Western Regional Aquaculture Center

Investigation into the Solution of Critical Problems of the Recirculation Aquaculture Industry in the Western Region

TERMINATION REPORT

2001-2006

TERMINATION REPORT

PART I: SUMMARY

REASON FOR TERMINATION: End of funding

PROJECT OBJECTIVES

Project objectives were:

- 1. To provide recirculation system design information specific to the needs of cool and cold water species cultured in the Western Region (WSU).
	- to develop nitrification design criteria for cool or cold temperature applications
	- to evaluate the fine solids removal performance of different biofilters
	- to optimize the design and operation of biofilters
- 2. To evaluate RAS design criteria at commercial production scales (WSU)
	- to evaluate the design criteria through a new commercial trout system
	- to evaluate the design criteria through a new commercial sturgeon system.
- 3. To develop methods to control off-flavor compounds in RAS (UCD)
	- to develop a treatment method for controlling off-flavor compounds that is compatible with commercial RAS and that is economically viable
- 4. To provide guidelines (Best Management Practices) for meeting the new regulations written by the EPA (**This is the primary outreach objective)** (UA, WSU).
	- to interview Western aquaculture producers and use existing literature to determine which effluent components will be most problematic with new EPA regulations.
	- to develop Best Management Practices that operators of RAS can utilize to improve their RAS and to meet effluent guidelines
	- to develop additional outreach materials that will inform users of RAS of improvements in equipment and techniques developed by the work group

PRINCIPAL ACCOMPLISHMENTS

*Objective 1***: To provide recirculation system design information specific to the needs of cool and cold water species cultured in the Western Region (WSU)**

Nitrification rate as a function of total ammonia nitrogen (TAN) concentration, with and without the interaction of organic matter, was investigated for three types of biofilters of laboratory scale: floating bead filter (FBF), fluidized sand filter (FSF), and submerged biocube filter (SBF). The performance of each type of biofilter was evaluated using a 5-reactor series with synthetic solutions containing different carbon/nitrogen ratios (C/N=0, 0.5, and 2.0). The tests were run at representative cold water aquaculture system temperatures of 10, 15 and 20[°]C respectively. In year 2004, data from tests at 15[°]C and 20[°]C, C/N=0, 0.5, and 2 were analyzed to determine the effect of substrate concentration, biofilter type, and temperature on the nitrification rate. Data from the 10° C tests were analyzed in 2005 and combined with the results at 15° C and 20° C to provide nitrification design information for cold and cool water systems. The highlights of these results are as follows:

- 1) The Nitrification rate of all three types of biofilters could be modeled linearly $(R^2>0.9)$ as a function of TAN concentration without the interaction of organic carbon at low TAN concentrations ([TAN] <6 mgl⁻¹).
- 2) Organic carbon had a significant (p<0.0001) inhibition on nitrification performance for all three types of biofilters. Key effects to nitrification performance were: as the C/N changed from 0 to 0.5, nitrification rates decreased dramatically and as C/N changed from 0.5 to 2 the nitrification rate of the floating bead filter (FBF) continued to decrease while the nitrification decrease was insignificant for the fluidized sand filter (FSF) and submerged biocube filter (SBF). With C/N=2, all three types of biofilters showed zeroorder nitrification kinetics over a substrate concentration of $3 \sim 11 \text{mgTANl}^{-1}$.
- 3) Without the interaction of organic carbon, the FBF had a nitrification rate of 12% and 50% higher than that of the FSF and SBF respectively; however the difference among them became less significant and even insignificant with an increase of organic carbon.
- 4) Temperatures of 15°C and 20°C had no significant impact (p=0.283, α =0.05, n=75) on the nitrification rate of the three types of biofilters tested.

The results of this study provide useful information for the design of nitrification biofilters for cold water RAS applications.

*Objective 2***: To evaluate RAS design criteria at commercial production scales (WSU)**

The cool/cold water RAS, served as a demonstration system and as the baseline system operated from January- July 2005 in the Aquaculture Education and Research Lab at WSU. The system was monitored for water quality, water consumption, energy consumption, and system performance.

Modeling of biofilm and Biofilter nitrification design recommendations for cold water RAS

A simplified analytical model was developed to solve the mass flux of ammonia and COD in multi-species biofilms. Based on the concept of equilibrium mass flux at the liquid-biofilm interface from the external and internal mass balance, this simplified model can be solved easily by an integrated interaction process within an Excel spreadsheet. A comparison of the performance of the simplified model against results from complex numerical solutions resulted in deviations of less than 10%. Comparison between bench scale data and the WSU cool/cold water RAS results indicated that the addition of organic matter can cause an 0ν 80% reduction in the nitrification rate of biofilters with the deficiencies associated with system scale-up causing another 10~80% reduction therefore, the ammonia nitrification rate in a commercial scale production system can be determined as,

R_A (actual) = $\alpha^* \beta^* R_L$ ($\alpha = 0.2 \sim 1.0$, $\beta = 0.2 \sim 0.9$)

Where, R_L = the TAN removal rate from a pure culture bench scale biofilter, mg m⁻² d⁻¹; α = reduction coefficient due to the effect of organic matter; β = reduction coefficient due to scale-up deficiency.

this model provides useful information for the design of cool and cold water RAS. A complete discussion on biofilm model parameters selection is provided for the end users of this model.

