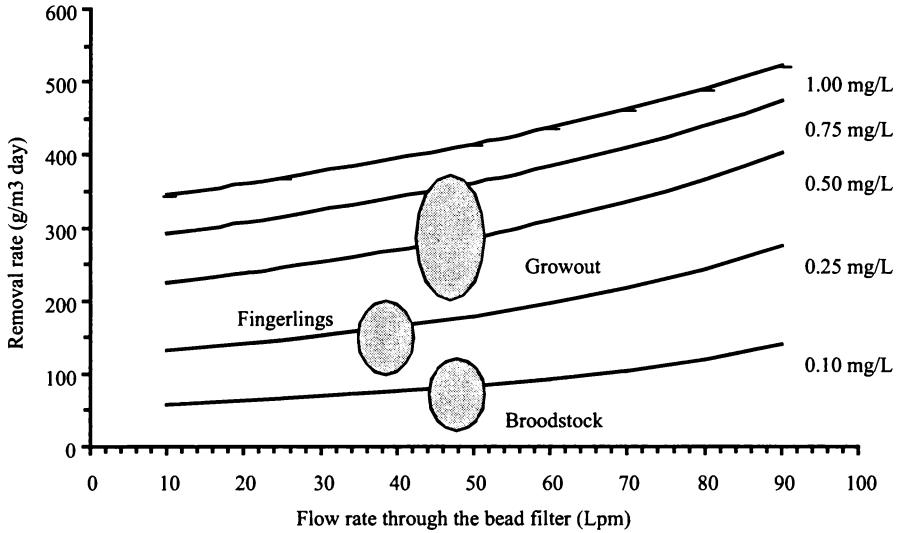

Figure 13. Performance characteristic curves for the bubble-washed bead filters, based on the experimentally determined Monod coefficients as a function of the flow rate through the bead filter.

CONCLUSION

There were no serious difficulties experienced in using a series of batch reaction rate experiments to determine the reaction rate kinetics for a commercially available pilot-scale bubble-washed bead filter. Empirical mathematical models for the nitrification of ammonia-nitrogen to nitrate-nitrogen were developed. The kinetics of nitrification were found to follow a simple first-order reaction model when the ammonia-nitrogen concentration was less than approximately 1.0 mg NH_4-N/L, and a zero-order reaction when the ammonia-nitrogen concentration was greater than 1.0 mg NH_4-N/L. The actual break-point between the two reaction regions was also found to be a function of the flow rate through the biofilter. In addition, the first-order kinetic reaction rate constants were also found to be a function of the flow rate through the filter, reflecting the influence of the fluid velocity on the mass transfer rate across the biofilm.

Using readily available graphical software, the Monod reaction parameters can quickly be determined and from them a series of performance characteristic curves developed as a function of the flow rate through the biofilter and the ammonia-nitrogen concentration. The chief advantage of these curves is that they are applicable over the entire range of ammonia-nitrogen concentrations. The end product of this evaluation technique is a set of design curves that can be used by engineers to properly size biofilters for a given intensive recirculation system design and production species. In addition, existing systems can be evaluated to determine if they are operating at the maximum removal rate for a given flow rate and operating ammonia-nitrogen concentration. From the performance curves, suggestions can be made as how to improve the overall removal rate or filter efficiency by modifying water flow rate through the biofilter or adjusting the ammonia-nitrogen concentrations in the production system.

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Two Warm-water Recirculating Hatcheries Used for Propagation of Endangered Species in the Upper Colorado River Drainage System

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Keywords: recirculating, hatchery, Colorado River, endangered fish species

ABSTRACT

The U.S. Fish and Wildlife Service (USFWS) has built two warm water recirculating hatchery facilities to enhance populations of endangered fish in the upper Colorado River Drainage System. The Grand Valley Propagation Facility in Grand Junction, Colorado, was built in 1996 inside a warehouse donated to the USFWS by the Bureau of Reclamation (USBR). In 1997, the hatchery was expanded, adding a second recirculating hatchery. The second hatchery more than doubled the capacity of the original facility. The Grand Valley Propagation Facility currently has the capacity to rear approximately forty thousand 200-mm endangered razorback suckers (*Xyrauchen texanus*) to stock into ponds for grow out to 300 mm. The resulting razorback suckers are stocked into the Colorado, Gunnison, and San Juan rivers.

In 1996, the Ouray National Fish Hatchery (ONFH) was constructed at Ouray National Wildlife Refuge (ONWR) to replace a small experimental facility practicing extensive culture. In 1998, the hatchery was completed and consisted of 36 lined ponds and a recirculating facility. Poor water quality, design flaws, and poor research have led to a nearly complete replacement of all water filtration components. The ONFH currently has the capacity to rear approximately twenty-five thousand 300-mm

International Journal of Recirculating Aquaculture 7 (2006) 43-52.

razorback suckers. The resulting razorback suckers are stocked into the Green River. As problems and limitations were encountered, both facilities were upgraded and improved to their current configurations. All of the modifications have led to insight into many types of filtration, filtration media, and intensive fish culture techniques.