*Objective 3***: To develop methods to control off-flavor compounds in RAS (UCD)**

Geosmin and 2-methylisoborneol (MIB) have been reported as the major compounds causing "earthy" and "musty" odor in aquaculture waters and related "off-flavor" in fish. A preliminary extended aeration study was successful for the removal of geosmin and MIB concentrations of 100 ng/L in less than 24 hours. Further degassing tests, with clean tap water in the laboratory and using effluent water from an aquaculture farm, were performed to measure geosmin and MIB removal in a 1.83 m high, 0.14 m diameter packed aeration column. The tests were carried out in water spiked with a geosmin and MIB stock solution prepared in methanol. Removal rates did not vary significantly for the gas to liquid flow rate ratios tested (approximately 1 to 10 on a volume basis), but there were differences between the results obtained in the laboratory with clean tap water and those obtained using effluent water from an aquaculture farm. Tests were carried out at a hydraulic loading rate of 78 m³ m⁻ ² h⁻¹. The K values obtained for geosmin and MIB were 0.33 ± 0.06 m⁻¹ and 0.66 ± 0.14 m⁻¹ for the laboratory and farm trials, respectively. These K values would result in approximate removals of 60 and 80% of influent concentrations for a 2.0 m tall column using the laboratory and farm results, respectively.

*Objective 4***: To provide guidelines (BMP) for meeting the new regulations written by the EPA (US, WSU)**

We have provided guidelines, blank forms, and example BMP's for meeting the new regulations at <http://ag.arizona.edu/azaqua/extension/BMPs/bmp.htm>. Development of outreach publications for individual states is continuing. We have developed and updated a comprehensive web site that describes the release of the effluent guidelines that were being proposed by EPA. http://ag.arizona.edu/azaqua/extension/BMPs/Final_EPA.html We also added documents from the National Sea Grant Law Center and the EPA regarding management practices to comply with the new regulations, links to compliance meetings held across the country are included in the website. Also, links to the recirculating aquaculture conferences and short courses and WRAC research reports were embedded as they were considered valuable sources of pertinent information. Guidelines (BMP's) for effluents in Arizona were developed and submitted to state agencies. Idaho has also developed a BMP document for intensive systems but does not include recirculating systems.

An additional aspect of the project is to develop an enterprise budget for a model recirculating system in the western US. The enterprise budget will be modified with two alternative scenarios incorporating the expenses and projected benefits of the off-flavor treatments developed at UC-Davis and the cool water nitrogen and biofilter technologies developed in the WSU aspect of the project. We anticipate having these models available by the time of the IAC-TC. Jim Durfey is planning to coordinate with the Idaho Research, Aquaculture Extension faculty and fish producers in Southern Idaho in early September to discuss findings of this project.

IMPACTS

Many industry members have been informed of the exact situation of the new effluent guidelines and requirements. There had been a large amount of mis-information and preliminary recommendations that were not implemented causing tremendous confusion. By providing accurate information and subsequently providing the tools for industry members to succinctly meet the requirements we have helped smooth the transition.

Growers are aware of the need to continually improve management strategies of their water resources. We no longer have the inexhaustible supply of water for single pass style production systems. As the need for fish products increases, recirculation technologies will provide producers the tools with which they can increase production with the same water resources. The opportunity to development recirculation technologies through joint operation of private producers and public land grant institutions creates a unifying pathway to help growers transition in these technologies. As growers transition into recirculation systems, their attention to detail of inputs and outputs of their system will be more critical. As growers adopt these management strategies as outlined in the BMP's <http://ag.arizona.edu/azaqua/extension/BMPs/bmp.htm> they will become more familiar with a standardization occurring throughout industry.

RECOMMENDED FOLLOW-UP ACTIVITIES

The use of recirculation systems continues to grow and new problems will need the attention of the research community to ensure the economic viability of recirculation systems. Some of these include the development of systems that are more energy efficient, the treatment of effluents, and improvements in water treatment effectiveness to allow for reductions in water use. Additional presentations at local, regional and national meetings will further inform the producers of how best to meet the regulations and implement new technologies that should improve their operations.

As a part of the extension and outreach, during the Fall of 2006 there will be visits with the growers to discuss these systems with Idaho research, extension and producers. A follow up contact with these growers will be done to see what progress is made to adopt these recirculation technologies.

PLAN OF WORK FOR REMAINING FUNDS

Remaining funds will be used to participate in conference and meetings (travel funds) and to develop outreach products based on results of the research carried out (i.e. student support, supplies, and computer equipment). This will include posting of additional information at the website and an outreach report on an enterprise budget for a typical recirculating system in the Western US.

In addition to the posting of information on the website, contact with the growers in a year following the termination will take place to see what adoption of these technologies has taken place.

SUPPORT

 $⁽¹⁾$ Washington State University</sup>

(2) **AquaKing** - Funds for intern to conduct research at recirculation tilapia farm in California.

(3) USDA-ARS - Funds for Dr. Kevin Schrader's participation in the Off-flavor work.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in Print and Manuscripts

Acuña-Rubio, S. 2004. Evaluation of methods for geosmin and MIB removal from recirculation aquaculture systems. M.S. thesis. University of California, Davis. 100p. Chen, S., Ling, J., Blancheton, J.P. 2006. Nitrification kinetics of biofilms as affected by water quality factors. Aquacultural Engineering 34:179-193.

Fitzsimmons, K. 2005. Tilapia culture. pp. 563-590. In: Kelly A.M. and Silverstein, J. eds. Aquaculture in the 21st Century. American Fisheries Society, Symposium 46, Bethesda, Maryland.

Ling, J. and Chen, S. 2005. Impact of organic carbon on nitrification performance of different types of biofilters. Aquacultural Engineering, 33, 150-162.

Ling, J., Huyser, M.J., Chen, S. 2005. Comparison and evaluation of solids removal efficiency between a floating bead filter and a trickling filter. International Journal of Recirculating Aquaculture (in review).

McIntosh, D., Ryder, E., Dickenson, G., and Fitzsimmons, K. 2004. Laboratory determination of a phosphorus leaching rate from trout (*Onchorhynchus mykiss*) feces. Journal of the World Aquaculture Society. 35: 506-512.