INTRODUCTION

Four native fish are currently endangered in the Upper Colorado River Basin: the razorback sucker (*Xyrauchen texanus*), the Colorado pikeminnow (*Ptychocheilus lucius*), the bonytail chub (*Gila elegans*), and the humpback chub (*G. cypha*). In 1987, the Recovery Implementation Program (RIP) was developed in a coordinated effort to recover these four endangered native fish species. One goal of the RIP was to conserve genetic variability of wild endangered fish stocks through recovery efforts that would reestablish viable wild stocks by removing or significantly reducing the limiting factors that caused population declines. Captive propagation was required for some species because of inadequate recruitment in the wild (Wydowski 1994) and near extirpation of certain species from their historical habitats.

Two fish culture facilities were established by the USFWS to hold endangered fish in refugia and for potential brood stock development. In 1987, the Colorado River Fisheries Project (CRFP), in Vernal, UT, USA, established a small, experimental pond-culture facility at ONWR. In 1992, the USFWS, CRFP, in Grand Junction, CO, USA, established the Horsethief Refugia Ponds located near Fruita, CO, USA, at Horsethief State Wildlife Area. The need for additional propagation facilities to produce endangered Colorado River fish was recognized in 1994 (Wydowski 1994), and these two facilities were expanded to meet this need.

The experimental facility at ONWR was expanded and became Ouray National Fish Hatchery in 1996. ONFH has continued to expand and currently consists of twenty-four 0.08-hectare and twelve 0.2-hectare lined ponds and an indoor water recirculating system. The propagation program in Grand Junction was expanded in 1996 with the addition of an indoor water recirculating facility. In 1997, this facility was expanded and a second recirculating hatchery was built. In addition to the hatchery expansion, numerous private ponds have been leased for grow-out purposes.

The purpose of this paper is to relate experiences with the various recirculating systems and filtration techniques at these facilities. Both facilities have been upgraded and improved over the years as problems and limitations have led to alteration of their original configurations. This has yielded insight into a wide variety of filtration, filtration media, and intensive fish culture techniques.

System Descriptions and Methods

While the facilities at Ouray have been in existence longer, when talking about the recirculating water systems, it is necessary to first look at the Grand Valley Propagation Facilities (GVPF). The design and construction of the facilities at Ouray, including the recirculating system, had major problems due to poor water quality, poor engineering, and poor construction. Most of the original components have been replaced or abandoned and a new system, modeled after the Grand Valley Facilities, has been installed and is currently in use.

Grand Valley Propagation Facilities

In 1996, the USBR donated an old warehouse to the USFWS and aided in the design and building of a warm-water recirculating intensive fish-culture facility. At full capacity, the system contains over 52,990 L of water, circulated at 795 Lpm to thirty 1.2 m-diameter circular fiberglass tanks (750-L capacity each and a flow rate of 19 Lpm), and six 2.4-m diameter circular tanks (3,550-L capacity each and a flow rate of 38 Lpm). In 1997, the hatchery was expanded with the addition of a second recirculating system. The second and separate recirculating system contains over 75,700 L of water, circulated at 1,254 Lpm to fifty 1.2-m diameter circular tanks and eight 2.4-m diameter circular tanks (same capacities and flow rates as mentioned previously).

Water Source

The hatchery uses domestic municipal water purchased from the Ute Water Conservancy District. The incoming water is chlorinated, and must be de-chlorinated by packed columns or by sodium thiosulfate. The 3,385 L of water in the original hatchery and 7,570 L of water in the expansion hatchery (more if needed) are replaced each day, requiring 10 to 14 days for full replacement. The incoming water is stored in holding tanks inside of the hatchery and reaches ambient temperature of the hatchery building

(23°C) in 24 hours. Water temperature is maintained by heating or cooling the hatchery building itself.

Nitrification

Nitrification in the original hatchery is accomplished by both a Water Management Technologies (WMT, Baton Rouge, LA, USA) 0.7-m^3 floating bead filter, and a 0.91-m diameter cyclonic sand filter. The floating bead filter was the original biofilter, but when the bag filters being used for clarification purposes were abandoned due to excessive clogging (resulting in system failure and alarm calls), the bead filter was employed for water clarification as well. The resulting loss in potential TAN removal led to the addition of another biofiltration device.

The cyclonic sand filter, from Marine Biotech (Beverly, MA, USA), has a 0.91-m diameter and is 4.88 m in height. It contains approximately 1.18 m^3 (static volume) of 20/40 (0.8 to 0.4 mm) silica sand, with a bed expansion of 60 percent to 70 percent at 950 Lpm. The maximum amount of feed needed at full capacity of this hatchery is approximately 27 kg/day or a 0.55-kg/day total ammonia-nitrogen (TAN) load. Using a nitrification rate of 1.0 kg/day/m^3 (Timmons and Summerfelt 1998), there is sufficient sand volume (1.18 m^3) to handle the heaviest loading, and the filter could theoretically handle 39 kg/day. Additionally, the bead filter used for clarification purposes also performs nitrification and therefore, TAN removal capacity is higher still. The maximum feed rate this hatchery has experienced is 11.4 kg per day and water quality has not been a concern (nitrites were 0.3 ppm or less, and ammonia was 0.02 ppm or less).

A rotating biological contactor, approximately 1.2 m in diameter and 1.83 m long, was the original biofilter for the expansion hatchery, but proved to be inadequate to handle the necessary feed rates. The construction of this filter was also substandard as the fiberglass holding tank would flex and the contactor would come off of its axis and jam. At 4.5 kg of feed/day nitrite levels were high (0.9 ppm and above) as were ammonia levels (0.3 ppm). This water quality was unacceptable and a new solution was sought.

Two 0.91-m diameter, 4.27 m tall, cyclonic sand filters were installed. The sand (same sand parameters as for the previously discussed cyclonic sand filter) was expanded 40 percent to 50 percent at 660 Lpm per filter. These filters (added together) have a potential of handling 78 kg of feed per day.

The maximum feed rate this hatchery has experienced is 20.4 kg per day and water quality has not been a concern (nitrites 0.25 ppm or less and ammonia 0.02 ppm or less).

Clarification

As previously mentioned, clarification in the original hatchery was first performed with bag filters that were abandoned in favor of the existing floating bead filter. The bag filters proved unable to handle the feed rate and clogged after a few hours of use. In the expansion hatchery, a self-cleaning PRA Rotofilter (PRA Manufacturing Ltd., Nanaimo, B.C., Canada), 1.98-m^2 filter screen area, fitted originally with a 30-μm screen and later a with a 60-μm screen, was responsible for clarification. Typical problems are leaking or improperly installed seals, fouling, holes in the screens, and water loss from cleaning, but overall, this filter performs well when properly maintained.

Sterilization

Both the original and expansion hatchery use UV filtration for water sterilization. The original hatchery makes use of a Wedeco-Ideal Horizons Inc. (Poultney, VT, USA) IH Series 10-bulb UV water treatment system, capable of disinfecting water at a rate of 985 Lpm. The expansion hatchery uses an Ideal Horizons IH Series 40-bulb UV water treatment system, capable of disinfecting water at a rate of 3,935 Lpm. No water quality data has been taken on these filters, it has just been assumed that they are doing their job, as there has been no major spread of the few disease outbreaks that have occurred (columnaris is the only disease experienced in the system).

Oxygenation and Degassing

Both hatcheries use packed columns to strip carbon dioxide and nitrogen gasses from the water as well as to re-oxygenate the water before recirculating to the fish. Dissolved oxygen levels greater than 5.0 ppm are maintained even at the highest loading (forty thousand 200-mm fish, approximately 3,200 kg, both hatcheries combined) prior to stocking. No oxygen injection or supplemental oxygen is added to the water at this facility.

Backup Systems

Both hatcheries have back-up oxygen systems that run off pressure switches and solenoid valves. If power is interrupted or the pressure drops due to pump or other equipment failure, a solenoid opens and distributes oxygen through air stones in each tank. At the same time the Sensaphone Express 6500 (Aston, PA, USA) alarm system is triggered and attempts to contact hatchery personnel by phone. The oxygen system can also be used to supply oxygen to fish during chemical treatments.

Ouray National Fish Hatchery

ONFH was established in 1996 to replace a small experimental extensive pond-culture facility. ONFH has continued to expand and now consists of twenty-four 0.08-hectare lined ponds, twelve 0.2-hectare lined ponds, and an indoor warm-water recirculating system.

Originally, up to 3,000 Lpm was to be pumped from the wells to the water treatment building and undergo sterilization by ozone, as well as sand filtration for iron and manganese removal. Of the 3,000 Lpm, 115 Lpm was to be used for the recirculating system, and the rest was split between the 36 lined ponds used for grow out and to hold broodstock.

The incoming water for the recirculating hatchery comes in at a temperature of 11°C and was to originally run through water heaters and be heated to 20°C. The heated water would then be continuously added to the system at 115 Lpm or 10 percent continuous make-up. The ozone system in the hatchery building was to sterilize the recirculating water as it passed through the mechanical room. The recirculating water was pumped at 1,500 Lpm through a 1.4-m^3 propeller-washed bead filter made by WMT (for biofiltration), through a degassing tower, out to the tanks by gravity flow, and then through a packed column for additional degassing (necessary due to high levels of nitrogen gas from the wells, and the heating of the incoming water with propane water heaters). There are twenty-one 2.4-m diameter circular tanks (3,030-L capacity and a flow rate of 38 Lpm) and thirty 1.2-m diameter circular tanks (380-L capacity and a flow rate of 19 Lpm). After circulating through the tanks, the water passed through a PRA Rotofilter (PRA Manufacturing Ltd., Nanaimo, B.C., Canada), with a 30-μm screen for clarification before returning to the sump.