Piedrahita, R.H., Acuña-Rubio, S., Schrader, K.K., and Rimando, A.M. 2006. Evaluation of Degassing for Geosmin and MIB Removal from Recirculation Aquaculture Systems. Aquacultural Engineering (in review).

Schrader, K.K., Acuña-Rubio, S., Piedrahita, R.H., and Rimando, A.M. 2005. Geosmin and 2 methylisoborneol cause off-flavors in cultured largemouth bass and white sturgeon reared in recirculating-water systems. North American Journal of Aquaculture 6:177-180.

Papers Presented

Acuña-Rubio, S., Piedrahita,R.H., Schrader, K.K., and Pace, R.K. 2004. Evaluation of methods for geosmin and MIB removal from recirculation aquaculture systems. Abstract and poster presented at the World Aquaculture Society meeting 2004, Honolulu, Hawaii (March 2-6, 2004).

King, C. and Fitzsimmons, K. 2004. Use of aquaculture effluents to fertilize tomatoes in an aquaponic system. Abstract from Asia-Pacific Chapter of WAS Annual Meeting. Sydney, AUSTRALIA.

Fitzsimmons, K. 2005. Advances in tilapia aquaculture. Idaho Aquaculture Association Annual Meeting, June 2005.

Fitzsimmons, K. 2005. Introduction to aquaculture in Arizona and the West. Extension Agents Professional Development Conference. August 2005.

Fitzsimmons, K. 2005. Strategies for Intensive Aquaculture. 2do Foro Internacional de Acuicultura. Hermosillo, Sonora.

Submitted: Rau*lki chalitale*. 2006, Sep, 8

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 2006, Sept. 8

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PARTICIPANTS:

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TECHNICAL SUMMARY AND ANALYSIS

*Objective 1***: To provide recirculation system design information specific to the needs of cool and cold water species cultured in the Western Region (WSU)**

The effectiveness of the nitrification process can be evaluated by nitrification kinetics. The rate of ammonia or nitrite oxidation depends strongly on the concentrations of the substrates in the bulk solution. Various parameters influence the nitrification process including dissolved oxygen (DO), temperature, pH, ammonia and nitrite concentration, organic loading, and hydraulic loading rate (Sharma and Ahlert, 1977).

The experimental system consisted of three types of biofilters: floating bead filter (FBF), fluidized sand filter (FSF) and submerged bio-cube filter (SBF). Each "type" series was composed of five biofilters in a sequence, each being connected to an individual aeration sump inside which a submersible pump recycled substrate solution through the biofilter. Each sump was provided with an air diffuser not only to maintain a sufficient oxygen level for the connected biofilter but also to obtain a completely mixed condition in the sump. A multichannel peristaltic pump (MASTERFLEX^R PUMPS) was used to supply a consistent volume of substrate solution to the first reactor of each biofilter series. Flexible tubes were set up between sumps to deliver the substrate solution through the entire reactor series by gravity flow. The whole system was placed in a water bath where temperature was maintained by a heater or chiller. Bead and sand filters were made of clear PVC, utilizing plastic beads (Aquaculture Systems Technologies) and 20/35 retaining mesh sieve size sand as filter media respectively. Submerged bio-cube filters were constructed from flat bottom cylindrical tanks with Bio-cube 650 (Keenton Industries, Inc) as the biofiltration media. The total surface area of the submerged bio-cube filter was maintained at twice the surface area of either the bead or the sand filter **(**Table 1**)**. The flow rate in Table 1 corresponds to the substrate flow rate provided by the peristaltic pump; i.e. the flow rate (solution deliver rate) through the reactor series. However, the flow rate through the biofilters, were generated from the submersible pump located inside the sump and were different from the substrate flow rate.

Nitrification kinetics of biofilters without the interaction of organic matter

In aquaculture systems, biofilters are operated at low TAN concentrations and TAN has been considered the rate-limiting factor for the nitrification process (Wheaton et al, 1994). The minimum TAN concentration was not considered for the nitrification kinetics, but it is very critical to incorporate S_{min} into the nitrification kinetics of aquaculture biofilters. Therefore, a modified Michaelis-Menten (M-M) model (Zhu and Chen, 1999) was used to predict the relationship between TAN removal rate and TAN concentration:

$$
R = R_{\text{max}} \frac{S - S_{\text{min}}}{K_s + S - S_{\text{min}}} \tag{1}
$$

where R_{max} is the maximum TAN removal rate (mgm⁻²d⁻¹), K_s is the half saturation constant (mgl⁻¹), and S_{min} is the minimum TAN concentration (mgl⁻¹). A minimum TAN concentration of 0.07mgl⁻¹ was determined in the previous study (Zhu and Chen, 1999) therfore by substituting into the above model.

$$
R = R_{\text{max}} \frac{S - 0.07}{K_s + S - 0.07}
$$
 (2)

The factors, R_{max} and K_s were calculated with experimental data by Lineweaver-Burke plots. The parameters are listed in table 2 for each of the biofilters under the three temperature levels. Since cold and cool water aquaculture systems are usually operated at a TAN lower than 3 mg $1⁻¹$, first order nitrification kinetics can be applied and equation 2 can be simplified as,

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

By substituting the obtained parameters, R_{max} and K_s , into equation (3), first order nitrification kinetics models can be developed for all the three types of biofilters (Table 2). These models are valuable tools for the design of floating bead filters, fluidized sand filters, and submerged bio-cube filters with the consideration of other impacting factors.

The experimental and model results of the affect of TAN concentration on nitrification rates of the three types of biofilters were plotted (Figure 1**)** without the impact of organic carbon (C/N=0). It can be seen that the experimental data had a strong correlation with results predicted by the modified M-M model for TAN concentrations ranging from 0 to12 mgl⁻¹ $(R^2 > 0.9$ for results at 15 and 20°C and $R^2 = 0.2 \sim 0.6$ for data at T=10°C).