The design and construction of the facilities at ONFH, including the recirculating system, had major problems due to bad water quality, poor engineering, and poor construction. These problems have led to the replacement and abandonment of most of the original components, yet if the water quality problem had been addressed first, many of the original components may have proved salvageable.

Water Source

The water at ONFH came from 6 shallow wells that pumped water to a lift station sump and were then pumped to the water treatment building using a variable frequency pump to control the quantity of water delivered. The well water contained high concentrations of the heavy metals iron (1.0 ppm) and manganese (0.2 ppm). An ozone system was in place to sterilize the incoming water. The iron and manganese were to be filtered out by the Commercial Hi-Rate Permanent Media Filter System, from Environmental Products Division (Rancho Cucamonga, CA, USA) in the water treatment building. These were a series of 8 filters with 0.56 m^3 of silica sand per filter. The sand filtration was able to reduce iron concentrations to 0.7 ppm, but did little to reduce manganese concentrations. Due to the problems caused by the iron and manganese in the water, many of the components proved unusable at that time. The heavy metals began to choke off pipes and small orifices, coat impellers, and slow flow in the re-use system. The ozone changed the manganese into permanganate at concentrations lethal to fish. The permanganate problem led to the abandonment of the ozone system, and replacement with an Ideal Horizons IH Series 40-bulb UV water treatment system (Wedeco Ideal Horizons, Poultney, VT, USA) for sterilization.

A small scale Burgess iron removal media (BIRM) filtration system was installed in the recirculating hatchery to further filter the water entering that system. This system consisted of 4 filters containing a total of 0.22 m^3 of BIRM with a flow of 115 Lpm (2-minute contact time), that reduces the iron concentration to 0.3 ppm, and manganese becomes undetectable. Due to the success of the small scale BIRM filtration system installed in the recirculating hatchery, the sand in the permanent media filters in the water treatment building was replaced with BIRM. The water has a 1.5-minute contact time (although 2 minutes is suggested by the manufacturer) with the BIRM at 3,030 Lpm. This reduces iron concentrations to 0.4 ppm and manganese to 0.1 ppm. At lower flow rates, contact time increases, and

more iron and manganese are removed. The BIRM is a manmade product that needs to be replaced periodically, but does not need regeneration like some other products used for iron and manganese removal. Proper flow and backflush rates are critical to the success of the media. Flows higher than recommended reduce contact time which reduces the BIRM's filtration ability. Backflushing rates higher than recommended flush the BIRM out of the filters, necessitating premature replacement of the media.

Recirculating System

The recirculating system contains 90,800 L of water at capacity. Currently, incoming well water enters the building at 11°C, passes through the previously mentioned small BIRM filtration system at 115 Lpm and into a 7,570-L make-up water storage tank where it warms up to the ambient hatchery temperature. Water temperature (23°C) is maintained by ambient temperature of the hatchery building itself. Each day 7,570 L of water is drained from the fish tanks in the daily cleaning processes and the 7,570 L of make-up water is added to the system.

Nitrification

A 1.4-m^3 propeller-washed bead filter was originally installed for biofiltration. A series of errors in the installation and the operation of the bead filter and other components of the system, led to the mothballing of the recirculating system and the hatchery was run as a cold-water pass through spawning building for a few years. Subsequently, the bead filter was replumbed and various other problems were worked out so that the re-use system could be put back into use. The bead filter eventually failed as colloidal iron and manganese clung to the beads, making them heavier, and causing them to be expelled during backflushing. Thus, biofiltration was poor to nonexistent.

The bead filter was replaced by two 0.091-m (3-foot) diameter, 4.88-m (16-foot) tall cyclonic sand filters from Marine Biotech containing 0.98 m^3 (static volume) of 20/40 silica sand. Bed expansion is ~ 60 percent at 750 Lpm. These 2 sand filters are theoretically capable of handling up to 78 kg of feed per day. The maximum feed rate this hatchery has experienced is 27.3 kg per day and water quality has not been a concern (nitrites were 0.25 ppm or less, and ammonia was 0.02 ppm or less).

Sterilization

Due to the previously mentioned water quality problems, the ozone system was removed and replaced with an Ideal Horizons IH Series 40-bulb UV water treatment system.

Water Delivery and Circulation

The gravity flow method of water delivery to the tanks was replaced by circulation pumps able to circulate up to 1,420 Lpm jetted into the tanks. After circulation through the tanks, the water is clarified by the original self-cleaning PRA Rotofilter, and returned to the sump.

Back-up Systems

There is a backup oxygen system that runs off pressure switches and solenoid valves. If the pressure drops due to pump failure or power failure, the solenoids open and oxygen is delivered to the tanks through air stones. At the same time, the Sensaphone Express 6500 alarm (Aston, PA, USA) is triggered and starts to call hatchery personnel on the phone.

RESULTS AND DISCUSSION

The modification of a recirculating hatchery is not unusual. The Grand Valley facility was a typical example of this. Problems were encountered and improvements were made that increased the abilities of the systems to maximize production potential. ONFH on the other hand, is an example of what can happen if the most basic factors of building a hatchery are ignored. In the book that every fish hatchery manager in the USFWS consults, *Fish Hatchery Management*, (Piper *et al.* 1982), it states in the second paragraph:

> Water quality determines to a great extent the success or failure of a fish cultural operation. Physical and chemical characteristics such as suspended solids, temperature, dissolved gases, pH, mineral content, and the potential danger of toxic metals must be considered in the selection of a suitable water source.

Had available water quantity and quality been considered prior to selection of this site, it most likely would have been disqualified as the final location. As a result, besides the problems normally associated with basic fish culture, the operation of this hatchery includes the operation of

a water treatment facility that would rival many municipal water treatment facilities. The cost of the choice of the site is incalculable in time, money and effort due to the poor water quality that must be dealt with. Had this choice been made by a commercial fish farmer rather than the federal government, it likely would have resulted in a financial failure.

Both facilities are now meeting their current production quotas. A large-scale recirculating system is a viable option for rearing warm-water endangered fish where water and space are a concern.

ACKNOWLEDGMENTS

The author would like to acknowledge the contribution of individuals involved in various aspects of the development and in some cases the redevelopment of the hatcheries mentioned above. Frank Pfeifer (USFWS) has been instrumental in the development and continued production of both hatcheries. Thanks to Thad Bingham and Brian Scheer for providing information and data for this paper. Thanks to Pat Kerins for the time and effort demanded to keep ONFH running and changing. Our principal engineers, Ram Dahm Khalsa (USBR) and Bob Norman (USBR), have been extremely helpful in designing our systems, and redesigning previous engineers' work. Thanks to Dave Irving for allowing me the time to write this paper.

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Sunshine Bass Fingerling Culture in Tanks

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Keywords: sunshine bass, striped bass hybrids, *Morone* species, tank culture

Year-round production is a top priority of hybrid striped bass producers. Most *Morone* culturists produce sunshine bass (white bass ♀ X striped bass ♂) that have very tiny fry and require rotifers as their first food. Almost 100 percent of the fingerlings are produced in ponds where high survival rates depend on fry being stocked at the right time – before rotifer concentrations peak and before copepods appear. Pond culture drawbacks include the inability to monitor growth and survival and seasonal limitations due to weather. Tank culture overcomes these problems and is necessary for year-round production. Little tank fingerling production has occurred because costs are higher than for pond culture. Supplying live food is a major expense. Sunshine bass larvae are stocked at 4 to 5 days post hatch (dph) and are fed enriched cultured rotifers. The rotifers require microalgae. Within a few days the fry are weaned to cultured *Artemia* nauplii. The culture of the larger palmetto bass and striped bass starts with feeding *Artemia* nauplii. By about 15 dph, weaning to an artificial diet begins and is completed by 26 dph. Grading at that time reduces cannibalism. Live food culture is risky, and requires time, space, costs, and expertise. Recent innovations may alleviate some of these problems. High-density (up to 16,000/mL) rotifer production methods are being developed. These systems require constant feeding, oxygen, pH and ammonia control, suspended particle removal, and proper harvesting. Fatty-acid enriched algae pastes can replace cultured algae. Ammonia and pH problems can be controlled with products like

International Journal of Recirculating Aquaculture 7 (2006) 53-68.

Chloram-X® and auto-sensing pH controllers. Water is conserved by utilizing recirculation systems for rotifer and fingerling production. Use of commercially available decapsulated brine shrimp eggs further reduces time and physical risk. Increased demand for fingerlings during the winter and reduced culture costs will increase tank fingerling production.

INTRODUCTION

Year-round production of sunshine bass is a top priority of hybrid striped bass producers (Anonymous 1998). Currently, fry and fingerling production is confined to March through June, the normal spawning periods of the parental stocks (Mike Freeze, Keo Fish Farm, personal communication; Becker 1983). Fish reach market-size in 10 to 20 months after hatching, depending on stocking rates, culture conditions, and diet (Carlberg *et al.* 1989). As a result, it is difficult for fish farmers to provide hybrid striped bass of uniform size and quality to markets year-round. Consequently, prices also vary considerably during the year. It is important to develop culture techniques that will provide for year-round availability of sunshine bass fingerlings.

The culture of striped bass (*Morone saxatilis*) and its hybrids with white bass (*M. chrysops*) for the food-fish market is a recent endeavor. The initial incentive for *Morone* culture was to replenish wild populations of striped bass whose stocks had been depleted by over-fishing and habitat degradation. However, hybrids between striped bass and white bass grow faster, have better survival, and tolerate pond culture conditions better than striped bass (Bishop 1968; Logan 1968; Ware 1974; Kerby *et al.* 1983; Smith 1988). The original cross, palmetto bass, with a striped bass female parent was stocked into many inland reservoirs. The establishment of hatcheries and inducement of spawning by hormone injection greatly facilitated propagation of these fish (Stevens and Fuller 1962), and by the early 1980s more cultured fish than wild fish were being caught (McCraren 1984). Commercial fishing for striped bass was also closed to allow recovery of the stocks. That action precipitated the birth of a food-fish industry during the mid 1980s (Harrell and Webster 1997).