It can be seen from Figure 1 and Table 2 that the nitrification rate (mg $m^2 d^{-1}$) of the submerged bio-cube filter was much lower than the floating bead filter or the fluidized sand filter. It is the authors' speculation that this was due to the difference in hydraulic conditions among the different biofilters. With a relatively low flow rate, the submerged bio-cube filter may have undergone insufficient turbulence within the reactor and was less efficient at transfering nutrients into the biofilm. Conversely, the high flow rate of the fluidized sand filter and the frequent backwashing of the floating bead filter contributed to the increase of the diffusion rate providing an active biofilm and resulting in a productive nitrifying bacterial population.

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

Statistical analysis indicated that the nitrification rate of the three types of biofilters were not significantly different at 15 or 20° C. However, all three types of biofilters exhibited significantly lower nitrification rates at 10° C. It is believed that the nitrification rate difference between biofilters decreased with temperature due to the higher dissolved oxygen (DO) concentration available at a lower temperature. The affect on the nitrification rate due to the

mass transfer through the biofilm created by the different flow regimes of the biofilters was assumed to be somewhat masked by the increase in DO concentration of the bulk solution.

Effect of organic matter on biofilter nitrification rates

The impact of organics on the biofilters nitrification performance was evaluated by operating the three biofilter types under two different C/N ratios (0.5 and 2). However, since the C/N ratios were not consistent through the five reactors in a biofilter series, the nitrification kinetics corresponding to TAN substrate concentrations were evaluated using the first reactor from each series under the same substrate concentration $(10 \pm 0.92 \text{ mg TAN} \, \text{L}^{\text{-}1})$.

Biofilters operated at a TAN concentration of 10 mg $1⁻¹$, demonstrated an exponential decrease of the nitrification rate with an increase of the C/N ratio under both temperature regimes. For this study the C/N ratios were converted to COD/N ratios for the convenience of comparison with previous studies. The C/N ratios of 0, 0.5 and 2, were equal to COD/N=0, 1.4 and 5.4, respectively, according to a ratio of COD/C=2.68 with sucrose as the organic carbon source (Zhu and Chen, 2001). The COD of sucrose can be determined by assuming a complete oxidation into $CO₂$ and water. The relationship between COD/N and nitrification rate in this study was very similar to the results obtained from an activated sludge system (Carrera et al., 2004). Figure 2 illustrates the effect of COD/N ratios on the nitrification rate for a bead filter (20° C) and an activated sludge system (25° C). The error bar was defined as the standard deviation of the mean value. The inhibition due to organic carbon on the nitrification rate is apparent and similar for both systems. As COD/N increased from 0 to 1.5 (C/N \approx 0.5), the nitrification rate declined rapidly but the degree of inhibition due to organics decreased as the COD/N ratio increased. If the COD/N ratio was higher than 3 ($C/N \approx 1$) the nitrification rates of both systems tended to remain unchanged at a minimum value. This indicates that the inhibition of organic matter on nitrification could be maximized as the growth of heterotrophic bacteria reached a saturation level corresponding to a certain COD/N ratio (COD/N=3 in this study). In the same manner, the relationship between the nitrification rate and influent COD/N ratio for the other two types of biofitlers can be defined by an exponential function $(r^2 = 1.0)$. Regression models of the effect of organic matter on biofilter nitrification rates are provided in Table 3. The results provide useful information for the design of nitrification biofilters which are operated in mixed culture conditions such as aquaculture systems. In order to clarify the negative effect of COD/N ratio on biofilter nitrification rates, the percentage TAN reduction rates of biofilters corresponding to the increase of COD/N ratio was calculated and plotted in Figure 3 for each temperature level. From Figure 3, the inhibition of organic matter on nitrification of different biofilters is clearly shown. The reduction in nitrification rate of the three types of biofilters was approximately 50~80% under the test conditions when the COD/N ratio increased from 0 to 5.4 (C/N=2). It also can be seen that the degree of impact on nitrification due to organics decreased as temperature decreased. A TAN removal rate reduction of $50~80\%$, $50~70\%$, and $45~50\%$ was seen at 20, 15, and 10 ° C respectively. It is thought that this was due to the higher DO saturation concentrations at lower temperatures possibly resulting in less competition between heterotrophs and autotrophs for oxygen sources.

Among the three types of biofilters, the floating bead filter was observed to exhibit a greater reduction in nitrification rate due to the presence of organic matter than the fluidized sand filter or submerged bio-cube filter at 15 and 20° C. However, the effect was similar for all three types of biofilters at 10° C. A possible reason for the greater effect on nitrification rate was due to the bead filters packed bed which was easily clogged by filamentous heterotrophs

at higher organic loading. Therefore, it appears that bead filters require more frequent backwashing at high COD/N ratios than provided during the study to decrease the negative effect of organic matter.

The relationship between the floating bead filter nitrification rate and influent COD/N ratios at 20^oC can be defined by an exponential function ($r^2 = 1.0$) according to equation 4, which is similar to the relationship developed in suspended growth systems by Carrera et al., 2004 **(**equation 5**)**.

$$
R = 0.67 + 2.27 e^{(-1.38(COD/N))}
$$
 (This study) (4)

$$
R_{nitrification} = 0.0323 + 0.334e^{(-1.660(COD/N))}
$$
 (Carrera et al., 2004) (5)

where the nitrification rate in the fixed film process was defined by g TAN removal per unit biofilm surface area per day, while the nitrification rate in the suspended growth system was defined by g TAN removal per g volatile suspended solids (VSS) per day (Equation 6).

$$
R_{nitrification} = \frac{Q_{in}([NH_4^+ - N]_{in} - [NH_4^+ - N]_{out})}{V_{reactors}[VSS]_{reactors}}
$$
(6)

Where Q_{in} Influent flow rate, $L^3 T^{-1}$, V_{reactors} Reactor working volume, L^3 . and [*VSS*] _{reactors} VSS concentration in reactor, M $L³$. In the same manner, the relationship between nitrification rates and COD/N ratios was developed for the other biofilters at at each temperature.