Evaluations of the hybrids for use in aquaculture indicated that they had higher potential than striped bass (Williams *et al.* 1981; Kerby *et al.* 1987; Woods *et al.* 1983) and by 1997, 87 percent of *Morone* producers cultured hybrids for market (Kahl 1997). Hybrid bass can be raised to commercial

sizes in ponds (Wawronowicz and Lewis 1979), net pens (Williams 1971), raceways and cages (Powell 1973), and tanks (Smith *et al.* 1985). Before 1995, most grow-out production resulted from intensive tank culture. Today that method produces 45 percent, while 55 percent is from pond culture systems (Carlberg, *et al.* 2000).

The two hybrids, palmetto bass and sunshine bass (*M. chrysops* X *M. saxatilis*), are difficult to distinguish as adults, but they differ significantly as fry. Sunshine bass fry are much smaller than palmetto bass fry and are more difficult to culture because they require rotifers or other very small size zooplankton for their first food (Ludwig 1993, 2004). In spite of that, more sunshine bass are produced because of brood stock considerations: white bass females mature a year earlier, have less spawning mortality, and are less susceptible to stress than striped bass females. White bass females are also more widely available than striped bass females and are smaller and more easily handled.

Food-fish production of striped bass and its hybrids with white bass has grown tremendously since its inception. Between 1986 and 1993 production increased from 10,000 to 6 million pounds (Hodson 1995). By 2000, the industry was growing at a 7 percent rate and had reached fifth in volume and fourth in value of all food fish grown in the U.S. with an estimated 10 million pounds production level (Carlberg *et al.* 2000).

Fingerling Pond Culture

Nearly 100 percent of sunshine bass fingerling culture is now done in ponds (Ludwig 2004). Early attempts found fingerlings of this hybrid difficult to culture. Survival rates were highly variable and averaged about 10 percent (Ludwig 1993) when farmers stocked 5-day-old larvae about 2 weeks after ponds were filled and fertilized and contained concentrations of large zooplankton (Geiger 1983a,b; Geiger *et al.* 1985; Geiger and Turner 1990). That procedure, however, provided good survival rates of about 45 percent by 30 to 45 dph for striped bass or palmetto bass (Hodson 1995). But, the much smaller sunshine bass larvae (ca. 3-mm total length) were being stocked into ponds that no longer held many rotifers (Ludwig 1993) and probably contained copepods that ate the larvae (Valderrama *et al.* 2000). Ludwig (1993) found that highest sunshine bass survival rates are achieved when larvae are stocked just before the rotifers reach their peak numbers, 3 to 19 days after pond

filling, depending upon temperature (Li *et al.* 1996; Ludwig 2000). Sunshine bass survival rates in commercial ponds where fry were stocked before the initial peak in rotifer concentration now average about 35 percent when harvested at 35 to 40 days (Jackson Currie, Small Fry Fish Farm, Wilmot, AR, USA, personal communication).

However, fingerling production in ponds has many limitations. It is often difficult to predict larvae acquisition times because brood stock are still mainly wild caught fish. Pond temperatures and zooplankton populations are also highly variable during the early part of the spawning season. High pH or un-ionized ammonia levels that accompany intense phytoplankton blooms, insect predation, temperature or chemical shock at the time of stocking, low dissolved oxygen concentrations, and other causes contribute to mortality and are difficult to control. Before harvest, fish mortality is also very difficult to determine. When mortality is high, ponds must be drained, refilled, refertilized, and restocked. Invasion of rooted macrophytes into ponds increases the amount of work necessary to harvest fingerlings and contributes to harvest mortality. Pond culture is also limited by weather conditions that are too cold during winter to allow production. Pond production also requires extensive level land area and particular soil types. Year-round culture of sunshine bass fingerlings in the U.S. requires indoor production facilities while water and energy costs require that recirculation systems be used.

Tank Culture of Fingerlings

Producing fingerlings indoors in tanks may overcome many of the difficulties of pond culture. Tank culture of striped bass fingerlings was first described by Snow *et al.* (1980), who fed freshwater rotifers *Brachionus calyciflorus* to the larvae. Lewis *et al.* (1981) provided a manual for tank culture of striped bass, and started feeding with *Artemia* nauplii at an initial rate of 50 to 60 L^{-1}. However, the small size of sunshine bass larvae requires the use of rotifers as a starting diet. The first report of sunshine bass fingerlings being raised in tanks was by Ludwig (1994) who used cultured freshwater rotifers, *B. calyciflorus*, before weaning the fry to salmon starter meal by 26 dph. Denson and Smith (1997) obtained better growth by starting with brackish water rotifers, *B. plicatilis*, followed by brine shrimp nauplii, and then weaning to a microencapsulated diet. Significant increases in survival and growth were found when rotifer and brine shrimp nauplii concentrations were

increased (Ludwig 2003). Freshwater rotifers and other zooplankton harvested from ponds with a rotating drum filter equipped with a 60-μm mesh screen were also used by Ludwig and Lochmann (2000) to raise sunshine bass larvae to the time they were weaned to dry feed.

The optimum feeding rates for live food or prepared feed for tank culture of sunshine bass have not been determined. Ludwig (1994) added *B. calyciflorus* to tanks until the concentration was 20/mL for 22 mornings. After 10 days, he supplemented the rotifers with a salmon starter meal (45 percent protein). Denson and Smith (1996) fed *M. chrysops* fry highly unsaturated fatty acid (HUFA) enriched rotifers once per day at 10/mL for 6 days before weaning the larvae to brine shrimp nauplii (3/mL/day) and later to a dry diet, and obtained up to 48 percent survival by 27 dph. Denson and Smith (1997) also cultured sunshine bass larvae with *B. plicatilis* at 10/mL, weaned the larvae to *Artemia* nauplii at 3/mL/day, and obtained 67 percent survival by the end of 8 days. Ludwig and Lochmann (2000) harvested rotifers from ponds with drum filters and fed them to sunshine bass fry at 10, 20, and 30/mL/day. After 5 days, the zooplankton was supplemented with a 50 percent protein microencapsulated larval feed. By age 22 days, survival rates were 3.1 percent, 14.2 percent and 24.3 percent respectively. Ludwig (2003) compared survival of larval fish fed at three levels of rotifers, brine shrimp nauplii, and microencapsulated feed. Larvae fed the highest amount (60 HUFA-enriched rotifers/mL/day, 6 *Artemia* nauplii/mL/day and then 3 g feed/day) had a 52.9 percent survival rate by day 21 post hatch. To summarize, the highest sunshine bass survival rates during these studies were obtained with a feeding protocol that started with enriched rotifers, changed to brine shrimp nauplii, and then to a high-protein dry feed.

Enrichment of rotifers and *Artemia* with HUFA before using them as live feed appears to increase growth and survival of a variety of fish larvae (Lubzens *et al.* 2001). Lemm and Lemarie (1991) found that larval striped bass survival increased greatly when the *Artemia* that they were fed were enriched with HUFA. Essential fatty-acid nutrition has been determined for larval striped bass and palmetto bass (Tuncer and Harrell 1992), but not for sunshine bass. For fingerlings, Harel and Place (2003) found that sunshine bass and striped bass weight gain was less affected by dietary changes in HUFA than were white bass. Clawson and Lovell (1992) found that palmetto bass and striped bass larvae required a supplementation of

n-3 HUFA during the time they are fed *Artemia*. Research is needed to determine optimum feeding rates and perfect live food enrichment.

Dependence on live microalgae cultures for rotifers has impeded the development of fingerling tank culture. Microalgae cultures require constant care, precise growing conditions, specialized equipment, and isolation to avoid contamination (Hoff and Snell 1997). Monocultures of rotifers are also very unstable, having sudden crashes in density, often from high pH and un-ionized ammonia fluctuations or contaminants introduced when live microalgae are used (Snell 1991). The commercialization of microalgae paste has greatly facilitated the culture of rotifers and reduced the risk of culture crashes. *Nannochloropsis* sp., *Isochrysus* sp., and other microalgae are concentrated and then refrigerated or frozen for long-term storage. During culture, they are diluted and can be supplied to the rotifer culture vessels via timer-controlled peristaltic pumps. The use of ammonia control chemicals (Chloram-X®, AmQuel®) particle traps, algae paste, and oxygen resulted in a fairly stable, semiautomated, high-density rotifer production system (Pfeiffer and Ludwig 2002). Ludwig (2003) successfully cultured rotifers with this system to produce sunshine bass fingerlings in tanks. The recent production of live, decapsulated brine shrimp eggs should also ease the difficulty in culturing sunshine bass fingerlings since it will eliminate the danger of using harsh chemicals and the time needed to decapsulate brine shrimp cysts before hatching them.

Eliminating the need for live feed would greatly enhance fingerling production. Webster and Lovell (1990) obtained 18 percent survival to 19 dph for striped bass fed only a commercially available dry diet. However, their results are equivocal because they did not have an unfed control: Rogers and Westin (1981) found that unfed striped bass larvae could survive up to 22 dph at 24°C and up to 32 dph at 15°C. Survival of sunshine bass fed only prepared feed has not been determined. Ludwig (1994) was able to wean 27-dph fry (21 percent survival) to a microencapsulated feed while no unfed larvae survived beyond 9 dph. Further research to determine the earliest fry can be weaned to a commercial diet is needed.