The fluidized sand filter exhibited less effect due to the addition of organic carbon compared to the other two types of biofilters, with a reduction of $46{\sim}54\%$ in nitrification rate at the three temperatures. The fluidization of the bed in the sand filter contributed to the self-cleaning of the sand media and provided more resistance to the negative effect due to organic matter. This also implies that the fluidized sand filter has an advantage for systems operated at high organic loadings.

Modeling and Design of nitrification in biofilms

The purpose of this part of the research was to develop a simplified but well constructed model by incorporating the major physical and chemical input parameters and biofilm kinetics of the reactor to provide useful information for the optimization of biofiter design. To achieve analytical solutions for the model, mathematical simplification was applied to determine the intermediate parameters in addition to the simplifications on the physical characteristics of the biofilm model. This model was developed as a spreadsheet so that it could be more practically utilized. The accuracy of the simplified model was evaluated in comparison to numerical solutions and parameter estimation guidelines were also provided for the application of this model in wastewater treatment and aquaculture systems. The biofilm model developed in this research is based on a one-dimensional steady-state model with consideration of multiple substrates and multiple species. From an engineering aspect, the 1D model is sufficient because the thickness of the biofilm is much smaller compared to the surface area. Scanning electron microphotographs demonstrating biofilm thickness and biofilm morphology under different loading conditions for floating bead filters at $T=20^{\circ}$ are shown in Figure 4. Because the substrate utilization and diffusion rate occurring within the biofilm are very fast compared to the bacteria growth rate in the biofilm, a steady-state mass balance on the substrate at any point in the biofilm is appropriate (Rittmann and Manem, 1992).

To achieve this goal, the simplified model was developed in two steps. First, mass balance equations of substrates (TAN, COD and DO) were developed for both the external and internal diffusion layers of the biofilm. Then, the mass balance equations from both layers were combined and solved for the mass transfer flux into the biofilm based on the fact that an equilibrium mass flux exists at the interface of the two layers (also called liquid-biofilm interface). The obtained substrate mass flux, the flux of TAN more specifically, was then used for nitrification biofilter design. The effect of temperature and hydrodynamic conditions on mass transfer was also included in the biofilm model so as to reflect the variation of flow regime in different types of biofilters as well as the variation in operating temperature. With its simplicity and convenience for application, this model is a useful tool in guiding the design and operation of biofilters in recirculating aquaculture systems. In the development of the model, COD was used for the substrate associated with organic matter for heterotrophs and TAN (total ammonia nitrogen) was used for the substrate of autotrophs. Also for model simplicity, the intermediate nitrite was not considered and the nitrifiers were treated as one "species" and that ammonia was oxidized directly to nitrate (Rittmann et al., 2004).

Model application for biofilter design

To design a biofilm reactor, the system parameters, such as flow rate, influent characteristics and effluent requirements are presumed to be provided and serve as the input for the calculation of the substrate mass fluxes into the biofilm, which can then be used to estimate the required biofilm surface area and the reactor volume. The kinetic parameters for heterotrophs and autotrophs (nitrifiers) were obtained from the literature and are summarized in Table 4 and 5. The values used for the biofilm model computation were based on the average of the literature results.

Biofilter design procedure with the simplified analytical model (SAM) spreadsheet

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

Validation of biofilm model with experimental data

To further verify the simplified model for biofilter nitrification prediction, experimental data from the reactor series system at 15°C were compared with the results of the simplified model based on the estimated parameters. As seen in Figure 5, the simplified model can provide a satisfactory prediction of nitrification rates under different C/N ratios. The correlation of determination (R^2) between predicted and observed values was low $(0.37 \text{ and } 0.18)$ for the tests of the fluidized sand filter and the submerged bio-cube filter at C/N=2. This may be due to the fact that the correlation of determination is oversensitive to extreme values. The scattered data points obtained from these two tests resulted in low $R²$ correlating to model predictions although the model curves fit within the range of observed data points. This model provided useful results for the effect of organic matter on the biofilter nitrification rate. For

*Objective 2***: To evaluate RAS design criteria at commercial production scales (WSU)**

WSU commercial scale cold/cool water RAS

A recirculating system for cold or cool water species was initiated and developed by the aquaculture engineering group at WSU. The WSU cold/cool water RAS consisted of the following components: (1) two "Cornell Dual-Drain" style culture units; (2) a radial/vertical flow clarifier; (3) a fluidized sand filter; (4) an oxygen cone; (5) a ultraviolet-disinfection unit (UV); and (6) two $CO₂$ stripping columns (Table 6).

The WSU RAS was stocked with approximately 10000 rainbow trout fry (size: 1.8 g/fish) in January, 2005. The system was stabilized for at least two months before water samples were collected to evaluate for fish growth and nitrification performance of the fluidized sand filter. The system was monitored for DO, pH, temperature, alkalinity and fish mortalities on a daily basis. Water samples were collected for the measurement of TAN, $NO₂$, BOD₅, and COD from the middle of March until the end of July.

1. System performance: fish growth rate, fish mortality, and feed conversion rate

Data from 6/21/2005 to 7/19/2005 were used to evaluate the system performance in terms of fish growth. During this period, the average fish weight was increased from 58.4 g/fish to 79.6 g/fish with a feeding rate of $2.0 \times 2.5\%$. The total biomass increased from 449 to 598 kg. The system was successful in raising rainbow trout at a high density of 0.7~0.9 lbs/gallon with a low mortality of 174 in total (about 6 morts/day). The feed to gain ratio was approximatley 1.47 during this period.

2. Fluidized Sand filter nitrification rates

Water samples were collected at the influent and effluent of the fluidized sand filter and measured for TAN, $NO₂$, $BOD₅$, and COD. The nitrification rate was then calculated and compared to the results of the bench scale fluidized sand filter from the reactor series system (Figure 7). Due to the high C/N ratio in the influent to the biofilter (BOD/N≈50), the fluidized sand filter was considered to encounter the maximum organic impact with a reduction in nitrification rate of 50 % according to Table 3 and Figure 3. As a result, the design equation of the sand filter in Table 2 was adjusted for a 50% reduction in nitrification rate and the design equation becomes,

$$
R = 278S - 19.5 \tag{7}
$$

As can be seen in Figure 7, the fluidized sand filter followed a first order nitrification rate similar to the first order nitrification kinetics of the bench scale fluidized sand filter and the volumetric nitrification rate was within the range of results from other commercial systems (Timmons et al., 2001) with a TAN removal rate of $0.46 \sim 1.4$ kg/m³-media/ day and $0.15 \sim 0.47$ kg/m³-expanded media/ day (based on a maximum expansion of 300%, actual expansion 150~300%). Additionally, the nitrification performance of the WSU cool/cold water RAS was compared with pilot and commercial scale RAS using the bench scale FSF as the standard for

performance (Table 7). It is clear that nitrification performance is quite variable between systems. The possible reasons for the discrepancy between systems are as follows;

- a. Scale up issues. The biofilters' nitrification performance was reduced due to less than optimal operating conditions. Ester et al. (1994) also reported that the nitrification of a commercial scale RBC was reduced by a factor of about 3 compared to a laboratory scale reactor. It was found at the end of the present study that about 2 inches of sand remained static at the bottom of the filter during operation, which may have contributed to the low nitrification rate of the biofilter.
- b. Differences in the influent wastewater composition. The bench scale sand filter was fed with a synthetic chemical solution. The fluidized sand filter was evaluated utilizing more complex fish culture wastewater.
- c. Problems with the media. Very fine sand with an average size of 185 µm and a high specific surface area of 23800 m^2/m^3 was utilized in the fluidized sand filter. Problems with the fine sand fluidized bed reactor included sand washing out of the reactor and bio-fouling possibly leading to negative effects on the nitrification performance.

3. Biofilter nitrification design recommendations for cold water RAS

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

$$
R_{A} (actual) = \alpha^{*} \beta^{*} R_{L} (\alpha = 0.2 \sim 1.0, \beta = 0.2 \sim 0.9)
$$

Where, R_L = the TAN removal rate from a pure culture bench scale biofilter, mg m⁻² d⁻¹; α = reduction coefficient due to the effect of organic matter; β = reduction coefficient due to scale-up deficiency.

For the design of floating bead, fluidized sand, and submerged bio-cube filters, α can be determined by Figure 3, while β has to be determined by comparing commercial scale data with laboratory scale data. Table 8 illustrates the necessary steps for the calculation of biofilter nitrification rates in a production scale system based on the design equations developed from the lab-scale series reactor system. The nitrification design equation was first selected from Table 2 after the biofilter type and operating temperature were determined. Then, the selected design equation was corrected according to α and β . Recommendations on the other operating parameters based on literature results (Chen et al., in press) are also presented in the same Table 8. The effect of DO concentration on biofilters' nitrification rates at different bulk TAN concentrations was developed in Figure 8 with the simplified biofilm model.The effect of DO/TAN ratio on nitrification rates was similar to all three types of biofilters. Based on the simulation results with the biofilm model, the DO concentration in bulk liquid of biofilters was recommended with a DO/TAN ratio above 2 for TAN = $1 \sim 2$ mg 1^{-1} or DO>2 mg 1^{-1} for TAN concentrations lower than 1 mg 1^{-1} in order to achieve over 80% nitrification compared to an ideal nitrification without DO limitation.

It was concluded that the nitrification design equations drawn from the study of the reactor series system were valuable in terms of providing guidelines for cold/cool water RAS design. However, in addition to the consideration of a reduction coefficient factor necessitated by organic impact, a supplementary coefficient corresponding to the difference between a commercial system and the bench scale system, and water quality parameters should be considered as well.

*Objective 3***: To develop methods to control off-flavor compounds in RAS (UCD)**

Aeration has been shown as a cost-effective method for the removal of organic contaminants from water (Nirmalakhandan et al, 1990). Although this technique is common in RAS for the removal of carbon dioxide, no data are available about mass transfer coefficients or removal rates of off-flavor compounds through aeration. A common degassing system is the packed column aerator (PCA) (Hackney and Colt, 1982; Colt and Bouck, 1984), and PCAs have been used for the removal of gases such as carbon dioxide (Grace and Piedrahita, 1994) or for low volatile organic contaminants (Nirmalakhandan et al., 1990).

Two types of aeration tests were carried out: extended aeration with diffusers and use of a packed column (PCA). In all cases, the work was carried out with water spiked with geosmin and MIB solutions. Samples for geosmin and MIB analysis were collected and stored at 3 ºC before being shipped overnight in insulated containers to the U.S. Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University, Mississippi, for analysis using solid-phase microextraction (SPME) and gas chromatographymass spectrometry (GC-MS) with a detection limit of 1 ng/L (Lloyd et al., 1998; Schrader et al., 2003).