Optimum physical, chemical and biological environment for effective tank culture of fingerlings has also not been resolved. Fingerlings grown at 22.6°C water temperature (Ludwig 2003) were shorter than those

grown at 25.6°C (Denson and Smith 1997). Woiwode and Adelman (1984) determined that 31°C was the optimum temperature for sunshine bass juvenile growth, while 26.8°C was optimum for juvenile palmetto bass (Woiwode and Adelman 1991). Optimum growth for striped bass fingerlings was determined to be 24°C by Cox and Coutant (1981), while Kellogg and Gift (1983) found the greatest growth of juvenile striped bass occurs at 28.5°C. Optimum temperatures for growth may be influenced by other factors. Woiwode and Adelman (1991) determined that optimum temperatures for growth of palmetto bass increased significantly when spring photoperiods were experienced and decreased when fish were exposed to decreasing photoperiods.

Stocking rates may have significant effects on growth and survival during tank fingerling culture but optimum stocking rates have not been determined for sunshine bass larvae. Lewis *et al.* (1981) recommended stocking striped bass larvae at 100 larvae/L, while Ludwig (1994) initially stocked sunshine bass at about 20/L but later increased the rate to 75/L (Ludwig and Lochmann 2000) and then to 80/L (Ludwig 2003). These rates are similar to the 75/L that Denson and Smith (1996) used for sunshine bass and white bass. No justification for the chosen stocking rate was given in any of the cited publications.

Most research on tank culture of fingerling sunshine bass has been performed in static or flow-through systems, but economics will most likely require that future indoor fingerling production systems will involve recirculation technology. Recently, a recirculation system for high-density rotifer production has been commercialized (Aquatic Ecosystems, Inc., Apopka, FL, USA). Commercial sunshine bass producers are attempting to develop economical recirculating fingerling culture systems (Lindell *et al.* 2004). In order for their efforts to be economically feasible, much of the research alluded to above will have to be carried out. In addition, it will be necessary to develop efficient ways to prevent cannibalism, grade fish, minimize and treat disease problems, minimize handling to avoid stress, and seamlessly convert from fingerling production to restocking for grow out in tanks.

Spawning

Year-round production of fingerlings requires year-round spawning of the parental species. That may be accomplished by compressing the annual

photothermal regime, a subject extensively reviewed by Bromage *et al.* (2001). By this technique, maturation was advanced by 2 to 5 months for striped bass, white bass, and palmetto bass (Blythe *et al.* 1994a, b; Kohler *et al.* 1994; Smith and Jenkins 1984, 1986). Smith *et al.* (1996) extended the spawning of captive white bass by 3 months by holding mature fish at reduced water temperatures. Tate and Helfrich (1998) also used photothermal compression to offset spawning and advance sexual maturity of sunshine bass. Although the parental stocks of striped bass and white bass have been induced to spawn out-of-season, none of these studies produced hybrid sunshine bass. Research is needed to determine if off-season spawning and production of sunshine bass can be sustained.

Broodstock Development

Development of improved and domesticated broodstock is a high priority in the hybrid striped bass industry. Improvement of heritable traits of fish stocks is a cost-effective means of increasing profits. The high fecundity and large genetic variation for growth rate and other desirable traits of striped bass and white bass should facilitate selection for increased production. Brown (1989) indicated that doubling the harvest size may triple the market. Tave (1993) cites production gains of 10 percent to 20 percent per generation for several fish species. However, genetic selection for the hybrid striped bass industry will be complicated because not only must desirable traits be selected for in both parental stocks, but they also must be expressed in the hybrid offspring. Genetic improvement will very likely involve a program of reciprocal recurrent selection. This program involves identifying, selecting traits, and maintaining parental stocks that produce desirable traits in hybrid offspring.

At present, the industry depends primarily on the capture of wild broodstock. However, for genetic improvement to occur, broodstock must be domesticated, a *de facto* form of selection for tolerance of hatchery conditions (Hallerman 1994, Harrell 1984, Smith and Jenkins 1984, Woods *et al.* 1990). This has been done on a very limited scale within the industry, at the University of Maryland Crane Aquaculture Facility, and at only a few research facilities (North Carolina Sate University, Southern Illinois University). Strain evaluation for desirable traits must occur concurrently with domestication. Both parental stocks have widespread natural distributions in eastern North America but appear to show limited morphological variation (Waldman *et al.* 1988, Waldman and Wirgin

1995). However, some northern strains of striped bass fry grow faster than fry from southern strains (Brown 1994, Brown *et al.* 1998). That gradient was not evident when Jacobs *et al.* (1999) evaluated 19 other families for growth rate and found Maryland and Florida strains grew faster than South Carolina and New York strains. Some white bass strains have also been domesticated (Kohler *et al.* 1994, Smith *et al.* 1996), and comparisons of sunshine bass production from these strains indicated greater fillet dress-outs for fish of northern decent (Kohler *et al.* 2001). Heritability of these traits is unknown but it is essential that baseline information of genetic correlations among commercially important traits such as growth rate, disease resistance, and dress-out percentages be determined for a selective breeding program to develop (Hallerman 1994).

ACKNOWLEDGEMENTS

The author wishes to thank Jason Brown, Nancy Ludwig, and Drs. Ken Davis, Tim Pfeiffer and Peter Perschbacher for reviewing the manuscript before it was submitted for publication. The use of trade names does not imply endorsement by the U.S. Department of Agriculture or the author.

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