Preliminary experiments were performed to determine the feasibility of using degassing to remove geosmin and MIB in aquaculture systems by measuring their concentrations as a function of time in an aerated water sample. The water was spiked with geosmin and MIB to an initial concentration of 100 ng/L before starting the air flow. Samples were collected at various time intervals up to 24 h. The concentrations of geosmin and MIB in the extended aeration tests decreased to non-detectable levels after 18 to 24 hours of aeration. Given the complete removal of geosmin and MIB, saturation concentrations were assumed to be 0 ng/L for calculations of mass transfer rate coefficients in the PCA tests.

Column experiments were carried out using a 1.83 m tall PCA (Grace, 1987) using clean tap water and using water from the effluent of an aquaculture farm. The concentrations of geosmin and MIB in the PCA influent were 1,000 ng/L for the laboratory tests and 200 ng/L for the farm tests. The performance of the column was evaluated for a hydraulic loading rate of 78 m³ m⁻² h⁻¹ at gas to liquid volumetric flow ratios (G/L ratios) between about 1:1 and 10:1 in the laboratory tests (24 °C) and between about 1:1 and 3.5:1 for the on-farm tests $(23-25)$ °C) (Hackney and Colt, 1982; Colt and Bouck, 1984; Grace, 1987).

There were no differences between the behaviors of geosmin and MIB, hence the results for the two compounds were combined. Figures 9 and 10 show the percentage of geosmin and MIB remaining at each of the ports with respect to the influent concentration for all G/L ratios for the laboratory and farm tests, respectively. As expected, off-flavor removal increased as the water flowed down the PCA, obtaining the highest off-flavor elimination at the lowest port (#6). For the laboratory tests, the maximum overall removal of 77% was achieved at a G/L ratio of 3.8, compared to a minimum of 41% at a G/L ratio of 9.4. Corresponding values for the farm tests were a maximum overall removal of 81% at a G/L ratio of 1.9 and a minimum of 59% at a G/L ratio of 1.4.

Removal between the reservoir and the first port was substantial and ranged from 29% of influent concentration for the 2.8 and 4.7 G/L ratios to 14% for the 1.9 and 7.9 G/L ratios for the laboratory tests, and from 39% of influent concentration for the 1.9 and 4.7 G/L ratios to 13% for the 2.4 G/L ratio for the farm tests. Removal prior to the first port is due to splashing in the distribution plate, hence in an effort to look at the degassing process in the packed column media, the data were recalculated to determine the percent of geosmin and MIB remaining with respect to the concentration in the first port (Figs. 11 and 12).

The overall mass transfer coefficient (K) (Hackney and Colt, 1982; Colt and Bouck, 1984) values ranged between 0.24 and 0.62 m^{-1} and between 0.40 and 0.87 m^{-1} for the laboratory and farm runs, respectively (Fig 13). The K values were also calculated separately for the combined laboratory and field data yielding values of 0.33 ± 0.06 m⁻¹ and 0.66 ± 0.14 m⁻¹, respectively.

There was wide variation in the results obtained (Figs. 9-12) and the results did not show a clear trend with respect to G/L ratio. A single factor ANOVA was carried out for each port depth (for the laboratory and field runs, Figs. 10 and 12) to determine if the differences among the percentages of geosmin and MIB remaining for the various G/L ratios were significantly different. The results indicate that the only case for which there was a statistically significant difference for the various G/L ratios was for Port 6 in the laboratory tests, although the significance was weak (F value of 2.62 vs. $F_{critical}$ of 2.33).

The mass of geosmin and MIB that could be transferred per hour was calculated (Fig. 14) assuming an influent concentration of 100 ng/L and using the overall K values determined for the combined laboratory and farm runs. The effluent concentration decreased and the amount of geosmin and MIB removed $[(\mu g/h)/(L/min)]$ increased as the column depth increased. The higher K value obtained for the farm runs resulted in higher calculated removal rates (Fig. 14). The ultimate effectiveness of a degassing system for controlling the concentration of geosmin and MIB in a RAS depends upon how the rate of production of the compounds compares to the rate of removal by degassing. The fact that off-flavor problems occur in systems in which strong aeration and degassing for carbon dioxide removal are used suggests that the rate of off-flavor removal by degassing is not necessarily sufficient to reduce the concentrations to eliminate off-flavor problems with the fish.

*Objective 4***: To provide guidelines (BMP) for meeting the new regulations written by the EPA (UA, WSU)**

We have provided guidelines, blank forms, and example BMPs for meeting the new regulations at <http://ag.arizona.edu/azaqua/extension/BMPs/bmp.htm>. EPA determined that the most practical method for aquaculture facilities to meet the effluent regulations was for farms to utilize Best Management Practices unique for each facility. By developing and following approved plans, each facility will reduce environmental impacts in the most cost effective manner.

The guidelines we posted include descriptions of the requirements of the effluent regulations developed by the EPA and how the Best Management Practices can be developed to meet those requirements. BMPs for Arizona facilities have been developed and submitted to the Arizona Department of Environmental Quality, the regulator for Arizona. Development of outreach publications for individual states is continuing.

We have developed and updated a comprehensive web site that describes the effluent guidelines that were published by EPA, and the impacts and methods to comply <http://ag.arizona.edu/azaqua/extension/BMPs/Final_EPA.html>. We also added documents from the National Sea Grant Law Center and the EPA regarding management practices to comply with the new regulations, links to compliance meetings held across the country are included in the website. Also, links to the recirculating aquaculture conferences and short courses and WRAC research report s were embedded as they were considered valuable sources of pertinent information. Guidelines (BMPs) for effluents in Arizona were developed and submitted to state agencies. Idaho has also developed a BMP document for intensive systems but does not include recirculating systems.

An additional aspect of the project is to develop an enterprise budget for a model recirculating system in the western US. The enterprise budget will be modified with two alternative scenarios incorporating the expenses and projected benefits of the off-flavor treatments developed at UC-Davis and the cool water nitrogen and biofilter technologies developed in the WSU aspect of the project. We anticipate having these models available by the time of the IAC-TC. Jim Durfey is planning to coordinate with the Idaho research, aquaculture extension faculty and fish producers in Southern Idaho in early September to discuss findings of this project.

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FIGURES

Figure 1 Impacts of TAN concentration on biofilters TAN removal rates (R-TAN) without the interaction of organic carbon; (a) $T=20^{\circ}C$; (b) $T=15^{\circ}C$; (c) $T=10^{\circ}C$.

Figure 2 Relationship between the nitrification rate and the influent COD/N ratio

Figure 3 Effects of COD/N ratio on biofilters TAN removal rates $(R-TAN)$: (a) T=20°C; (b) T= 15° C; (c) T= 10° C.

Figure 4 Scanning electron microphotographs demonstrating biofilm thickness and biofilm morphology under different loading conditions for floating bead filters at $T=20^{\circ}C$ a), b), c): measurement of bead filter biofilm thickness with $C/N= 0$, $C/N= 0.5$, $C/N= 2$ (500x); d), e), f): bead filter biofilm morphology with $C/N = 0$, $C/N = 0.5$, $C/N = 2$ (5000x).

Figure 5 Comparison between experimental results and biofilm model prediction for the effect of C/N ration on nitrification rates of: a) floating bead filter; b) fluidized sand filter; c) submerged bio-cube filter.

Figure 6 A schematic of the cold/cool water recirculating system recommended by WSU

Figure 7 Comparison of fluidized sand filter nitrification efficiency at a commercial scale and a bench scale

Figure 8 Nitrification rates (R) of biofilters relative to maximum rates (R_m , defined as nitrification rates without DO limitation) as affected by DO/TAN ratio.

Figure 9. Geosmin and MIB remaining at each port for all G/L ratios for the laboratory tests. Concentrations are expressed as a percentage of the influent concentration.

Figure 10. Geosmin and MIB remaining at each port for all G/L ratios for the farm tests. Concentrations are expressed as a percentage of the influent concentration.

Figure 11. Geosmin and MIB remaining at each port for all G/L ratios for the laboratory tests. Concentrations are expressed as a percentage of the concentration at Port 1.

Figure 12. Geosmin and MIB remaining at each port for all G/L ratios for the farm tests. Concentrations are expressed as a percentage of the concentration at Port 1.

Figure 13. Overall mass transfer coefficient (K) values for geosmin and MIB for the G/L ratios tested.

Figure 14. Effluent concentration and mass of geosmin and MIB removed as calculated with K values obtained for the laboratory and farm tests. The left and right arrows indicate the scale corresponding to the lines on the graph.

TABLES

Biofilter type	Floating bead filter	Fluidized sand filter	Submerged bio-cube filter
Water volume (1)	0.51	0.29	3.24
Expansion		50%	
Media specific surface area (SSA, m^2m^{-3})	1310	6070	361
Total biofilm area (m^2)	0.40	0.40	0.80
Flow rate $(l \text{ min}^{-1})$	1.78	2.26	1.11
Cross-sectional area $(cm2)$	20	11	182
Feeding rate $(m^3 d^{-1})$	0.216	0.216	0.216

Table 1. Specifications of three biofilter series.

Temperature $(^{\circ}C)$	Biofilter type	R_{max}	K_{s}	$\mathbf R$
		$(mgm-2d-1)$	(mgl^{-1})	$(mgm-2d-1)$
20	Floating bead	5000	8.5	$R = 588 * S - 41$
	Fluidized sand	3330	5.3	$R = 625 * S - 44$
	Submerged biocube	1670	5.5	$R = 345 * S - 24$
15	Floating bead	5000	9.5	$R = 526 * S - 37$
	Fluidized sand	3330	6	$R = 556 * S - 39$
	Submerged biocube	1670	6	$R = 278 * S - 19$
10	Floating bead	1000	2.4	$R = 417 * S - 29$
	Fluidized sand	1429	7.1	$R = 201*S-14$
	Submerged biocube	1250	$\overline{4}$	$R = 312 * S - 22$

Table 2. Biofilter nitrification kinetic constants and first order reaction rates at low ammonia concentration (TAN <3 mgl⁻¹).

		Temperature (
Biofilter type	$T=20$	$T=15$	$T=10$
Bead filter	$R=0.67+2.27e^{(-1.38(COD/N))}$	$R=0.85+1.88e^{(-1.36(COD/N))}$	$R=0.52+0.47e^{(-0.84(COD/N))}$
Sand filter	$R=1.0+1.16e^{(-0.92(COD/N))}$	$R=0.90+0.93e^{(-1.21(COD/N))}$	$R=0.48+0.40e^{(-1.75(COD/N))}$
Bio-cube filter	$R=0.33+0.65e^{(-1.38(COD/N))}$	$R=0.51+0.62e^{(-2.22(COD/N))}$	$R=0.40+O.38e^{(-1.14(COD/N))}$

Table 3. Expressions of the COD/N effect on nitrification rate (gm^2d^1) .

Table 4. Kinetic parameters for autotrophs.

Table 5. Kinetic parameters for heterotrophs.

System Components	Volume (gal)	$V(m^3)$	Media SSA $(m^2 m^{-3})$	Media volume (m^3)	Comments
Culture tank	1450	5.48			
Fluidized sand filter	238	0.90	23800	0.32	Model: FBB-50, Aquaneering Inc.
Floating bead filter	127	0.48	1310	0.17	Model: PBF-10S, Aquaculture Systems Technologies, LLC
Clarifier	250	0.95			15° cone bottom tank
Sump 1	375	1.42			
Sump 2	220	0.83			
Cone oxygenator	30	0.11			Aquatic-Eco Systems, Inc.
$CO2$ stripping column	40	0.15	160	0.11	

Table 6. Specifications of WSU cold water RAS components.